ASTRINGENCY CHARACTERIZATION OF TANNAT RED WINE

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ABSTRACT

Tannat is a red variety of *Vitis vinifera* that has become the emblematic red wine variety of Uruguay. Tannat wine is characterized by its high total phenolic content, which provides a good potential for aging and differential sensory characteristics. The aim of the present thesis was to characterize the astringency of Uruguayan Tannat wines from both a physicochemical and sensory perspective. Astringency is a complex and time-dependent attribute, which exhibits buildup and carry over effects upon repeated ingestions, and is related to several subtle sensations that can be perceived simultaneously. This poses several challenges for the accurate sensory characterization of Tannat wine astringency, which have been addressed during the development of the thesis. First, qualitative studies with consumers were carried out to understand their conceptualization of astringency and the vocabulary they use for describing this complex attribute. Basic research about the applicability of different palate cleansers for wine astringency evaluation was also conducted. The astringency of 40 commercial wines was characterized using static and dynamic sensory methods: Time-Intensity (TI), check-all-that-apply (CATA) questions and Temporal Dominance of Sensations (TDS). The TI evaluations performed by a panel of trained assessors showed that commercial Tannat wines mainly differed in intensity-related parameters rather than in astringency development over time, although the variability was moderate. CATA questions involving 16 astringency sub-qualities proved to be a valuable tool to describe and differentiate a large sample set of Tannat wine samples based on their astringency characteristics. Different styles of Tannat wine were identified, which were described using a wide range of sub-qualities, from *silky* and *velvety* to *harsh* and *aggressive*. The phenolic profile of the 40 commercial Tannat wines was also characterized using reversed phase high performance liquid chromatography coupled with a mass spectrometer (HPLC-MS) and its relationship with sensory astringency was explored using boosted regression trees. The total content of condensed tannins as well and some specific phenolic compounds were identified as the main predictors of sensory astringency. Finally, the comparison of astringency evaluation of trained assessors and experts showed that their intensity assessments were partly related, but their description of astringency sub-qualities differed. Insights on the astringency sub-qualities related to expert's perception of astringency quality were also revealed. Results from the thesis provide both methodological insights about astringency evaluation, as well as applied information about the astringency of Tannat wine, which could be highly valuable for the Uruguayan wine industry.

RESUMEN

La uva Tannat es una variedad de *Vitis vinifera* para elaborar vinos tintos, que se ha convertido en la cepa emblemática del Uruguay. El vino Tannat se caracteriza por su elevado contenido de compuestos fenólicos, que le confiere un gran potencial para el envejecimiento y características sensoriales diferenciales. El objetivo de la presente tesis fue caracterizar la astringencia de vinos Tannat uruguayos desde una perspectiva sensorial y fisicoquímica. La astringencia es un atributo complejo y dependiente del tiempo, que presenta efectos de acumulación por ingestas repetidas, y que involucra diversas sensaciones que son percibidas simultáneamente. Estas particularidades representan diversos desafíos para caracterizar adecuadamente la astringencia del vino Tannat, los cuales han sido abordados durante el desarrollo de la tesis. En primer lugar, se realizaron estudios cualitativos con consumidores para entender su conceptualización de astringencia y el vocabulario que utilizan para describir esta compleja sensación. También se realizó investigación básica sobre la aplicabilidad de distintos borradores para la evaluación de astringencia en vino tinto. Se caracterizó la astringencia de 40 vinos Tannat comerciales utilizando metodologías sensoriales estáticas y dinámicas: tiempo-intensidad (TI), preguntas “marque todo lo que corresponda” (CATA) y Dominancia Temporal de Sensaciones (TDS). Las evaluaciones de TI realizadas por un panel de jueces sensoriales entrenados reveló que los vinos Tannat comerciales se diferenciaron en parámetros vinculados a la intensidad de astringencia, más que a su evolución en el tiempo, aunque la variabilidad fue moderada. Las preguntas CATA incluyendo 16 sub-cualidades de astringencia resultaron ser una valiosa herramienta para describir y diferenciar un conjunto numeroso de muestras de vino Tannat en función de sus características de astringencia. Se identificaron distintos estilos de vino Tannat, los cuales fueron descriptos por una amplia gama de sub-cualidades de astringencia, desde *sedoso* y *aterciopelado* hasta *que raspa* y *agresivo*. Además se determinó el perfil fenólico de los 40 vinos Tannat comerciales utilizando cromatografía líquida de alta performance en fase reversa, acoplada a un espectrómetro de masas (HPLC-MS), y se exploró su relación con la astringencia sensorial utilizando árboles de regresión (Boosted Regression Trees). El contenido total de taninos condensados, así como algunos compuestos fenólicos específicos, fueron identificados como los principales predictores de la astringencia sensorial. Finalmente, la comparación de la evaluación de astringencia de jueces entrenados y expertos reveló que las medidas de intensidad estuvieron parcialmente relacionadas, pero su descripción de las sub-qualidades de astringencia fue diferente. Además se identificaron características de astringencia relacionadas a la percepción de calidad de astringencia

de los expertos. Los resultados de la tesis aportan conocimiento sobre aspectos metodológicos de la evaluación de astringencia, así cómo información aplicada sobre la astringencia de vino Tannat, lo que podría ser sumamente valioso para la industria vitivinícola uruguaya.

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LIST OF PUBLICATIONS

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INTRODUCTION

Tannat is a red variety of *Vitis vinifera* that is currently cultivated in few places in the world and has become the emblematic wine variety of Uruguay. In the past years, several studies have been conducted in the country to better characterize its wine quality potential, and to better understand the chemical composition of the grapes and wines (Alcalde-Eon, Boido, Carrau, Dellacassa, & Rivas-Gonzalo, 2006; Boido, Alcalde-Eon, Carrau, Dellacassa, & Rivas-Gonzalo, 2006; Boido, Fariña, Carrau, Dellacassa, & Cozzolino, 2013; Boido et al., 2011; Boido et al., 2003; González–Neves et al., 2004; González-Neves, Ferrer, & Gil, 2012; González–Neves, Gómez-Cordovés, & Barreiro, 2001; Lloret et al., 2003), as well as the impact of vineyard managing and winemaking practices on the quality of the grapes and wines (Coniberti et al., 2012; Fariña, Carrau, Boido, Disegna, & Dellacassa, 2010; Favre et al., 2014; Gámbaro et al., 2001; González-Neves, Favre, & Gil, 2014; González-Neves, Gil, Barreiro, & Favre, 2010; González-Neves, Gil, Favre, & Ferrer, 2012). However, research on the sensory characteristics of Uruguayan Tannat wine is still scarce (Fariña et al., 2015; Gámbaro et al., 2003; Varela & Gambaro, 2006). In particular, although astringency is one of the differential sensory attributes that contribute to the tipicity of Tannat wines, research on the astringency characteristics of this wine variety is still lacking.

Astringency is a complex sensory characteristic, which develops slowly and evolves during wine consumption, and is related to several subtle sensations that are simultaneously perceived (Jackson, 2014). It can be defined as a set of sensations related to drying, roughing and puckering of the mouth epithelium (ASTM, 2004). Astringency intensity determined by a trained sensory panel was shown to positively contribute to Tannat wine quality as perceived by a group of regular fine wine consumers (Varela & Gámbaro, 2006), which stresses the importance of conducting extensive research on the astringency characteristics of this wine variety. This information can largely contribute to better characterize the quality of Tannat wine and to better communicate its sensory characteristics to both national and international consumers.

The most direct method to evaluate red wine astringency is sensory analysis, which should be ideally performed by a group of trained assessors in order to obtain accurate and reliable results (Lesschaeve & Noble, 2010). Trained assessors usually rate the intensity of total astringency or specific astringency sub-qualities using scales. However, in order to account for the time dependency of astringency, dynamic methods such as time-intensity are required (Ishikawa & Noble, 1995; Noble, 1995). The complexity of this sensory perceptual phenomenon makes its study extremely

challenging. Rigorous experimental protocols and exhaustive assessors' training are needed to obtain reliable sensory information, which can be expensive and time-consuming for both researchers and wineries (Cheynier & Sarni-Machado, 2010; Ma et al., 2014). Thus, alternative techniques for astringency assessment, based on physicochemical and instrumental techniques have been proposed.

The astringency of red wine has been mainly attributed to its phenolic profile, in particular the content and composition of condensed tannins (Gawel, 1998; Lesschaeve & Noble, 2005). The capacity of such compounds to interact, complex and precipitate salivary proteins has been related to astringency (Bajec & Pickerin, 2008). In line with this, the majority of the physicochemical assays used to estimate astringency are based on the determination of phenolic compounds and/or their reaction with proteins. However, the chemical basis and the mechanisms underlying astringency perception are not fully understood yet (Cheynier & Sarni-Machado, 2010; Ma et al., 2014). The combination of sensory assessments with physicochemical and instrumental measures can contribute to the understanding of the chemical and physiological bases of astringency perception (Cheynier & Sarni-Machado, 2010).

Research on the relationship between the astringency characteristics of Tannat wines with their phenolic composition is of great interest to the Uruguayan wine industry, as it could enable the selection of vineyard managing and winemaking practices to obtain Tannat wines with specific astringency characteristics. Most of the studies that have explored the role of phenolic compounds and other wine components in astringency perception have used model systems, addition of specific compounds to wine samples or fractionation-reconstitution studies (Scollary, Pásti, Kállay, Blackman, & Clark, 2012). However, as argued by Scollary et al. (2012), the use of model solutions or the perturbation of the wine matrix do not take into account the interaction between the different wine components and the potential molecular assembly between them. Instead, the use of statistical tools to link analytical measurements to sensory data using actual wines seems a much more promising approach.

In this context, the general aim of this thesis was to characterize the astringency of commercial Uruguayan Tannat wines, from both a sensory and physicochemical perspective. The following literature review provides the background to the experimental work undertaken during the development of the thesis.

LITERATURE REVIEW

TANNAT WINE

Tannat is a red variety of *Vitis vinifera*, originally from the grapevines in the French Pyrénées (Viala & Vernorell, 1903). It has been cultivated since ancient times in the southwest of France, and nowadays most Tannat grapevines are located in the region of Maridan (Blanchard, 1999). Tannat vines were first introduced in Uruguay in 1874 by Pascual Harriague, a French immigrant (Carrau, 1997), and currently they are widely cultivated in the country, representing 26% of the total national grape production (INAVI, 2017). In the last decades, the Uruguayan wine-making industry decided to embrace Tannat as the national emblematic wine, using state-of-the-art viticultural technology to produce high quality wines as a strategy to compete in the international varietal wine market (Carrau, 1997).

Wines produced with Tannat grapes have great tipicity, and in general present relatively intense colour, high astringency and acidity in comparison with other red varieties (Blanchard, 1999; Boidron et al., 1995). Comparative studies with other red varieties from Uruguay have shown that Tannat grapes and wines have a particular and differential phenolic composition (Alcalde-Eon, Boido, Carrau, Dellacassa, & Rivas-Gonzalo, 2006; Boido et al., 2011; González-Neves, Gil, Favre, & Ferrer, 2012; González–Neves, Gómez-Cordovés, & Barreiro, 2001; Lloret et al., 2003). In general, Tannat wines have higher phenolic contents and are on average more colourful than Carbernet and Merlot red varieties. These characteristics position Tannat as a variety with great potential to produce high quality wines with a rich structure and suitability for long-term ageing.

Astringency is one of the differential sensory attributes of Tannat wines, which stresses the importance of studying this complex sensory characteristic to better characterize the quality of this wine variety.

ASTRINGENCY

Astringency is a sensory attribute which can be experienced during the consumption of a wide range of food and beverages, including fruits and fruit products (Joslyn & Goldstein, 1964), tea (Scharbert, Holzmann, & Hofmann, 2004), soymilk (Courregelongue, Schlich, & Noble, 1999), cocoa (Misnawi, Jinap, Jamilah, & Nazamid, 2004), legumes (Troszyńska, Amarowicz, Lamparski, Wołejszo, & Baryłko-Pikielna, 2006), spinach (Brock & Hofmann, 2008), dairy products (Beecher, Drake, Luck, & Foegeding, 2006; Lemieux & Simard, 1994) and wine (Gawel, 1998). In many of these

products, astringency is regarded as an unpleasant attribute as it is negatively associated to liking. However, in the specific case of red wine, astringency has long been recognized as one of the most important sensory characteristics that define its quality, complexity and persistence (Peynaud, 1987; Cheynier & Sarni-Manchado, 2010). It is widely acknowledged that a balanced level of astringency is desirable in high quality red wines (Gawel, 1998).

Astringency is a complex sensation generally related to the drying of the mouth, roughing of oral tissues and shrinking or puckering of the cheeks and face muscles. According to the American Society for Testing and Materials, astringency can be defined as "*the complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins*" (ASTM, 2004).

Astringency has been shown to be a strongly time-dependent sensation (Guinard, Pangborn, & Lewis, 1986), which develops slowly increasing its concentration (Ishikawa & Noble, 1995) and presents a lingering aftertaste that can last up to six minutes after swallowing or expectoration (Lee & Lawless, 1991). Besides, it exhibits build-up effect upon repeated ingestions, which means that perceived astringency intensity significantly increases with repeated ingestions of astringent stimuli (Colonna, Adams, & Noble, 2004; Courregelongue et al., 1999; Lee & Vickers, 2008; 2010; Noble, 2002; Ross, Hinken, & Weller, 2007).

Another characteristic of astringency sensation is that it cannot be considered as a unique attribute. A wide range of subtle sensations which are simultaneously perceived are involved in the complex perceptual phenomenon synthesized by the word astringency (Bajec & Pickering, 2008; Green, 1993). For instance, Lee and Lawless (1991) assessed the time-course of *dry*, *rough*, *puckering* sensations and overall *astringency* and suggested that these attributes may not be interchangeable. Experienced wine tasters and wine-makers also use different words to describe astringency sub-qualities, such as *fine*, *sappy*, *harsh*, *woody*, *round/smooth*, *coarse*, and *green*, among others (Peynaud, 1987; Sáenz-Navajas et al., 2016). In view of this, Gawel, Oberholster and Francis (2000) proposed a Mouth-feel wheel to precisely and comprehensively characterize the mouthfeel of red wine, which includes 33 astringency descriptors grouped into 7 categories (*particulate*, *surface smoothness*, *complex*, *drying*, *dynamic*, *harsh*, *unripe*).

Astringency can be induced by different types of substances, including tannins and other phenolic compounds, salts of multivalent metallic cations (e.g. aluminium salts, -alums-), dehydrating agents (e.g. ethanol and acetone) and organic and mineral acids

(Joslyn & Goldstein, 1964). In the specific case of red wine, astringency has been mainly attributed to the presence of phenolic compounds, particularly tannins (Gawel, 1998; Lesschaeve & Noble, 2005). In fact, the word astringent comes from the Latin term *ad stringere*, which means "to bind", because astringency was generally believed to be elicited by compounds capable of binding with proteins (Joslyn & Goldstein, 1964). Although the mechanisms of astringency perception have not been fully unveiled yet (Ferrer-Gallego, Hernández-Hierro, Rivas-Gonzalo, & Escribano-Bailón, 2014; Ma et al., 2014), the interaction of polyphenolic compounds with salivary proteins, leading to a decrease in the lubrication of the oral ephythelium, is thought to be one of the ways in which astringency is elicited (Lyman & Green, 1990).

MECHANISMS OF ASTRINGENCY PERCEPTION

Research attempting to uncover the mechanisms responsible for astringency perception date from decades ago (Bate-Smith, 1954; Joslyn & Goldstein, 1964). However, this continues to be an active area of research (Ma et al., 2014; Schöbel et al., 2014; Kishi, Sadachi, Nakamura, & Tonoike, 2017), and it is now theorized that different mechanisms are likely to be involved in the perception of this extremely complex sensation (Gibbins & Carpenter, 2013).

Astringency perception is not limited to a particular area of the mouth, but it is perceived throughout the oral cavity as a diffuse sensation (Joslyn & Goldstein, 1964). In order to understand the different mechanisms that may be involved, a basic understanding of the oral physiology is necessary. Sensory perception arises from the stimulation of different receptors, such as chemoreceptors, mechanoreceptors and thermoreceptors, each of which respond to a particular stimulus (Jacobs et al., 2002). In the oral cavity there are four different types of papillae: filiform, fungiform, foliate and circumvallate. The latter three host taste receptor cells, while filiform papillae are sensitive to mechanical and thermal stimuli (Laguna, Bartolomé, & Moreno-Arribas, 2017). Receptors transfer sensory information into the brainstem through different nerves that innervate the oral cavity: the facial nerve (including the *chorda tympani branch*) and the glossopharyngeal nerve are linked to taste buds, while the trigeminal nerve provides somatosensory innervation, i.e. responds to thermal, mechanical and nociceptive stimuli (Bajec & Pickering, 2008).

The oral cavity is covered by a mucous membrane, which is lubricated by the saliva secreted by salivary glands (Joslyn & Goldstein, 1964). Saliva is composed of over 99% water and around 0.5% of solids which are mainly proteins and other inorganic substances. A vast diversity of proteins is present in human saliva, including proline-rich-

proteins (PRPs), histatins (histadine-rich proteins), α-amylase, lactoferrin (Lf) and mucins (Bajec & Pickering, 2008). Salivary proteins bind to the oral epithelium, forming a salivary pellicle on the mucosa, which acts as a physical barrier to protect the mouth surfaces from possible abrasion and contributes to the lubrication of the oral cavity. A mobile salivary film, with different thickness and flow velocity according to the location in the mouth, is formed on the salivary pellicle, and also contributes to the lubrication of the oral cavity (Gibbins & Carpenter, 2013). When no food or beverage is present in the mouth, this system provides what Gibbins and Carpenter (2013) call "*the normal mouthfeel*", and define as "*a tactile feeling with no abrasion between rubbing surfaces (e.g. tongue or palate) aided by hydrated surfaces maintained by a thin film of saliva*". However, the salivary pellicle, the salivary film, and the amount and composition of saliva present in the oral cavity (which affects its rheology) are modified by the consumption of food and beverages (Gibbins & Carpenter, 2013). This is the base for many of the proposed mechanisms of astringency perception.

It has been debated whether astringency should be considered a taste, such as the five basic tastes (sweet, sour, bitter, salty and umami), or a tactile or trigeminal sensation. Researchers who claim that astringency is a taste sensation have provided evidence that, in rodents, astringent stimuli activate the *Chorda tympani* (Schiffman, Suggs, Sostman, & Simon, 1992) and the glossopharyngeal nerves, but do not evoke responses of the lingual nerve fibers that are sensitive to mechanical and thermal stimuli (Kawamura, Funakoshi, Kasahara, & Yamamoto, 1969). Critchley and Rolls (1996) also supported that astringency should be considered as a taste after evaluating the neuronal response of primates to tannic acid and substances eliciting sweet, sour, bitter and salty tastes. More recently, Kishi et al. (2017) explored the mechanisms responsible for the recognition of astringency, as compared to bitter and sweet tastes, using functional magnetic resonance imaging (fMRI) in humans. They found that astringent stimuli caused the activation of some parts of the primary gustatory cortex, and suggested that the human brain might recognize astringency as a taste, although stressed that further research is necessary to determine whether astringency is a taste or an oral sensation.

In spite of the results discussed above, it is widely accepted that astringency is a tactile sensation, as was first suggested by Bate-Smith (1954). Evidence to support this theory was provided by Breslin, Gilmore, Beauchamp, and Green (1993), who showed that astringency sensation could be perceived when the stimulus was applied on oral tissues which lack taste receptors. More recently, Schöbel et al. (2014) demonstrated that when human subjects had their taste nerves blocked (by taste transection or local anesthesia), salty, sweet, sour and bitter taste were almost completely suppressed, while

astringency was still perceivable. The subjects were unable to perceive astringency only when both taste and trigeminal nerves were blocked, suggesting that astringency is more likely to be a trigeminal sensation. Another argument supporting that astringency is not a taste is the fact that, unlike some of the basic tastes, which present an adaptation effect under continuous stimulation, astringency presents a build-up effect upon repeated ingestions (Green, 1993).

The most widely accepted mechanism of astringency perception postulates that the sensation is caused by a decrease in the lubrication of the oral ephythelium (Breslin et al., 1993; Kallithraka, Bakker, & Clifford, 1998; Lyman & Green, 1990; Thorngate & Noble, 1995) and an increase in the friction between mouth surfaces (Gawel, 1998), which are consequence of the interaction of some phenolic compounds, especially tannins, with salivary proteins. In line with this, the interaction between tannins and other phenolic compounds with the different proteins present in saliva, as well as with other proteins such as casein, gelatin and bovine serum albumin, have been extensively studied (Bajec & Pickering, 2008). This interaction is affected by several factors apart from the specific molecular structure of the protein, such as the molecular structure of the phenolic compound (degree of polymerization, degree of galloylation), the ratio protein:polyphenol, and the environmental conditions of the reaction (Bajec & Pickering, 2008).

Charlton et al. (2002) proposed a 3-stage model to explain the interaction and precipitation of polyphenols and PRPs, involving: i) the binding of proteins and phenolic compounds through, possibly multiple, hydrophobic associations as well as hydrogen bonds; ii) association between protein-phenol complexes through further hydrogen bonding forming larger aggregates; and iii) the precipitation of the complexes once they are large enough to be insoluble (Ma et al., 2014). Such precipitates of protein-phenol complexes might contribute directly to astringency by causing a gritty sensation, or by disrupting the salivary film (Gibbins & Carpenter, 2013). However, the interaction between tannins and salivary proteins does not necessarily lead to the precipitation of the formed complexes; it depends on the colloidal state of the tannins (Cala et al., 2012; Scollary, Pásti, Kállay, Blackman, & Clark, 2012). Besides, phenolic compounds which are incapable of precipitating proteins have been reported to induce astringency (Rossetti, Bongaerts, Wantling, Stokes, & Williamson, 2009).

The precipitation of salivary proteins-tannins complexes does not fully explain all aspects of astringency (Ferrer-Gallego, Gonçalves, Rivas-Gonzalo, & Escribano-Bailón, 2012), which indicates that the aggregation and precipitation of the complexes is likely to be only one of the factors that contribute to astringency development (Gibbins &

Carpenter, 2013). In fact, it has been recently suggested that the interaction between tannins and proteins is more closely related to astringency than their precipitation (Obreque-Slier, López-Solís, Peña-Neira, & Zamora-Marín, 2010).

Therefore, it is currently acknowledged that astringency is probably elicited through multiple mechanisms that may occur simultaneously (Bajec & Pickering, 2008; Gibbins & Carpenter, 2013; Ma et al., 2014). For instance, the interaction between phenolic compounds and proteins is thought to provoke the disruption of the salivary film, causing changes in its rheological and lubricating properties. Although the decrease in the lubrication properties of saliva is widely accepted as a mechanism of astringency, some authors claim this may not be the main mechanism (Lee & Vickers, 2012; Rossetti et al., 2009). Furthermore, the disruption of the salivary film or pellicle possibly leads to the exposure of the oral mucosa, enabling both free phenolic compounds and protein-phenol aggregates to interact directly with the oral tissue and reach different receptors (Ma et al., 2014) It has been shown that tannins are able to interact with oral epithelial cells (Payne, Bowyer, Herderich, & Bastian, 2009), and that monomeric flavan-3-ols strongly interact with lipids (Furlan, Jobin, Pianet, Dufourc, & Géan, 2014), suggesting that phenolic compounds might bind directly to the membranes of the oral cavity (Ma et al., 2014). The receptors involved in gathering information from astringent stimuli might be mechanoreceptors (identifying the changes in friction and lubrication), or taste receptors (Bajec & Pickering, 2008; Gibbins & Carpenter, 2013). Schöbel et al. (2014) have recently postulated that astringency sensation is generated by both the chemosensory detection of astringent phenols and the stimulation of trigeminal mechanosensors.

ASTRINGENCY PERCEPTION IN RED WINE

Red wine is a complex food matrix that contains a vast diversity of compounds, such as ethanol, organic acids, carbohydrates, non-volatile phenolic compounds and a wide range of volatile compounds (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006). Among these, phenolic compounds have historically attracted the attention of wine researchers and oenologists (Kennedy, Saucier, & Glories, 2006), because they are considered to be responsible of key wine sensory characteristics, including colour, aroma, flavour, bitterness and astringency (Garrido & Borges, 2013). Non-volatile phenolic compounds are without doubt the most cited with respect to astringency perception (Cheynier & Sarni-Manchado, 2010). Several studies have reported positive correlations of total tannin concentration with astringency intensity (Bindon et al., 2014; Kallithraka, Kim, Tsakiris, Paraskevopoulos, & Soleas, 2011; Monteleone, Condelli, Dinnella, & Bertuccioli, 2004; Preys et al., 2006; Robichaud & Noble, 1990; Vidal, Courcoux, et al., 2004). The specific chemical structure of phenolic compounds also influence how astringency is perceived (Bajec & Pickering, 2008; Ma et al., 2014).

Red wine astringency, as well as wine colour, are known to evolve during wine ageing. This has been related to the fact that phenolic compounds are very reactive, and undergo different oxidation and condensation reactions, leading to the formation of more stable compounds or complexes of compounds (Cheynier & Sarni-Machado, 2010; Ribéreau-Gayon et al., 2006). The decrease or softening of astringency that is observed during wine ageing has been attributed to reactions between the different phenolic compounds present in wine, particularly tannin polymerization and condensation with anthocyanins (Ma et al., 2014).

Other constituents of the wine matrix, such as ethanol, polysaccharides, proteins, organic acids, as well as wine's pH, are known to affect astringency perception. Increasing ethanol concentration has been found to reduce overall astringency and to modify astringency sub-qualities in wines and model wines (Fontoin, Saucier, Teissedre, & Glories, 2008; Demiglio & Pickering, 2008; Vidal, Courcoux, et al., 2004). This effect has been attributed to an interference of ethanol in the binding between tannins and salivary proteins limiting the aggregation of protein-phenol complexes, an increase in viscosity, as well as to higher lubricity in the oral cavity with higher ethanol concentrations (Fontoin et al., 2008; Demiglio & Pickering, 2008; Smith, June, & Noble, 1996).

The effect of pH on astringency has also been reported in wine and model wines. Astringency intensity and the association between tannins and proteins has been reported to increase at lower pH values (Fontoin et al., 2008; Kallithraka, Bakker, &

Clifford, 1997a). The effect of pH was found to be more relevant than increasing the concentration of malic, lactic or tartaric acids (Fontoin et al., 2008; Kallithraka, Bakker, & Clifford, 1997c).

Wine polysaccharides also affect astringency perception. Rhamnogalacturonan II, mannoproteins and arabinogalactoproteins have been found to decrease the astringency caused by tannins in model solutions (Vidal, Courcoux, et al., 2004; Vidal, Francis, Williams, et al., 2004). This effect has been attributed to the ability of these macromolecules to compete or interfere in the binding of tannins with salivary proteins or preventing their aggregation (Cheynier & Sarni-Machado, 2010).

Astringency perception is also modulated by interactions with other taste qualities or aromas. It is important to note that astringency and bitterness are often confused because many phenolic compounds are perceived as both bitter and astringent (Bajec & Pickering, 2008). However, it has been shown that they can be distinguished and rated separately (Lee & Lawless, 1991).

Brannan, Setser, and Kemp (2001b) evaluated the interaction between astringency (using alum and tannic acid) with the basic tastes, and reported that all of them modulate astringency depending on the concentration of the compounds eliciting each sensation. Other studies have focused on the cross-modal effects between astringency and sweetness. The addition of sucrose to tannic acid solutions (Lyman & Green, 1990) or wine (Ishikawa & Noble, 1995; Valentová, Skrovánková, Panovská, & Pokorný, 2001) decreased the perceived astringency. However, it has been suggested that this effect is more related to an increase in viscosity or the stimulation of saliva secretion caused by the sweetener, than to sweet taste. In this sense, the addition of a non-viscous artificial sweetener such as aspartame has been found to be less effective than sucrose in reducing astringency (Lyman & Green, 1990; Smith et al., 1996).

The influence of aroma on red wine astringency has been less studied. Sáenz-Navajas, Campo, Fernandez-Zurbano, Valentin, and Ferreira (2010) evaluated astringency modulation by aromas in reconstituted wines, using different combinations of volatile and non-volatile fraction, and reported a decrease of the astringency and bitterness of the samples related to the addition of volatile fruity extracts. On the other hand, Ferrer-Gallego et al. (2014) reported that the addition of certain aroma compounds to flavanol solutions increased their perceived astringency intensity. More recently, an enhancement of alum astringency due to the addition of an aroma compound with green characteristics at certain concentration levels was reported (Niimi, Liu, & Bastian, 2017).

PHENOLIC COMPOUNDS RELATED TO RED WINE ASTRINGENCY

Wine phenolics comprise a huge and heterogeneous family of compounds, which are characterized by having in their structure at least one aromatic ring with one or more hydroxyl groups attached (Crozier, Jaganath, & Clifford, 2006; Kennedy et al., 2006). They range from very simple low molecular-weight compounds with a single aromatic ring, to large polymers with diverse substituents, which can additionally exist in their free form or conjugated with acid of sugar molecules. Phenolic compounds are usually classified into flavonoids and non-flavonoids based on their number and arrangement of carbon atoms (Crozier et al., 2006; Garrido & Borges, 2013). Flavonoid compounds, which are the most widely distributed phenolic compounds in plants, share a basic skeleton of 15 carbon atoms in which two aromatic rings are bound through a 3 carbon chain (C6-C3-C6) (Fig.1; Crozier et al., 2006). Differences in the arrangement, degree of oxidation of the central pyran ring and substitution to this carbon skeleton gives rise to a wide diversity of compounds, which can be further divided into several classes, such as flavones, flavanones, flavonols, flavan-3-ols, isoflavones, anthocyanidins, dihydroflavonols, chalcones, dihydrochalcones and coumarins (Crozier et al., 2006; Garrido & Borges, 2013). Non-flavonoids, on the other hand, include other phenolic compounds that do not present the flavonoid skeleton in their structure, such as phenolic acids, ellagic acid and stilbenes (Kennedy et al., 2006). Only some classes of phenolic compounds which have been reported to be relevant for red wine astringency will be covered in this literature review.

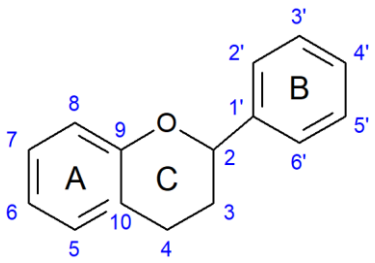

Fig.1. Structure and numbering of the carbons of the basic flavonoid skeleton.

Phenolic acids

Phenolic acids are the main non-flavonoid phenolic compounds found in grapes and wine, and can be divided into two main groups: hydroxybenzoic acids and hydroxycinnamic acids (Ribéreau-Gayon et al., 2006). Benzoic acids have a basic structure of seven carbon atoms (C6-C1) while cinnamic acids present nine (C6-C3), and for both types different substitutions of their benzene ring can occur (Garrido & Borges, 2013; Ribéreau-Gayon et al., 2006). Examples of phenolic acids found in grapes and wine are shown in Fig. 2. Gallic acid is one of the principal phenolic acids, because it is the precursor of hydrolyzable tannins and can also form part of condensed tannins (Garrido & Borges, 2013). Hydroxycinnamic acids may occur in their free form in small quantities, but more generally they appear as esters with tartaric acid (Ribéreau-Gayon et al., 2006). Phenolic acids have been reported to induce astringency in wines by different authors (Ferrer-Gallego et al., 2014; Hufnagel & Hoffman, 2008; Preys et al., 2006; Sáenz-Navajas, Avizcuri, Ferreira, & Fernández-Zurbano, 2012; Sáenz-Navajas, Tao, Dizy, Ferreira, & Fernández-Zurbano, 2010).

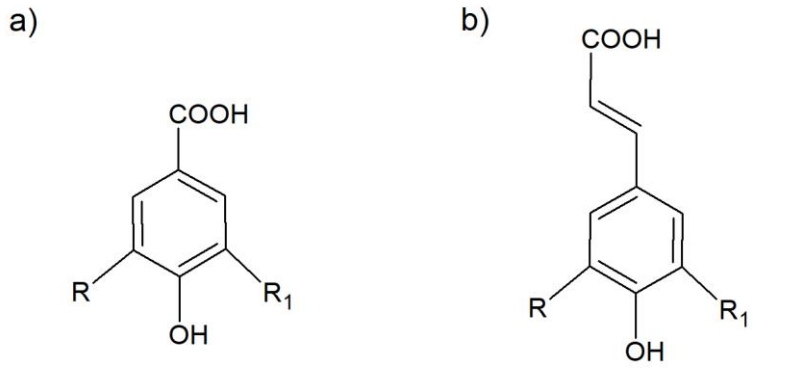

Fig. 2. Chemical structure of some hydroxybenzoic (a) and hydroxycinnamic (b) acids found in grape and wine.

Flavonols

Flavonols are the most widespread class of flavonoids (Crozier et al., 2006) and are characterized by having a double bond between carbons 2 and 3, a carbonyl group in carbon 4 and a hydroxyl group in carbon 3 (Garrido & Borges, 2013). In grapes, they commonly appear as *O*-glycosides of different sugars, producing glucosides, galactosides and glucuronides, while in wine the aglycone form is present because glycosides are hydrolyzed during fermentation (Garrido & Borges, 2013; Ribéreau-Gayon et al., 2006). Some of the flavonols present in grapes and wines are shown in Fig. 3. Flavonols have been reported to contribute to astringency perception (Cliff, King, & Schlosser, 2007; Ferrer-Gallego et al., 2016; Hufnagel & Hoffman, 2008). In particular, Hufnagel and Hoffman (2008) have suggested that some flavonol glycosides are responsible for eliciting a velvety astringency.

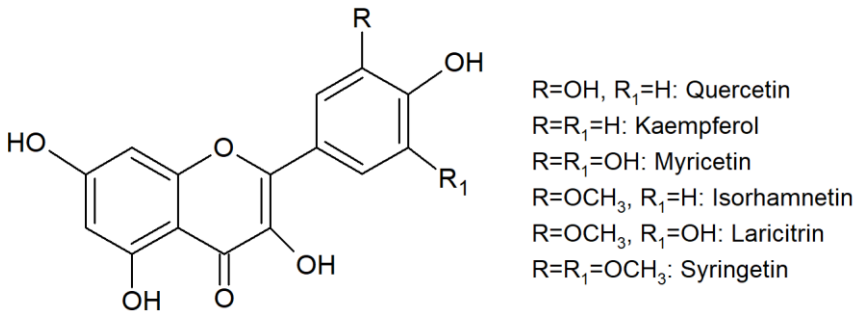

Fig. 3. Chemical structure of some flavonols found in grape and wine.

Flavan-3-ols

Flavan-3-ols are the most complex family of flavonoids, with compounds ranging from simple low-molecular monomers to large and complex polymers (Crozier et al., 2006). In these compounds, the basic flavonoid skeleton presents a saturated carbon chain between carbons 2 and 3 and a hydroxyl group in carbon 3 (Garrido & Borges, 2013). The flavan-3-ols monomers most widespread in nature are (+)-catechin and its enantiomer (-)-epicatechin (Crozier et al., 2006), but other derivatives have been identified in grapes and wines (Fig. 4; Garrido & Borges, 2013). While the astringent character of monomeric flavan-3-ols has been reported (Kallithraka, Bakker, & Clifford, 1997b; Thorngate & Noble, 1995), some authors have claimed that they are not the main compounds involved in red wine astringency (Hufnagel & Hoffman, 2008; Sáenz-Navajas et al., 2012). However, it is generally accepted that the polymers of flavan-3-

ols, called proanthocyanidins, are closely related to astringency (Arnold, Noble, & Singleton, 1980; Bajec & Pickering, 2008; Broussaud, Cheynier, & Noble, 2001; Gawel, 1998; Lesschaeve & Noble, 2005; Noble, 2002).

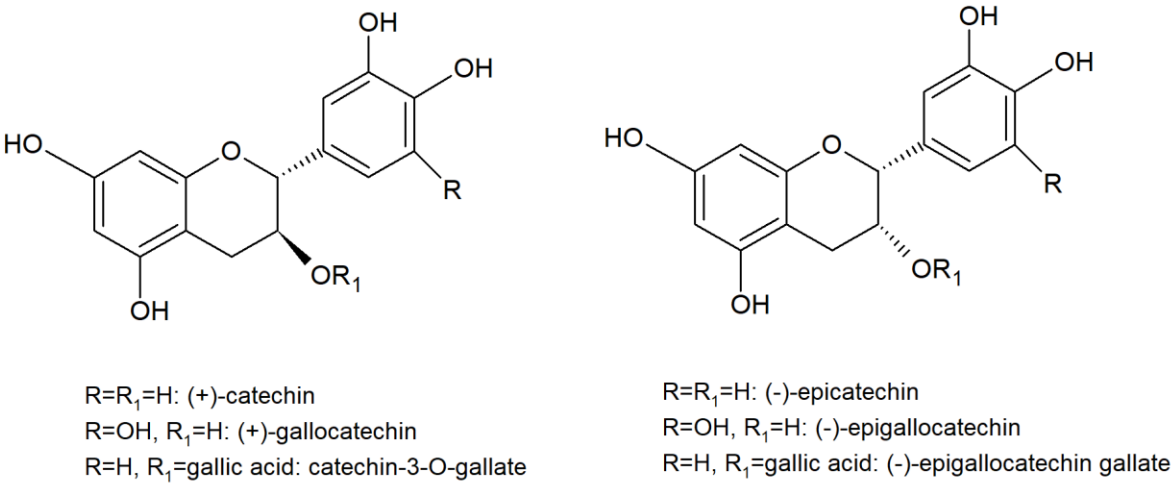

Fig. 4. Chemical structure of some monomeric flavan-3-ols found in grape and wine.

The proanthocyanidins found in grapes and wine are mainly dimers, oligormers and polymers of (+)-catechin and (-)-epicatechin, or their galloylated derivatives, linked through bonds C_4-C_8 or C_4-C_6. A wide range of structurally different proanthocyanidin molecules have been identified, with different combinations of monomer subunits and α or β C_4-C_8 or C_4-C_6 (Garrido & Borges, 2013). They are called proanthocyanidins because when they are heated in acidic medium they give rise to anthocyanidins (Ribéreau-Gayon et al., 2006); procyanidins and prodelphinidins hydrolyze to cyanidin and delphinidin, respectively (Garrido & Borges, 2013). Dimeric procyanidins can be divided into two categories: type B procyanidins are formed by two flavan-3-ol monomers linked by C_4-C_8 or C_4-C_6, while type A procyanidins have an additional ether bond between C_5 or C_7 of one of the subunits and C_2 of the other (Fig. 5). A vast number of isomers both of dimers and oligomers (three to ten flavanol units) are possible, which makes the isolation of these compounds a very difficult task (Ribéreau-Gayon et al., 2006).

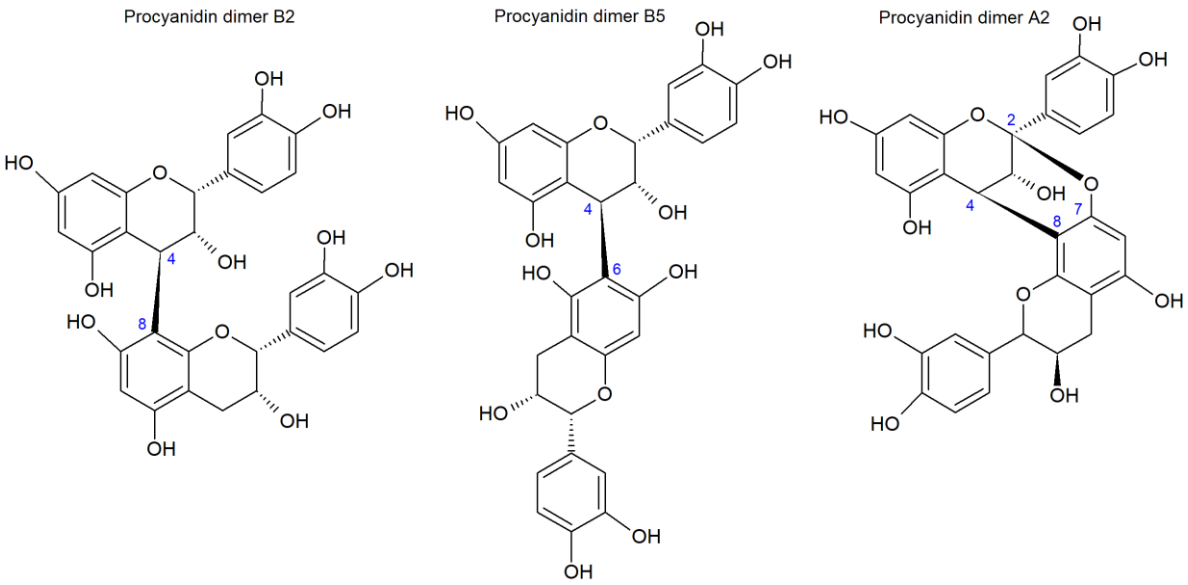

Fig. 5. Chemical structure of some procyanidin dimers found in grape and wine.

Tannins

Tannins are by definition substances capable of binding with proteins and other polymers, and correspond to polymers of simpler monomeric phenolic compounds (Ribéreau-Gayon et al., 2006). They are usually classified into hydrolyzable and non-hydrolyzable or condensed tannins. Tannins that are naturally present in grapes and wine are predominantly of the condensed type (Garrido & Borges, 2013), while wine hydrolyzable tannins are usually extracted from oak barrels during the process of wine ageing (Smith, McRae, & Bindon, 2015) or come from the addition of oenological tannins (Ribéreau-Gayon et al., 2006).

Hydrolyzable tannins are polymers of gallic acid and hexahydroxydiphenoyl acid, and as their name suggest, they can be degraded though pH changes and through enzymatic or non-enzymatic hydrolysis into smaller fragments (Crozier et al., 2006; Garrido & Borges, 2013). They can be divided into gallotannins and ellagitannins, depending on whether they release gallic of ellagic acids when hydrolyzed. They also contain glucose in their molecular structure (Fig.6; Ribéreau-Gayon et al., 2006).

Non-hydrolyzable or condensed tannins are large proanthocyanidins, i.e. polymers of flavan-3-ols. As for dimers and oligomeric proanthocyanidins, there is a huge diversity in the molecular structure of condensed tannins, and their structure and colloidal status confers them different properties in terms of flavour and mouthfeel (Ribéreau-Gayon et al., 2006). Individual characteristics of proanthocyanidins, such as their mean degree of polymerization and their subunit composition and distribution, have been

reported to largely influence astringency perception (Chira, Jourdes, & Teissedre, 2012; Chira, Pacella, Jourdes, & Teissedre, 2011; Preys et al., 2006; Quijada-Morín et al., 2012; Vidal et al., 2003). The astringency intensity elicited by pure proanthocyanidins has been reported to increase with an increase in their degree of polymerization (Peleg, Gacon, Schlich, & Noble 1999; Leeschaeve & Noble, 2005; Chira et al., 2012; Chira et al., 2011; Vidal et al., 2003). The identity and location of flavan-3-ol monomers in proanthocyanidin molecules also affect astringency perception (Lesschaeve & Noble, 2005). For example, Vidal et al. (2003) reported significant correlations between *coarse*, *dry* and *chalky* astringency sub-qualitites and the degree of galloylation of proanthocyanidin fractions, while a decrease in *coarse* was related to the presence of epigallocatechin. On the other hand, Quijada-Morín et al. (2012) suggested that proanthocyanidins with higher proportions of epicatechin subunits in their terminal positions are perceived as more astringent.

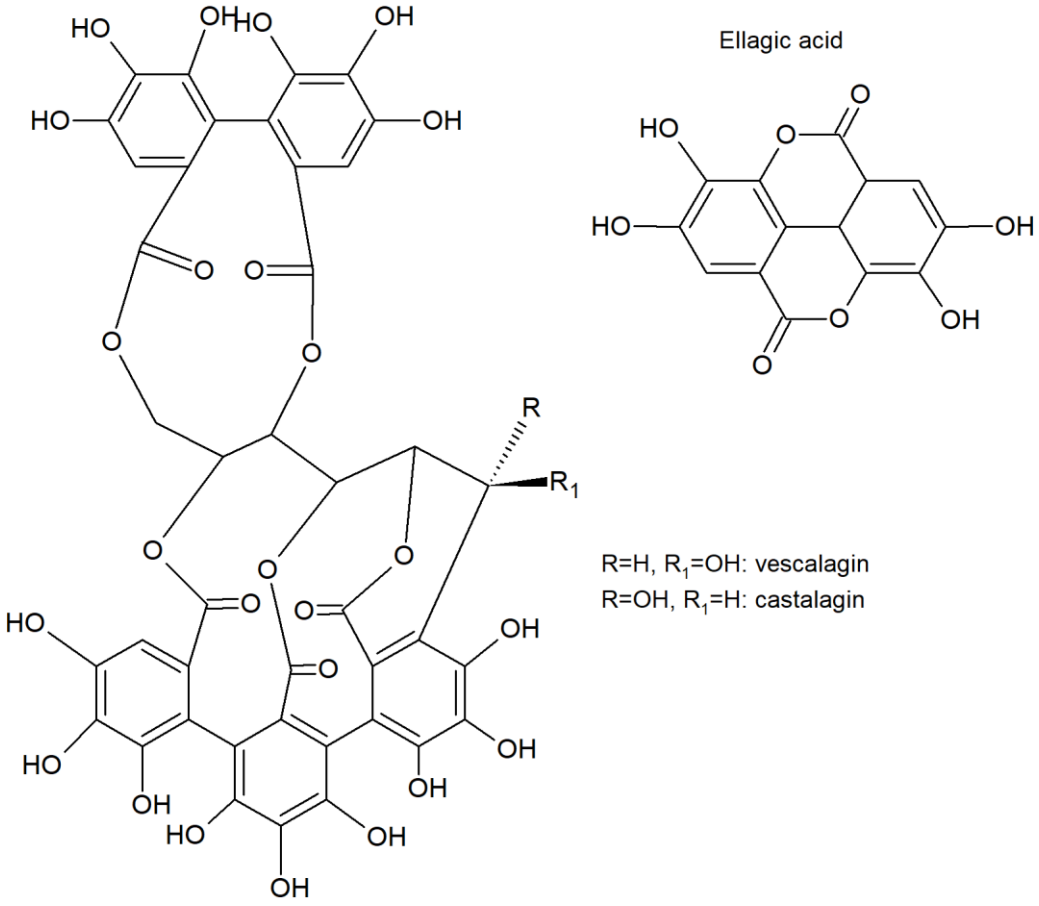

Fig. 6. Chemical structure of ellagic acid and some ellagitannins found in grape and wine.

Anthocyanins

Anthocyanins are widely distributed in the plant kingdom, and are the pigments responsible for the colours of various flowers and fruits (Crozier et al., 2006). Anthocyanins are the glycosylated form of anthocyanidins, flavonoid compounds whose structure is based on the flavylium cation (Ribéreau-Gayon et al., 2006). The five anthocyanidins that are found in grapes and wine are shown in Fig. 7. They are usually found as 3-monoglucosides, or as acylated monoglucosides, which are much more stable than the aglycone anthocyanidin form. Traces of 3,5- and 3,7-diglucosides have also been reported in *V. vinífera* grapes (Garrido & Borges, 2013; Ribéreau-Gayon et al., 2006). There is contradictory evidence in the literature regarding the contribution of anthocyanins to wine astringency, with some authors reporting a positive contribution of anthocyanin fractions (Vidal, Francis, Williams, et al., 2004) or individual anthocyanins (Gonzalo-Diago, Dizy, & Fernández-Zurbano, 2014), while others finding no or little contribution (Kallithraka et al., 2011; Vidal, Courcoux, et al., 2004). Still, it has been proposed that anthocyanins could modulate wine astringency through their reaction with wine tannins, or directly (Vidal, Francis, Williams, et al., 2004). Anthocyanins are thought to be particularly relevant in the process of astringency "softening" during wine ageing, through their condensation with tannins to produce pigmented tannins. The fact that astringency increases with tannin degree of polymerization (Vidal et al., 2003) and that fractions containing soluble tannin–anthocyanin adducts did not contribute to wine astringency (Vidal, Francis, Noble, et al., 2004) suggest that the formation of pigmented tannins is more likely to cause the loss of astringency during wine ageing than tannin polymerization (Cheynier & Sarni-Machado, 2010; Ma et al., 2014).

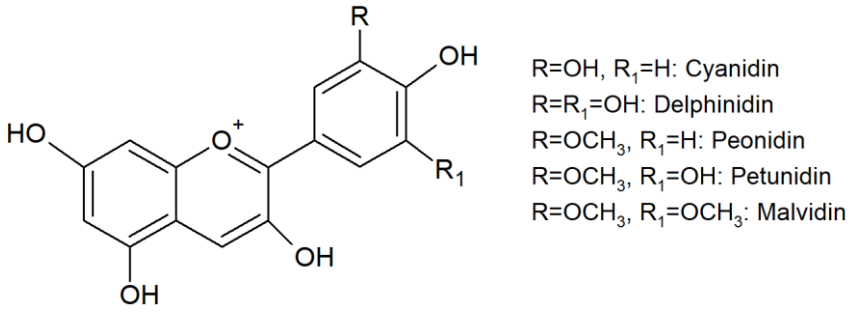

Fig. 7. Chemical structure of anthocyanidins found in grape and wine.

SENSORY APPROACHES FOR MEASURING ASTRINGENCY

Sensory analysis is the most common and direct method to evaluate the sensory attributes experienced during wine consumption, including astringency (Cheynier & Sarni-Manchado, 2010; Ma et al., 2014). Wine tasting has traditionally been one of the central activities of the wine industry, being mainly performed by wine experts, such as winemakers, oenologists and sommeliers. Experts have in general better abilities that consumers to describe and evaluate wine's sensory characteristics (Gawel, 1997; Lawless, 1984), and are capable of detecting wine defects and assessing if a wine typifies a specific style (Gawel & Godden, 2008). Hence, experts' assessments constitute a valuable source of information for both wineries and wine consumers. However, there is sometimes large variability in wine experts' assessments (Gawel & Godden, 2008; Hodgson, 2008), as they tend to base their evaluations in both objective and subjective considerations. Thus, in order to obtain accurate and reliable measurements, wine sensory analysis should ideally be carried out by a trained sensory panel (Lesschaeve & Noble, 2010).

Trained sensory panels have been traditionally used when objective measures of the sensory characteristics of products are needed, either for product discrimination or description (Lawless & Heymann, 2010). The members of a trained panel (8 to 20) are usually selected for having sensory acuity and ability to discriminate among products superior to the average population (Stone & Sidel, 2004). Furthermore, a training process is conducted to familiarize the assessors with the sensory methods and the specific products, and to improve their ability to recognize, describe and quantify the product's sensory attributes (Lawless & Heymann, 2010). The use of standardized protocols and controlled conditions, together with an appropriate panel training, seek to minimize both psychological and physiological biases in order to obtain objective and reliable information about the sensory characteristics of food products (Lesschaeve & Noble, 2010).

Assessors' training usually includes a series of tasks which depend on the objective of the study. When intensity ratings of specific sensory attributes are needed, extensive training is required to ensure that assessors are able to recognize an attribute and distinguish it from others, and to rate its intensity using a specific scale (Lawless & Heymann, 2010). Different types of scales exist, with category and unstructured line scales being the most commonly used in wine sensory evaluation (Lesschaeve & Noble, 2010). Intensity scales are commonly anchored with intensity-related words in their

extremes (“none”/”low” and “high”), and these extremes should be clearly defined using specific references (Lawless & Heymann, 2010).

Training is particularly important for astringency measurement as perception of this specific sensory characteristic has been reported to be largely affected by individual characteristics of the person who is assessing the wine. Differences in salivary flow rate of individuals have been reported to influence astringency perception of different solutions of astringent compounds and in wine matrices, with larger flow rates being associated to a decrease in astringency (Condelli, Dinnella, Cerone, Monteleone, & Bertuccioli, 2006; Fischer, Boulton, & Noble, 1994; Ishikawa & Noble, 1995). However, this effect has not been observed in other studies, where no influence of salivary flow rate on astringency perception was found (Guinard, Zoumas-Morse, & Walchak, 1998; Smith et al., 1996). Saliva composition in terms of individual salivary proteins is also likely to affect astringency perception (Cheynier & Sarni-Machado, 2010), as the affinity to bind phenolic compounds differs according to the type of protein (Bajec & Pickering, 2008). Furthermore, it has been suggested that sensitivity to astringency is related to the ability of the subjects to restore their saliva characteristics to their basal condition after tasting an astringent stimulus (Dinnella, Recchia, Fia, Bertuccioli, & Monteleone, 2009). Several studies have tried to relate individual differences in sensitivity to 6-n-propylthiouracil (PROP), which is known to be genetically inherited, to differences in astringency perception. However, conflicting results have been obtained, with some authors reporting that PROP status does not affect astringency perception (Ishikawa & Noble, 1996; Smith et al., 1996) and other studies suggesting it does (Pickering & Robert, 2006; Pickering, Simunkova, & DiBattista, 2004).

There are various characteristics of astringency sensation that make it a particularly challenging sensory attribute to assess. Astringency presents buildup effect upon repeated ingestions, and may present carry over effect as a result of its strong persistence. Besides, it is a sensation that develops slowly; astringency intensity immediately after tasting a sample is not the same as after 13-15 s, or after swallowing/expectorating the sample (Jackson, 2014). This implies that experimental designs and tasting protocols are of extreme importance when conducting sensory studies to evaluate astringency (Bajec & Pickering, 2008). Different strategies have been proposed in experimental protocols for astringency evaluation, including sip/spit (or swallow) protocols with a fixed timeline, forced waiting time between samples and the use of palate cleansers to prevent carry-over effects (Colonna et al., 2004; Condelli et al., 2006; Guinard et al., 1986; Lee & Vickers, 2010; Ross et al., 2007).

Another important consideration for the assessment of red wine astringency is the selection of the proper sensory methods, which will depend on the objective of the study. The methods that have been used to evaluate the sensory characteristics of wine can be broadly classified into static and dynamic. In static methods, a single assessment of the sensory characteristics of a given product is obtained. For example, assessors are asked to rate the intensity of each sensory attribute only once. However, the sensory perception of food products is a dynamic phenomenon as the perceived sensory characteristics of the products change during consumption (Lawless & Heymann, 2010). For this reason, dynamic sensory methods have been introduced for astringency measurement to take into account the changes in perception that occur as the wine is consumed.

Static methods for astringency evaluation

Descriptive analysis is one of the most established tools to provide a detailed sensory characterization of food products (Lawless & Heymann, 2010). This method involves the selection and definition of the sensory attributes that are relevant for describing the differences among the samples that will be assessed. Trained assessors are asked to rate the intensity of each of attribute for each sample, usually using an unstructured line scale. Average intensities across assessors are obtained for each sensory attribute, and significant differences among samples can be evaluated using statistical tools such as analysis of variance. Hence, the method provides information on the size and nature of the sensory differences among products. Astringency has been included as one of the sensory attributes for red wine characterization in a vast number of studies (Bindon et al., 2014; Hufnagel & Hofmann, 2008; Preys et al., 2006; Sáenz-Navajas et al., 2012; Varela & Gámbaro, 2006). Studies involving basic research on astringency, or looking for correlations of astringency perception with physicochemical or instrumental measures have also rated astringency intensity as a single attribute, using different scales (Arnold et al., 1980; Cáceres-Mella et al., 2013; Chira et al., 2011; Condelli et al., 2006; Fontoin et al., 2008; Llaudy et al., 2004; Monteleone et al., 2004; Obreque-Slier, Peña-Neira, & López-Solís, 2010b; Pickering et al., 2004; Quijada-Morín et al., 2012; Quijada-Morín, Williams, Rivas-Gonzalo, Doco, & Escribano-Bailón, 2014; Rossetti et al., 2009; Sáenz-Navajas et al., 2015).

The evaluation of total astringency intensity is usually insufficient to characterize all the subtle sensations that are simultaneously experienced when consuming red wine (Bajec & Pickering, 2008). The specific sub-qualities of astringency related to a specific wine are closely linked to the product's quality (Sáenz-Navajas et al., 2016; Sáenz-

Navajas, Ballester, Pêcher, Peyron, & Valentin, 2013). In line with this, in the last decades several authors have started to include the evaluation of specific astringency sub-qualities in their research, using mainly descriptive analysis (Cáceres-Mella et al., 2014; del Barrio-Galán, Pérez-Magariño, & Ortega-Heras, 2011; Ferrer-Gallego et al., 2016; Ferrer-Gallego et al., 2014; Francis et al., 2002; Gawel, Francis, & Waters, 2007; Gawel, Iland, & Francis, 2001; Oberholster et al., 2015; Ortega-Heras, Pérez-Magariño, Cano-Mozo, & González-San José, 2010; Pickering & Robert, 2006; Vidal, Courcoux, et al., 2004, Vidal, Francis, Noble, et al., 2004; Vidal, Francis, Williams, et al., 2004).

Rating the intensity of astringency sub-qualities can be a difficult task for assessors and may require an extensive training process. Therefore, alternative methods for sensory characterization may provide a simpler and quicker approach for astringency characterization (Valentin, Chollet, Lelievre, & Abdi, 2012; Varela & Ares, 2012). Check-all-that-apply (CATA) questions (Adams, Williams, Lancaster, & Foley, 2007) may be particularly suitable for this objective. The application of CATA questions for sensory characterization consists on presenting assessors with a list of terms and asking them to select all those that apply to describe the focal sample. CATA questions have proven to be a simple task that does not require extensive training, allowing to capture the main sensory attributes that are perceived in a product (Ares & Jaeger, 2015). Recently, Fleming, Ziegler, and Hayes (2015) have used CATA questions including, among other attributes, several astringency sub-qualities, to characterize the sensory properties of different types of astringent compounds (multivalent salts, organic acids and phenolic compounds).

CATA questions give rise to binary data (0/1), that indicates whether an attribute has been selected or not, for each sample and assessor. The frequency of use of each attribute to describe each sample, expressed as count or as percentage, is computed and arranged in a contingency table. Although the frequency of use of a sensory attribute is not a direct and quantitative measure of its intensity, both measures are usually correlated (Ares & Jaeger, 2015).

Dynamic methods for astringency evaluation

Static measurements can only capture either the maximum astringency intensity or the average astringency intensity perceived over a certain period of time (Ma et al., 2014). Therefore, considering that astringency perception is strongly time-dependent, dynamic methods are necessary to fully characterize red wine astringency (Ishikawa & Noble, 1995; Noble, 1995).

One of the most popular temporal methods is time-intensity (TI), which relies on the continuous measurement of an attribute's intensity over a period of time (Cadena, Vidal, Ares, & Varela, 2014; Lawless & Heymann, 2010). TI has been widely used to provide a detailed characterization of astringency development during consumption (Boulet et al., 2014; Colonna et al., 2004; Ishikawa & Noble, 1995; Kallithraka et al., 2011; Lee & Vickers, 2010; Lee & Lawless, 1991; Noble, 1995; Ross et al., 2007; Valentová et al., 2002). The data obtained with this method correspond to TI curves, which represent astringency intensity as a function of time, for each sample and assessor. Several parameters can be extracted from each curve, such as the maximum intensity, the time to reach the maximum intensity, the total duration of the sensation and the area under the curve. Differences among samples on each of the TI parameters can be assessed using the same statistical approaches used for analyzing intensity rating from descriptive analysis (Cadena et al., 2014). Rating the intensity of an attribute over time is a complex task, so in general assessors require more training to perform TI tasks than to provide static intensity ratings (Lesschaeve & Noble, 2010).

Another important matter that should be taken into account is that the time-dependency of some astringency sub-qualities and total astringency have been reported to be different (Lee & Lawless, 1991). Thus, the potential of multi-attribute temporal methods, such as Temporal Dominance of Sensations (TDS; Pineau et al., 2009), to fully characterize the dynamics of astringency perception also deserve exploration. TDS is a relatively novel sensory methodology that enables the simultaneous evaluation of various attributes over a certain period of time. Assessors are presented with a list of attributes, and are asked to select the dominant attribute at each time of the evaluation, until the perception is over or the time assigned to the task expires (Cadena et al., 2014). The dominant attribute is defined as the one that most catches the attention at a given time, not necessarily the most intense (Pineau et al., 2009).

Similar to CATA questions, the raw data provided by a TDS task is binary, and indicates whether an attribute has been selected as dominant or not, at each time of the evaluation, for each sample and assessor. In order to visualize the temporal dominance profile of each sample, TDS curves are constructed. For each sample, dominance rates of each attribute at each time of the evaluation are obtained by computing the proportion of judgments (assessors x replicates) in which the attribute was selected as dominant. The curves representing the dominance rates as a function of time of all attributes are superimposed to obtain the temporal dominance profile of a sample.

Disadvantages of sensory methods

Although sensory analysis is the only alternative to measure the actual physiological and psychological responses to food products, rigorous protocols are needed to select and train a sensory panel in order to obtain reliable sensory information, which can be expensive and time-consuming (Cheynier & Sarni-Machado, 2010; Ma et al., 2014). Both researchers and wineries might not be able to face the costs associated with sensory evaluation of astringency, which has motivated the development of alternative analytical approaches to assess red wine astringency in vitro (Scollary et al., 2012). Physico-chemical and instrumental measures are also necessary to understand the chemical and physiological bases of astringency perception (Cheynier & Sarni-Machado, 2010).

PHYSICOCHEMICAL AND INSTRUMENTAL APPROACHES FOR MEASURING ASTRINGENCY

Physicochemical assays have been used for several decades to provide an approximate measure of astringency in vitro. Given that red wine astringency has been mainly attributed to the wine's phenolic composition, analytical measures of the concentration of total phenolics, classes of phenolic compounds, and more recently individual phenolic compounds, have been used to predict astringency or understand its chemical basis (Bindon et al., 2014; Cáceres-Mella et al., 2014; Cáceres-Mella et al., 2013; Chira et al., 2011; Kallithraka et al., 2011; Quijada-Morín et al., 2012; Quijada-Morín et al., 2014; Sáenz-Navajas et al., 2012).

The most common assays to estimate total phenolic content in wine are the total polyphenol index (absorbance at 280 nm) and the Folin–Ciocalteau assay, both based in spectrophotometric measures (Ribéreau-Gayon et al., 2006). Condensed tannin concentration can be estimated using the method proposed by Ribéreau-Gayon & Stonestreet (1966), which is based on the property of proanthocyanidins of releasing carbocations that are partially converted into cyanidin when they are heated in an acid medium (Ribéreau-Gayon et al., 2006).

The advances in chromatrographic techniques have allowed to obtain a more detailed characterization of wine phenolic composition. Reversed phase high performance liquid chromatography (HPLC) coupled with diode-array-detector (DAD) and/or mass spectrometers (MS) has been used to separate, identify and quantify individual phenolic compounds. Total proanthocyanidin concentration and their characteristics, such as mean degree of polymerization and subunit composition, can be

analysed by acid catalyzed cleavage of these polymers, followed by HPLC analysis of the reaction products (Cheynier & Sarni-Machado, 2010).

Chemical assays based on the ability of tannins to bind and precipitate proteins have also been proposed, and used to estimate or predict wine astringency (Cáceres-Mella et al., 2013; Cliff et al., 2007; Llaudy et al., 2004). Among these methods, one of the most commonly used is the Gelatin Index (Scollary et al., 2012), which is based on tannin precipitation with gelatin. However, it has been pointed out that the method only provides an approximate estimation of tannin content, and that the large heterogeneity of commercial gelatin composition makes results subject to large variability and imprecision (Llaudy et al., 2004). In fact, Goldner and Zamora (2010) reported that the gelatin index is a good predictor of red wine astringency only at low phenolic contents. Thus, methods using proteins with higher similarity to the salivary proteins that are thought to be more relevant for astringency perception have been proposed, including bovine serum albumin (BSA), ovalbumin and mucins (Llaudy et al., 2004; Ma et al., 2013; Monteleone et al., 2004; Scollary et al., 2012). Methods based on tannin-protein precipitation which use real saliva have also been introduced (Obreque-Slier, Peña-Neira, et al., 2010b).

Furthermore, taking into account that astringency perception is probably linked to friction and modifications of saliva properties in the oral cavity, instrumental measures have also been proposed to assess astringency perception and the effect of astringent stimuli on saliva, including rheology and tribology studies (Laguna, Bartolomé, et al., 2017). Rheology has been used to measure the flow properties (such as viscosity) of wine, saliva, and mixtures of wine and saliva (Laguna, Bartolomé, et al., 2017; Laguna, Sarkar, et al., 2017). Tribology studies have been conducted to measure the friction coefficients of saliva and saliva mixed with specific phenolic compounds, wine or model wine, as an approximation of the friction that would occur between oral surfaces when wine is consumed (Brossard, Cai, Osorio, Bordeu, & Chen, 2016; Laguna, Sakar, et al., 2017; Rossetti et al., 2009). Instrumental techniques that account for the interaction of saliva with wine components, such as dynamic light scattering to measure the particle size of aggregates of polyphenol-saliva complexes, and Transmission Electron Microscopy to visualize qualitatively the microstructure of such complexes, have also been considered (Laguna, Sakar, et al., 2017). Still, up to now neither individual instrumental methods nor combinations of them have proven to fully account for all aspects of astringency perception.

OBJECTIVES

The general objective of the thesis was to characterize the astringency of commercial Uruguayan Tannat wines using both sensory and physicochemical methods.

The specific objectives of the present thesis were the following:

- To determine an appropriate vocabulary to characterize the astringency of red wine, based on the perception of Uruguayan fine-wine consumers, which can be applicable to the Uruguayan wine marketplace.
- To develop a methodology for the sensory characterization of red wine astringency, taking into account both astringency total intensity and specific astringency sub-qualities.
- To obtain information on the sensory astringency of commercial Uruguayan Tannat wines.
- To study the relationship between physicochemical variables of the wines (pH, ethanol content, total acidity and phenolic profile) and the sensory astringency of Tannat wine.
- To compare astringency sensory characterization of Tannat wine of trained assessors and wine experts.
- To identify which astringency characteristics influence wine experts' perception of Tannat wine astringency quality.

STRUCTURE OF THE THESIS

In order to accomplish the objectives set forth, a series of activities were undertaken, which can be divided into three stages. The first stage was focused on the fulfillment of the first specific objective, i.e. to generate an appropriate vocabulary to characterize red wine, which should be relevant for Uruguayan wine consumers. A qualitative study was conducted to understand consumer's conceptualization of red wine astringency and identify specific terms that are relevant for them to describe the different astringency-related sensations. The results and implications of this study are presented in **CHAPTER 1**.

The second stage of the thesis involved all the activities related to the tune up of methods that were necessary to characterize the astringency of Tannat wines. It included the selection and training of a panel of trained assessors, and the definition of the sensory methodologies that were used in the formal tasting sessions of the commercial samples. Details of these activities can be found in the materials and methods section of **CHAPTERS 2, 3 and 5**. Not only was it necessary to establish standardize protocols for the evaluation of astringency intensity of red wine, but an appropriate palate cleanser to avoid carry-over effects had to be selected (**CHAPTER 2**).

The third stage of the thesis included all the activities that were undertaken to fulfill the general aim, i.e. to characterize the astringency of commercial Uruguayan Tannat samples. A detailed description of the sensory characterization of the astringency of 40 commercial samples, considering both astringency intensity and the different associated sub-qualities is presented in **CHAPTER 3**. The characterization of the basic composition of the commercial samples, and the determination of their phenolic composition are explained in detail in **CHAPTERS 3 and 4**. In particular, **CHAPTER 4** deals with the relationship between these physicochemical variables and the intensity and characteristics of the astringency of the 40 commercial Tannat wines. **CHAPTER 5** describes the application of Temporal Dominance of Sensations (TDS), a relatively novel temporal methodology, to evaluate the dynamics of astringency description. The sensory characterization of Tannat wine astringency described in **CHAPTERS 3 and 5** was performed by a highly trained panel of assessors. However, in Uruguay most wineries still rely on the judgment of wine experts for decision making. In this context, a study was conducted to compare wine astringency characterization of experts and trained assessors, using a subset of 6 commercial Tannat wine samples (**CHAPTER 6**). At the end of the thesis, the general conclusions from all the experimental chapters are summarized.

CHAPTER 1

How do consumers describe wine astringency?

ABSTRACT

Astringency is one of the most important sensory characteristics of red wine. Although a hierarchically structured vocabulary to describe the mouthfeel sensations of red wine has been proposed, research on consumers' astringency vocabulary is lacking. In this context, the aim of this work was to gain an insight on the vocabulary used by wine consumers to describe the astringency of red wine and to evaluate the influence of wine involvement on consumers' vocabulary. One hundred and twenty-five wine consumers completed and on-line survey with five tasks: an open-ended question about the definition of wine astringency, free listing the sensations perceived when drinking an astringent wine, free listing the words they would use to describe the astringency of a red wine, a CATA question with 44 terms used in the literature to describe astringency, and a wine involvement questionnaire. When thinking about wine astringency consumers freely elicited terms included in the Mouth-feel wheel, such as dryness and harsh. The majority of the specific sub-qualities of the Mouth-feel wheel were not included in consumer responses. Also, terms not classified as astringency descriptors were elicited (e.g. acid and bitter). Only 17 out of the 31 terms from the Mouth-feel wheel were used by more than 10% of participants when answering the CATA question. There were no large differences in the responses of consumer segments with different wine involvement. Results from the present work suggest that most of the terms of the Mouth-feel wheel might not be adequate to communicate the astringency characteristics of red wine to consumers.

1.1. INTRODUCTION

Astringency can be defined as “the complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins” (ASTM, 2004). Red wine astringency is mainly attributable to the phenolic compounds, particularly proanthocyanidins (tannins) (Lesschaeve & Noble, 2005), and is one of its most important sensory characteristics (Peynaud, 1987).

Astringency has been shown to be a complex perceptual phenomenon that involves several sensations that are simultaneously perceived (Green, 1993; Lee & Lawless, 1991). Therefore, the evaluation of total astringency is not enough to characterize perceived astringency when consuming red wine (Bajec & Pickering, 2008). For this reason, a standardized and well-defined vocabulary to describe wine astringency is necessary (Gawel, 1997). This type of vocabulary allows accurate description of wine and facilitate communication across different wineries or trained panels (Lawless & Civille, 2013).

Wine tasters have traditionally used descriptive terms such as *sappy*, *harsh*, *woody* and *green* to describe wine astringency (Peynaud, 1987). Several authors have proposed lexicons to describe astringency sub-qualities of wine and other alcoholic beverages. Lee and Lawless (1991) generated terms to describe solutions of allum, gallic acid and tartaric acid using focus groups: *drying*, *puckering*, *sour*, *astringent*, *bitter* and *rough*. Similar terms were used by Lawless, Corrigan, and Lee (1994) to describe the mouthfeel sensations of several compounds. Besides, other terms such as *stickiness*, *powdery*, *sappy*, *harsh* and *gritty* have been used by different authors to describe the astringency of beer and brewing products (Langstaff, Guinard, & Lewis 1991; Meilgaard & Muller, 1987).

Gawel, Oberholster, and Francis (2000) proposed a Mouth-feel wheel to precisely and comprehensively characterize the astringency of red wines. It comprises a hierarchical vocabulary of 53 terms to describe the mouthfeel characteristics of red wine, including 33 astringency descriptors grouped into 7 categories (particulate, surface smoothness, complex, drying, dynamic, harsh, and unripe). Although the Mouth-feel wheel provides valuable information to describe the astringency of red wine, some of the terms include a hedonic component in their definition and are related to other flavour characteristics (e.g. *complex*, defined as "a positive hedonic grouping consisting of an amalgam of pleasing astringency sensations, flavour and balanced acidity") (Lawless & Civille, 2013). This makes it necessary to refine the vocabulary used for describing wine astringency (Kielhorn & Thorngate, 1999). Besides, one of its main drawbacks is that it

was constructed considering the perception of wine experts with extensive experience in wine tasting. Therefore, the terms of the Mouthful Wheel do not necessarily include the terms consumers normally use for describing wine astringency.

One of the biggest challenges in consumer research is understanding consumer vocabulary (Lawless & Civille, 2013). Although standardized astringency vocabulary may allow an accurate description of wines and facilitate communication across different panels and companies, they do not necessarily reflect how consumers would describe them (Lawless & Civille, 2013). Understanding consumers' astringency vocabulary can contribute to identify the most relevant characteristics for consumers' quality perception and to reduce differences with the descriptions provided by experts (Carr, Graig-Petsinger, & Hadlich, 2001). This approach can also be useful for improving communication with non-technical staff and to develop marketing and communication strategies based on sensory information (Lawless & Civille, 2013; Swahn, Öström, Larsson, & Gustafsson, 2010). Providing information about the sensory characteristics of products has been reported to improve consumers' expectations and purchase intention and has been increasingly used by food companies (Smith, Møgelvang-Hansen, & Hyldigc, 2010; Wansink & Painter, 2001).

Involvement is a motivational state that determines how relevant a person perceives a product within their personal needs, values, interests and motivations for a given situation (Marshall & Bell, 2004). People involved with a product usually invest more time and effort for making their purchase decisions (Bell & Marshall, 2003). Wine involvement has been shown to influence consumers' consumption frequency, as well as the relative importance they give to different product characteristics when making their wine purchase decisions (Hollebeek, Jaeger, Brodie, & Balemi, 2007; Lockshin, 1998; Lockshin, Quester, & Spawton, 2001; Lockshin, Spawton, & MacIntosh, 1997). High involvement has been also related to demand for knowledge and variety seeking (Dodd, Pinkelton, & Gustafson, 1996; Goldsmith & d'Hauteville, 1998). Therefore, wine involvement is expected to influence the vocabulary used for describing wine astringency: consumers involved with wine may use a higher number of concrete, technical terms than low-involved consumers. In this sense, research has shown that wine expertise affects perceived quality and the vocabulary used for communicating and describing wine (Ballester, Patris, Symoneaux, & Valentin, 2008; Hopfer & Heymann, 2014). Expertise has been reported to improve the communicative value of wine descriptions (Lehrer, 1975; Lawless, 1984; Solomon, 1990)

The aims of the present work were to: (a) gain an insight on the vocabulary used by wine consumers to describe the astringency of red wine, (b) compare consumer vocabulary with the terms included in the Mouth-feel wheel, and (c) evaluate differences in the vocabulary of consumer groups with different wine involvement.

1.2. MATERIALS AND METHODS

1.2.1. Consumers

A total of one hundred and twenty-five consumers participated in the study (56% female). Their ages ranged from 21 to 69 years old (average = 40.0 years old, standard deviation = 13.3 years old). Participants were recruited from the consumer database of the Food Science and Technology Department of Universidad de la República (Uruguay), according to their wine consumption (at least once a month) and interest to participate in the study. Participants signed an informed consent form prior to completing the study.

1.2.2. Questionnaire

Participants were asked to complete a questionnaire that comprised five tasks. First, they were asked to provide a definition of the term “astringency” (“astringencia” in Spanish) by answering the following open-ended question: *“How would you define the astringency of red wine?”*.

Then, they had to complete two free listing tasks. Free listing is a simple qualitative technique widely used in anthropology, which consist of asking participants to list all the terms that fit into a certain criterion (Rusell Bernard, 2005; Hough & Ferraris, 2010). In the present study, participants were asked to *list all the sensations they perceive when drinking an astringent red wine* and *all the words they would use to describe the astringency of a red wine*.

After completing the free listing tasks participants were asked to answer a check-all-that-apply (CATA) question which comprised 44 terms used in the literature to describe astringency. Participants were asked to select all the terms they considered appropriate to describe the astringency of a red wine. Thirty-one of the terms were included in the Mouth-feel wheel (*harsh, hard, aggressive, abrasive, dry, numbing, parching, pucker, chewy, adhesive, complex, soft, fleshy, mouthcoating, fine emery, velvet, suede, silk, talc, powder, plaster, dusty, grainy, chalky, sawdust, unripe, resinous, sappy, green, full, viscous*) (Gawel et al., 2000). The rest of the terms were *rough, irritant, sand paper, hessian, fine grain, coarse grain, smooth, lush, long, round, even, sticky,*

and *oily*. These additional terms were selected based on pilot testing with wine professionals.

Then, participants completed a wine involvement questionnaire composed of 21 statements. The items of the questionnaire (Table 1.1) were selected considering published literature (Lockshin et al., 1997; Mittal & Lee, 1989). Participants had to rate their degree of agreement with each of the statements using a 7-point scale ranging from "totally disagree" to "totally agree".

Finally, participants were asked to indicate their age, gender, wine frequency consumption, type and price range of wines usually consumed.

The questionnaire was implemented using a web interface (Google Doc®). Consumers were asked to answer all questions spontaneously and explained that there were no right or wrong answers. The software imposed consumers to answer the questions one at a time in the specified order.

Table 1.1. Items of the questionnaire used to evaluate wine involvement.

	Item
1	I enjoy selecting the adequate wine for each occasion
2	Wine purchase is irrelevant for me
3	I am interested in wine
4	Deciding what wine to buy is an important decision for me
5	I care about what wines I buy
6	I carefully choose the wines I buy
7	It is worth investing extra time when buying wine to get discount prices
8	I think carefully about the wines I drink
9	Wine consumption gives me social status
10	I usually read wine magazines and leaflets
11	I always look at the colour of wine before trying it
12	I always evaluate the aroma of wine before drinking it
13	I usually go to wine tastings or courses
14	Drinking wine has a positive effect on my quality of life
15	I enjoy going to wine fairs or expositions
16	I enjoy drinking a good wine
17	I like my image when I drink wine
18	I indulge myself when I buy wine
19	Drinking wine is beneficial
20	Knowing what type of wine a person drinks tells you a lot about the type of person she/he is
21	Wine makes my life easier

1.2.3. Data analysis

1.2.3.1. Astringency definition

All valid words mentioned by participants were considered for data analysis. Astringency definitions were analysed following the methodology proposed by ten Kleij

and Musters (2003) to analyse open-ended questions. The first step of the analysis consisted of deleting stop words such as “a,” “and,” “or” or “the”, and words that were included in the proposal such as “wine” or “astringency”. Frequency of mention of each word was calculated. Then, data were analysed by grouping the phrases and words mentioned by participants into categories using inductive coding (Krippendorff, 2004). In this process the categories are determined by the researchers as they read the data. Three different researchers with a minimum of 2 years of experience in consumer research performed the analysis (Modell, 2005). Then, a consensus between the three researchers was reached and final categories were determined to balance out the subjective influences of individuals (Denzin, 1978). Frequency of mention of each category was determined. Categories mentioned by more than 5% of the participants were considered (Guerrero et al., 2010).

1.2.3.2. Free listing tasks

First, the number of terms elicited by each participant was counted. Then, the elicited terms were qualitatively analysed by grouping responses in categories, following the procedure described in 1.2.3.1.

1.2.3.3. Check-all-that-apply question

The number of terms selected by each consumer was determined. Frequency of use of each of the terms included in the CATA question was determined by counting the number of consumers who selected each of the terms.

1.2.3.4. Wine involvement questionnaire

Exploratory factor analysis was performed on data from the wine involvement questionnaire. Prior to performing the analysis, psychometric adequacy of the data matrix was assessed using Bartlett’s test of sphericity (Bartlett, 1950) and the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy (Kaiser, 1970; Kaiser & Rice, 1974). Bartlett’s test of sphericity was significant (χ^2 = 1497, p<0.0001), and KMO measure of sample adequacy was 0.88, suggesting that the correlation matrix was suitable for factor analysis. Parallel analysis (Horn, 1965) was used to determine the number of factors to keep in the solution. Responses for Item 2 (Table 1.1) from the questionnaire were reverse-scored prior to running the factor analysis. Internal reliability of each factor was tested using Cronbach’s alpha (Cronbach, 1951). Items which showed factor loadings higher than 0.40 were considered for the interpretation of the factor solution.

1.2.3.5. Evaluation of differences between consumer groups with different wine involvement

Cluster analysis was performed on the average scores of the items correlated to each of the identified factors in order to identify groups of consumers with different wine involvement. Hierarchical cluster analysis with Euclidean distances and Ward's agglomeration method was used.

Data from the first four tasks of the questionnaire were analysed separately for each of the identified consumer groups. Global chi-square analysis was used to assess differences between consumer groups in the frequency of mention of the categories used in each task. When the global chi-square test was significant, a chi-square per cell analysis was performed to identify its source of variation (Symoneaux & Galmarini, 2014).

All statistical analyses were performed with R (R Core Team, 2017), and packages tm (Feinerer, Hornik, & Meyer, 2008) and psych (Revelle, 2013) were used.

1.3. RESULTS

1.3.1. Astringency definition

Consumer responses to the open-ended question which required them to define astringency are shown in Table 1.2. Only 2% of the participants were not able to define astringency and indicated that they didn't know the term, while a small percentage referred to astringency as a flavour attribute and related it to bitterness and sourness. However, the great majority of wine consumers were able to accurately define astringency. As shown in Table 1.2, the majority of the consumers referred to astringency as a *rough* or *dry sensation*, felt on the *mouth*, *palate* and *tongue* when or *after drinking* wine. Besides, a small percentage of consumers mentioned tannins and phenols as specific astringent compounds in their definition.

1.3.2. Sensations felt when drinking an astringent wine

When consumers were asked to list the sensations they perceive when drinking an astringent wine they mainly mentioned words related to *dryness* and *roughness* (Table 1.3). Consumers referred to intensity or time-related characteristics of astringency, such as *strong* and *persistent*, as well as a hedonic term (*disgust*). Participants also elicited terms that are not related to astringent sensations, such as *bitterness*, *acidity*, *taste,* and *aroma*.

Table 1.2. Categories identified in the open-ended question in which consumers were asked to define the astringency of a red wine, and percentage of consumers who mentioned responses within each of the categories.

Category	Examples	Percentage of mention (%)
Sensation	Sensation, feeling, feel	62
Mouth	Mouth	42
Dry	Dry, dryness	41
Rough	Rough, roughness	38
After	After, end	30
Drinking	Drinking, having, tasting	26
Palate	Palate	18
Tongue	Tongue	17
Harsh	Harsh	14
Flavour	Taste, Flavour	10
Bitter	Bitter	9
Astringent compounds	Tannins, phenols	8
Acid	Acid, Sour	6
Strong	Strong	5
Throat	Throat	5

Table 1.3. Categories identified in the free-listing task in which consumers were asked to list all the sensations perceived when drinking an astringent red wine, and percentage of consumers who mentioned responses within each of the categories.

Category	Percentage of mention (%)
Dryness	53
Roughness	42
Bitterness	20
Taste	18
Acidity	17
Strong	17
Harsh	16
Rugosity	10
Persistent	9
Body	8
Disgust	8
Aroma	6

1.3.3. Terms for describing astringency

When consumers were asked to freely write down words to describe the astringency of red wines they elicited an average of 3.0 terms and a maximum of 9.0. The most frequently mentioned terms were related to dryness and roughness (Table 1.4). Apart from the two terms *dry* and *rough*, the only astringency sub-qualities elicited by consumers were *harsh*, *hard*, *smooth* and *sand paper*.

Consumers listed terms commonly used for describing astringency intensity (i.e. *strong, low, moderate, high*) as well as time-related terms (*persistent*). Besides, participants elicited several terms that are not astringency descriptors, such as *acid, bitter, colour,* and some non-specific terms (*body, astringent, tannin*). In addition, a small percentage of the consumers related astringency to wine quality, either as a positive or negative characteristic for the overall quality.

Table 1.4. Categories identified in the free-listing task in which consumers were asked to list all the terms they used for describing the astringency of red wine, and percentage of consumers who mentioned responses within each of the categories.

Category	Percentage of mention (%)
Rough	34
Dry	33
Strong	24
Astringent	18
Acid	14
Harsh	13
Body	11
Hard	11
Tannin	10
Bitter	10
Don'tknow	10
Taste	10
Intensity (low, modearte, high)	8
Colour	8
Smooth	7
Quality (Good, Bad)	6
Young	5
Persistent	5
Sand paper	5

1.3.4. Frequency of use of the terms included in the CATA question

When participants were presented with 44 astringency terms and were asked to select all those that applied to describe wine astringency, few terms were considered applicable, and only 25 were selected by at least 10% of the participants.

As shown in Fig. 1.1, the only terms selected by more than 50% of the consumers were *rough, harsh* and *dry*. Other relevant terms were *aggressive, sand paper, abrasive, hard, coarse grain*, and *irritant,* which were selected by more than 25% of the participants.

Regarding the terms of the Mouth-feel wheel, only 17 out of the 31 included in the CATA question were selected by more than 10% of the participants, as shown in Fig. 1.1.

1.3.5. Influence of involvement on consumers' astringency vocabulary

Parallel analysis performed on the data collected through the wine involvement questionnaire suggested an underlying structure of three factors. Exploratory factor analysis performed with principal axes extraction method and oblimin rotation allowed to identify three factors that together explained 50% of the variance. Factor loadings of the 21 items on the three factors are shown in Table 1.5. The first factor involved items mainly related to wine purchase (Cronbach $\alpha = 0.91$), whereas the second one was related to associations with wine (Cronbach $\alpha = 0.79$). The third factor was associated with the items of the questionnaire that involved wine-focused activities (Cronbach $\alpha = 0.82$), such as wine tastings, fairs and expositions (i.e. Tables 1.1 and 1.5).

Two consumer segments were identified using cluster analysis on the average scores in each of the dimensions identified in the factor analysis of the wine involvement questionnaire. Cluster 1 ($n = 73$) showed the highest scores in the three factors ($p<0.001$), suggesting higher wine involvement (Table 1.6). There was no difference in gender composition of the two clusters identified ($p=0.217$), nor in the type of wine consumed ($p=0.722$), as the majority of the participants indicated they usually consume red wine. However, there were significant differences among consumer groups with different wine involvement in age ($p=0.005$), wine frequency consumption ($p=0.002$) and price range of the wines usually consumed ($p=0.005$). Consumers with higher wine involvement tended to be older (mean age Cluster 1 = 42.7, mean age Cluster 2 = 36.0), to consume wine more frequently and purchase more expensive wines than consumers with lower wine involvement.

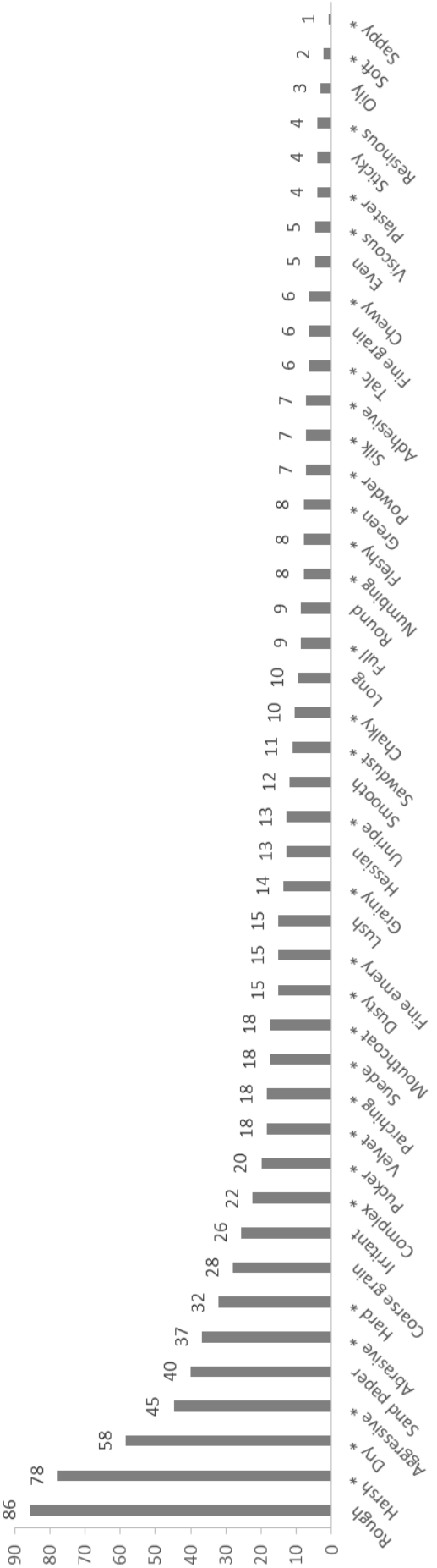

Fig. 1.1. Percentage of use of the terms included in the CATA question in which consumers were asked to select the terms they consider appropriate to describe the astringency of red wines. Terms included in the Mouth-feel wheel are indicated with (*).

No significant differences were identified between the two consumer segments with different wine involvement in the responses when asked to provide an astringency definition and in the two free listing tasks. However, when consumers were asked to list all the terms they would use to describe the astringency of red wine, consumers with higher wine involvement tended to generate a higher number of different terms (average = 3.3, maximum = 9) than consumers with less wine involvement (average = 2.4, maximum = 5).

Table 1.5. Factor loadings from the exploratory factor analysis performed on item scores of the wine involvement questionnaire.

Item	Factor 1 Purchase involvement	Factor 2 Associations with wine	Factor 3 Involvement in wine-focused activities
1	**0.77**	0.08	0.02
2 *	**0.52**	0.00	-0.15
3	**0.63**	0.07	0.19
4	**0.87**	0.04	-0.03
5	**0.95**	0.02	-0.05
6	**0.91**	-0.03	0.02
7	**0.51**	0.01	0.13
8	**0.68**	-0.09	0.17
9	-0.07	**0.40**	0.35
10	0.06	0.12	**0.63**
11	0.36	-0.10	**0.52**
12	0.24	-0.01	**0.46**
13	-0.08	0.07	**0.72**
14	0.18	**0.43**	0.28
15	0.20	0.06	**0.58**
16	**0.47**	0.16	0.04
17	-0.04	**0.57**	0.18
18	0.30	**0.57**	-0.16
19	0.10	**0.57**	0.05
20	-0.09	**0.42**	0.03
21	-0.05	**0.74**	0.00

Factor loadings with absolute value higher than 0.40 are highlighted in bold.
*Item 2 was reverse-scored.

The frequency of use of the astringency descriptors included in the CATA question significantly differed between the two consumer groups (χ^2 =59.8, p= 0.046). However, only 5 of the 44 terms were responsible for such a difference. Consumers with higher wine involvement used more frequently *unripe, silk* and *round*, and less frequently *dry* and *parching*.

Table 1.6. Average scores of the factors identified in the exploratory factor analysis performed on consumers' responses to the items of the wine involvement questionnaire, for the two consumer clusters identified using hierarchical cluster analysis.

Cluster	Purchase involvement (Items 1-8, 16)	Associations with wine (Items 9, 14, 17-21)	Involvement in wine-focused activities (Items 10-13, 15)
1 (n=73)	6.0[a]	3.9 [a]	5.2 [a]
2 (n=52)	4.3[b]	3.0[b]	2.6 [b]

Values within the same column with different superscript are significantly different according to Tukey's test for a 0.05 significance level.

1.4. DISCUSSION

Astringency is one of the most important sensory characteristics for the quality of red wine (Peynaud, 1987). Considering the multidimensionality of astringency perception, it is important for the industry to determine how to communicate astringency characteristics to consumers. The present work provided an insight on how consumers describe astringency and the vocabulary they would use to describe the astringency of red wine.

Consumers showed a good understanding of what the astringency of red wine is. They provided precise terms related to the sensations that are typically experienced when consuming astringent products (Lesschaeve & Noble, 2005). According to consumers' responses, astringency can be defined as *dry and rough sensations that are experienced in the mouth, palate and tongue when consuming astringent products*. This definition was similar to that used by trained assessors panels in several published articles (e.g. Ferrer-Gallego, Hernández-Hierro, Rivas-Gonzalo, & Escribano-Bailón, 2014; Lee & Vickers, 2008; Ross, Hinken, & Weller, 2007; Sáenz-Navajas, Avizcurri, Ferreria, & Fernández-Zurbano, 2014).

The sensations that consumers mostly associated with astringency and that were most frequently mentioned to describe the astringency of red wine were related to dryness and roughness (c.f. Tables 1.2-1.4), which have been reported to be essential to astringency perception (Green, 1993). Besides, according to Lee and Lawless (1991) dry and rough sensations evolve in time similarly than astringency.

Consumers mentioned bitterness and sourness when asked to describe the sensations they perceive when drinking astringent wines or the terms they use to describe astringency (Tables 1.3 and 1.4). Astringency has been reported to be accompanied by sourness and bitterness sensations (Arnold, Noble, & Singleton, 1980; Lyman & Green, 1990; Sowalsky & Noble, 1998). In particular, bitterness and astringency are often confused in red wines because almost all phenolic compounds elicit these two sensations (Lesschaeve & Noble, 2005). However, astringency,

bitterness, and sourness can be distinguished and rated separately (Lea & Arnold, 1978; Lee & Lawless, 1991; Ishikawa & Noble, 1995). Several authors have argued the need to improve assessors' ability to separate bitterness and sourness from astringency perception during training (Lee & Vickers, 2010; Ross et al., 2007). Besides, Lee and Vickers (2008) removed the term *puckery* from the definition of astringency to prevent assessors from confusing sourness and astringency.

Consumers spontaneously elicited an average of 3.0 terms when asked to write down the terms they would use to describe wine astringency. Besides, they did not spontaneously referred to a large number of terms, suggesting that consumers use a limited vocabulary for describing wine astringency. The most relevant terms were *dry, rough, harsh, hard, smooth and sand paper* (Table 1.4). These terms have been previously considered by several authors to describe wine astringency (Ferrer-Gallego et al., 2014; Lawless et al., 1994; Lee & Lawless, 1991; Peynaud, 1987). The majority of the terms of the Mouth-feel wheel were not deemed applicable for describing wine astringency by the great majority of the participants (Fig. 1.1). This suggests that the vocabulary proposed by Gawel et al. (2000) contains too many technical terms and may not be appropriate to communicate the astringency characteristics of wine to consumers. The terms not included in the Mouth-feel wheel that were more relevant for consumers were c*oarse grain*, *sand paper* and *rough*, which was the one with the highest frequency of use among all the terms included in the CATA question (Fig. 1.1).

Although wine involvement has been reported to affect consumers' purchase decisions and wine knowledge (Dodd et al., 1996; Goldsmith & d'Hauteville, 1998; Hollebeek et al., 2007; Lockshin, 1998; Lockshin et al., 2001), in the present work it did not significantly affect consumers' astringency vocabulary. Responses differed between groups of consumers with different wine involvement only in the CATA questions, and this difference was due to five of the 44 terms considered. This result can be related to the fact that astringency description is not a common activity among consumers, being more associated with wine experts. Previous research has shown that experts describe wines using more concrete, precise and accurate terms than consumers (Ballester et al., 2008; Lehrer, 1975; Lawless, 1984; Solomon, 1990).

It is important to stress that culture is expected to have a large influence on consumers' astringency vocabulary and how it is mediated by wine involvement, as previously reported for other complex wine characteristics, such as minerality (Parr, Ballester, Peyron, Grose, & Valentin, 2015). Wine conceptualization has been reported to be influenced by culture, which may affect how wine attributes are perceived (Mouret, Lo Monaco, Urdapilleta, & Parr, 2013). The astringency vocabulary reported in the

present work is expected to largely differ from that of consumers in countries where wine has a long social, economic and political tradition, such as France (Parr et al., 2015). Cross-cultural research on consumers' astringency descriptions can contribute to the development of an accurate astringency vocabulary.

1.5. CONCLUSIONS

Results from the present work showed that consumers accurately understand the meaning of wine astringency. This sensory characteristic is mainly related to dry and rough sensations. However, consumers' vocabulary to describe astringency seems limited. Therefore, most of the terms of the Mouth-feel wheel do not seem adequate to communicate the astringency characteristics of red wine to consumers. This indicates the need for further research on how consumers perceive and describe wine astringency. Future studies should aim at exploring how consumers understand the different sub-qualities of astringency and what sensations they expect from wines described with those characteristics. Further research should also be carried out exploring consumers' perception and description of wine astringency when evaluating samples. Descriptive studies of astringent wines can contribute to the selection of astringency terms and to determine the number of astringency characteristics that can be evaluated when performing sensory analyses involving consumers. Also, research comparing consumers' and trained assessors' descriptions of wine astringency seems necessary.

CHAPTER 2

Evaluation of palate cleansers for astringency evaluation of red wines

ABSTRACT

Astringency has been reported to be strongly time-dependent and to exhibit build-up upon repeated ingestions. A common approach to deal with this phenomenon during astringency evaluation is the use of palate cleansers. The aim of this work was to evaluate palate cleansers for red wine astringency evaluation, considering both reduction of astringency build-up and sample discrimination. Fourteen trained panellists evaluated two sets of four Tannat wine samples using time-intensity methodology. In the first sample set the same wine was presented four times while the second one comprised four samples with different added concentrations of grape seed tannins. Each assessor evaluated both sample sets with five different palate cleansers (still mineral water, plain unsalted crackers, skimmed milk, drinkable plain sweetened yogurt and 2 g/L pectin solution) in triplicate. None of the evaluated palate cleansers could prevent the occurrence of astringency buildup, while sample discrimination ability differed among the five palate cleansers considered.

PRACTICAL APPLICATIONS

Astringency is one of the most relevant sensory characteristics involved in wine quality and complexity, which makes its evaluation very important for wine industry and wine research. Results from this study provide evidence for the selection of best practices in astringency evaluation. The fact that none of the evaluated palate cleansers was efficient in preventing astringency buildup over repeated ingestions highlights the importance of limiting the number of wine samples evaluated in a single session and/or considering a pre-determined waiting time between samples. Regarding sample discrimination, drinkable plain sweetened yogurt, followed by a water rinse, provided the best results compared to other usual alternatives, such as water, pectin solutions or plain crackers. In fact, plain crackers, which have been recommended in other studies, showed the lowest discrimination.

2.1. INTRODUCTION

Astringency is one of the most important sensory characteristics that define the complexity and quality of red wine (Peynaud, 1987). According to the ASTM, astringency can be defined as "*the complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins*" (ASTM, 2004).

Astringency perception has been reported to be strongly time-dependent (Guinard, Pangborn, & Lewis, 1986). Perceived astringency increases linearly during the first 13-15 seconds after ingestion, regardless of the astringent compound and its concentration (Ishikawa & Noble, 1995). Besides, astringency exhibits build-up effect upon repeated ingestions, i.e., perceived astringency significantly increases with repeated ingestions of astringent stimuli (Colonna, Adams, & Noble, 2004; Courregelongue, Schlich, & Noble, 1999; Lee & Vickers, 2008; 2010; Noble, 2002; Ross Hinken, & Weller, 2007). Guinard et al. (1986) reported that maximum astringency intensity and total duration of the astringent sensation significantly increased with repeated red wine ingestions taken at 20 s intervals. Similarly, Lyman and Green (1990) showed that astringency intensity continuously increased with repeated intakes of 10 mL of tannic acid solution when placed in mouth for 10 s/min, for a total of 20 min.

These characteristics make astringency a complex sensory attribute and poses challenges for is evaluation by sensory panels (Ross et al., 2007). For this reason, different experimental protocols have been proposed to prevent the influence of buildup and carry over effects upon repeated ingestions when conducting sensory studies (Lee & Vickers, 2010).

One of the possibilities is increasing the interval of time elapsed between the evaluation of samples (Lee & Vickers, 2010). Guinard et al. (1986) showed that increasing inter-sample period from 20 to 40 s reduces carry-over effects, whereas Gillespie (2000) reported no astringency buildup when 30 s were elapsed between sips. However, astringency has been reported to last for up to 6 min after ingestion in some situations (Lee & Lawless, 1991). Considering this waiting time is not practical and limits the number of samples that can be evaluated by trained assessors in a single session, the most common approach to prevent build-up and carry over effects has been the use of palate cleansers (Bajec & Pickering, 2008).

Palate cleansers aim at aiding the removal of residual materials from previous samples to re-establish baseline conditions (Lawless & Heymann, 2010). In the specific case of astringency, several palate cleansers have been proposed based on their ability

to compete with salivary proteins for interacting with poyphenols (Bajec & Pickering, 2008). These include gum (e.g. carboymethyl cellullose, pectin) and protein (e.g. gelatin, casein) solutions (Colonna et al., 2004; Ross et al., 2007). Besides, considering that astringency is a tactile sensation that has been related to changes in mouth lubrication, palate cleansers that lubricate the mouth have also been proposed, including crackers, water, sucrose, artificial saliva and a combination of oil and xanthan gum (Breslin, Gilmore, Beauchamp, & Green, 1993; Lee & Vickers, 2010).

Although several studies have been performed to compare palate cleansers for the evaluation of different astringent stimuli, no consensus has been reached on the most efficient alternative (Lawless & Heymann, 2010). Based on their ability to reduce astringency buildup different palate cleansers have been identified as the most efficient, including corn oil with xantham gum (Breslin et al., 1993), xanthan gum rinses with or without oil (Brannan, Setser, & Kemp, 2001a), pectin solutions (Colonna et al., 2004) and crackers (Ross et al., 2007).

Most studies on palate cleansers have focused on the reduction of astringency buildup over repeated ingestions. However, as stressed by Lee and Vickers (2010), an ideal palate cleanser should enhance discrimination among samples. Brannan et al. (2001a) showed that palate cleansers can mask astringency sensation during subsequent tastings, reducing assessors' ability to discriminate among samples. Lee and Vickers (2010) compared six palate cleansers (nothing, water, crackers, skimmed milk, carboxymethylcellullose, and wax plus lemons) based on sample discrimination and their efficacy to prevent astringency buildup during repeated ingestions. They concluded that although the palate cleansers did not differ in their ability to prevent astringency buildup, water or nothing improved discrimination among samples.

In this context, the aim of the present work was to evaluate palate cleansers for red wine astringency evaluation, considering both reduction of astringency build-up and sample discrimination.

Five palate cleansers were evaluated: water, crackers, 2g/L pectin solution, skimmed milk and drinkable plain sweetened yogurt. Water, crackers and pectin solutions have been reported to be the most efficient palate cleansers for astringency evaluation by different authors (Colonna et al., 2004; Lee & Vickers, 2010; Ross et al., 2007). Skimmed milk was included in the study considering that casein and bovine serum albumin have been reported to bind with tannins (de Freitas & Mateus, 2001; Luck et al. 1994). Besides, skimmed milk was found to be the most efficient alternative to reduce the astringency of antioxidant extracts of Uruguayan native plants (Ares, Barreiro, Deliza,

& Gámbaro, 2009). The selection of yogurt was motivated by the fact that, although it has been used as palate cleanser in other food products, it has never been evaluated for wine astringency evaluation (Lucak & Delwiche, 2009). This product has potential to serve as an efficient palate cleanser due to the potential interaction between milk proteins and tannins, and its ability to increase salivation due to its acidity and sweetness (Froehlich, Pangborn, & Whitaker, 1987).

2.2. MATERIALS AND METHODS

2.2.1. Trained assessor panel

The panel consisted of 14 trained assessors (9 females, ages ranging between 24 and 48 years old). Assessors had been recruited among students and workers of Universidad de la República, following the guidelines provided by ISO (2012). They signed an informed consent form before starting the study. The study was approved by the Ethics Committee of Facultad de Química, Universidad de la República (Uruguay).

2.2.2. Assessors training

Assessors attended a total of fifteen 20-min training sessions before starting the study, in which they were introduced to astringency evaluation, intensity measurement and time intensity methodology. In the first session assessors were familiarized with astringent sensations, by presenting an alum solution (5.0 g/L alum, McCormick, Hunt Valley, MD) as reference standard. Astringency was defined as the "tactile sensation felt in mouth and characterized by dryness and roughness". Assessors were also trained to differentiate between astringency, bitterness and sourness by presenting additional reference standards of the last two tastes (1.5 g/L citric acid and 0.8 g/L caffeine, respectively).

In subsequent sessions assessors were familiarized with the evaluation protocol and trained to quantify astringency using a line scale, anchored with the terms "low" and "high". Alum solutions of different concentration (0.5 g/L to 5.0 g/L), commercial red wines with different astringency level and red wines with added grape seed and skin tannins (0.5 g/L to 2.5 g/L) were used. The 5.0 g/L alum solution was considered as the reference for "high" astringency. The evaluation protocol required assessors to take a sip (15 mL) in their mouth, to swish the sample gently for 10 s while performing a standardized vertical tongue movement. Then, assessors were asked to spit the sample. Astringency ratings were collected using a line scale.

Finally, assessors were introduced to the time-intensity methodology and to the software used for data collection. A total of six sessions were considered in which they evaluated samples with different astringency intensity.

2.2.3. Samples

2.2.3.1. Wines

A Uruguayan Tannat wine (Don Pascual 2013, Establecimiento Juanicó) was used as astringent stimuli. Palate cleansers where evaluated for the evaluation of two sample sets.

The first sample set was composed of the same wine presented four times, which aimed to simulate repeated ingestions of the same sample. Assessors were not aware that all samples corresponded to the same wine.

The second sample set comprised wine samples with different astringency, generated by adding different concentrations of grape seed tannins (Abastecimientos, Uruguay) to the base wine. Four concentrations were considered: 0, 0.1, 0.2 and 0.3%. Samples were presented following a Willliams' Latin square experimental design.

Samples (15 mL) were served in 50 mL plastic cups, labelled with random 3-digit codes.

2.2.3.2. Palate cleansers

Five palate cleansers were used in the study: still mineral water (pH=7.5, 35 mg/L calcium, 9.5 mg/L magnesium, 6.8 mg/L sodium, 0.7 mg/L potassium, 6.99 mg/L chlorides, 2.52 mg/L nitrates, 2.47 mg/L sulphates), plain unsalted crackers (ingredients: enriched flour (wheat flour, folic acid, iron), high oleic sunflower oil, malt extract, glucose syrup, yeast, ammonium bicarbonate, soy lecithin, flavourings), skimmed milk (3.1% protein), drinkable plain sweetened yogurt (10% added sugar, 3.3% protein, 3.2% fat) and 2 g/L low methoxyl pectin solution (Sabores e Ingredientes, Montevideo, Uruguay).

Palate cleansers were used following an ad libitum protocol, i.e. the exact amount of palate cleanser used by assessors was not controlled. Lee and Vickers (2010) reported that controlled ingestions or ad libitum protocols did not influence the efficiency of palate cleansers. Liquid palate cleansers were served in 50 mL plastic cups, whereas 2 crackers were served. Assessors were instructed to take a sip/bite of the palate cleanser, according to the timing defined in the evaluation protocol.

2.2.4. Evaluation protocol

The evaluation protocol for the two sample sets was similar.

Assessors were asked to make click on the start button of the software and to simultaneously take a sip of palate cleanser in their mouth. No instructions were given regarding swallowing or expectoration. After 20 seconds they had to take a sip of still mineral water, as suggested by Colonna et al. (2004). These authors reported that palate cleansers were most efficient when residuals were removed by water rinses before the evaluation of the next sample. The amount of water in this step was determined ad-libitum by each assessor to assure removal of the palate cleanser, as previously suggested by Lee and Vickers (2010). Then, after 40 seconds they had to take a sip of sample (15 mL) and to start the time-intensity task. They were asked to swish the sample gently for 10 s while performing three standardized up and down tongue movement, without pressing the tongue against any mouth surface. After the 10 s they expectorated the sample. Assessors were asked to continuously rate astringency intensity (defined as tactile sensation felt in mouth and characterized by dryness and roughness) using the line scale for a total of 40 s. The timeline for sample evaluation is shown in Fig. 2.1. Once assessors completed the evaluation protocol for one of the samples, they immediately started the evaluation of the next sample.

Palate cleansers were evaluated by the assessors following a Williams' Latin square design. Three replications of each sample/palate cleanser combination were evaluated by each assessor for each sample set. In each session assessors evaluated one sample set using one palate cleanser, meaning that a total of 30 sessions were needed to complete the study.

Data collection was carried out using Compusense-at-hand (Compusense Inc., Guelph, Ontario, Canada). Testing took place in standard sensory booths in a sensory laboratory that was designed in accordance with ISO 8589 (ISO, 2007), under artificial daylight and temperature control (22ºC).

2.2.5. Data analysis

Maximum astringency intensity over the 40 s evaluation period, time to maximum astringency and astringency at the end of the evaluation period were determined for each sample, assessor and replicate. Data from each sample set were analysed using linear mixed modelling to evaluate the influence of palate cleansers on astringency buildup over repeated ingestions.

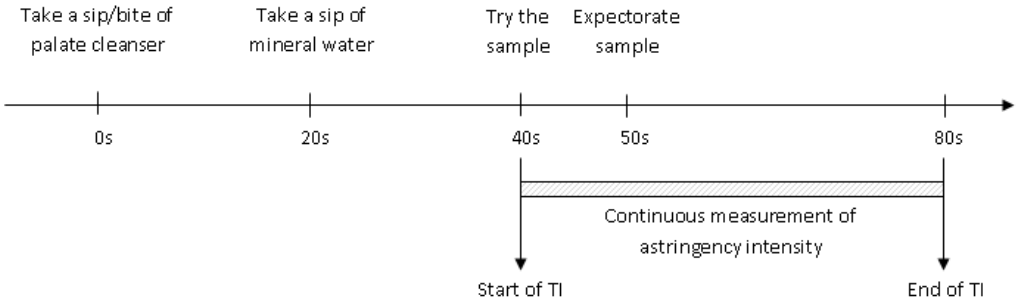

Fig. 2.1. Schematic representation of the evaluation protocol used to evaluate palate cleansers for wine astringency evaluation using time-intensity (TI).

For the analysis of data from set 1 palate cleanser, serving position in a tasting session and their interaction were specified as fixed effects, whereas session and assessor were considered as random effects. The variable serving position refers to the serving position in a single tasting session, and takes the values 1, 2, 3 or 4 because four samples were evaluated in each session. The Serving position * Palate cleanser interaction was used to evaluate the influence of palate cleanser on astringency buildup. Tukey's test was used for post-hoc pairwise comparisons of palate cleanser and serving position means.

For the analysis of data from set 2 sample, palate cleanser, serving position in a tasting session were specified as fixed effects, whereas session and assessor were considered as random effects. Two-way interactions between fixed effects were also considered in the model. Tukey's test was used for post-hoc pairwise comparisons of palate cleanser, serving position and sample means. Also, a linear mixed model was used separately on data from each palate cleanser considering serving position and sample as fixed effects and assessor and session as random effect. Two-way interactions were also considered in the model. Tukey's test was used for post-hoc pairwise comparisons of sample means.

All statistical analyses were performed with R (R Core Team, 2017).

2.3. RESULTS

2.3.1. Astringency buildup

Maximum astringency intensity significantly increased with serving position for both sample sets, which evidences the occurrence of astringency buildup (Table 2.1). When assessors repeatedly evaluated the same sample (sample set 1), maximum astringency did not significantly increase between the first and the second serving positions (Fig. 2.2.a). However, significant differences in maximum astringency intensity were identified from the first to the third position, as well as between the second and the fourth. For sample set 2, in which assessors evaluated wine samples with different concentration of added tannins, astringency buildup occurred between the first and the rest of the serving positions (Fig. 2.2.b).

Time to maximum astringency was not significantly affected by serving position in any of the studies, as show in Table 2.1.

Astringency intensity at the end of the evaluation did not significantly increase with serving position for the evaluation of sample set 1, in which assessors repeatedly evaluated the same wine four times (Table 2.1). However, a trend towards an increase in astringency intensity at the end of the evaluation with serving position was observed (Fig. 2.2.a).

Astringency build up was evidenced through the increase in astringency intensity at the end of the evaluation in sample set 2. When assessors evaluated samples with different astringency intensity serving position significantly affected astringency intensity at the end of the evaluation (Table 2.1). As shown in Fig. 2.2.b, astringency at the end of the evaluation was significantly lower for the first sample than for the third and fourth samples.

The effect of the interaction between serving position and palate cleanser on maximum astringency intensity, time to maximum astringency and astringency intensity at the end of the evaluation was not significant for both sample sets (Table 2.1).

2.3.2. Average astringency intensity

Palate cleanser significantly affected average maximum astringency intensity ratings for the evaluation of the first sample set (Table 2.1). Cleansing with crackers resulted in significantly lower average maximum astringency ratings across all sessions and samples than for the rest of the evaluated palate cleansers (Table 2.2). For this sample set astringency intensity at the end of the evaluation was also affected by palate

cleanser (Table 2.1). As shown in Table 2.2, the lowest astringency intensities at the end of the evaluation were observed for plain crackers and yogurt.

In the evaluation of the second sample set average maximum astringency intensity, as well as astringency intensity at the end of the evaluation were not significantly affected by palate cleanser (Table 2.1). A similar result was observed for time to maximum intensity for both sample sets, which was not significantly affected by palate cleanser (Table 2.1).

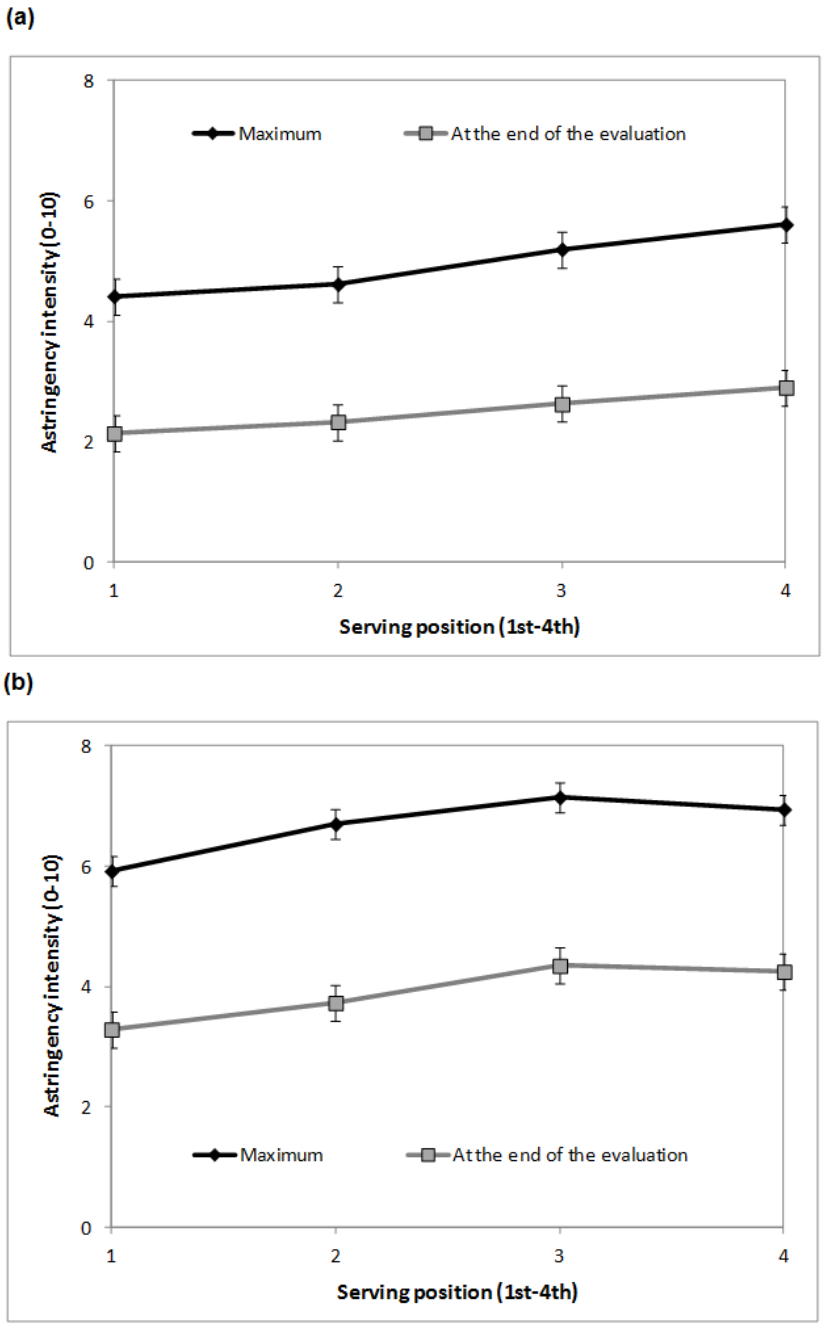

Fig. 2.2. Maximum astringency intensity and astringency intensity at the end of the evaluation as a function of serving position when assessors evaluated two sets of wine samples using time-intensity: (a) sample set 1 (one wine sample presented four times) and (b) sample set 2 (four samples of wine with varying concentration of added tannins).

Table 2.1. F and p-values (between brackets) of the fixed effects considered in the analysis of variance performed on maximum astringency intensity, time to maximum astringency and astringency intensity at the end of the evaluation in the time-intensity task in which assessors evaluated wine samples using different palate cleansers.

Sample set (*)	Fixed effect	Maximum astringency intensity	Time to maximum astringency (s)	Astringency intensity at the end of the evaluation
1	Serving position	6.49 (<0.001)	1.70(0.167)	1.74 (0.159)
	Palate cleanser	2.76 (0.037)	1.59 (0.177)	3.31 (0.011)
	Serving position x Palate cleanser	0.97 (0.481)	0.857 (0.591)	0.99 (0.458)
2	Serving position	4.88 (0.003)	1.42 (0.237)	5.03 (0.002)
	Palate cleanser	1.26 (0.288)	0.46 (0.773)	1.06 (0.379)
	Sample	72.14 (<0.001)	0.87 (0.455)	29.47 (<0.001)
	Serving position x Palate cleanser	0.59 (0.853)	1.21 (0.273)	0.93 (0.516)
	Serving position x Sample	1.17 (0.315)	0.91 (0.520)	0.60 (0.797)
	Palate cleanser x Sample	1.02 (0.435)	1.17 (0.308)	0.57 (0.867)

(*) Sample set 1: the same wine sample presented four times. Sample set 2: four samples of wine with different concentration of added seed tannins

Table 2.2. Average maximum astringency, time to maximum astringency and astringency at the end of the evaluation across all samples, assessors and sessions for the five palate cleansers.

Palate cleanser	Sample set 1			Sample set 2		
	Maximum astringency (*)	Time to maximum astringency (s)	Astringency at the end of the evaluation (*)	Maximum astringency (*)	Time to maximum astringency (s)	Astringency at the end of the evaluation (*)
Plain crackers	4.3^{a}	6.7^{a}	1.9 ^{a}	6.5^{a}	8.2^{a}	3.6 ^{a}
Pectin	5.2^{b}	7.6^{a}	2.8 ^{b}	7.0^{a}	8.9^{a}	4.3 ^{a}
Yogurt	5.0^{b}	7.2^{a}	2.0 ^{a}	6.3^{a}	9.0^{a}	3.9 ^{a}
Milk	5.2^{b}	6.1^{a}	2.7 ^{b}	6.6^{a}	8.6^{a}	3.6 ^{a}
Water	5.1^{b}	7.8^{a}	2.8 ^{b}	6.3^{a}	9.9^{a}	4.1 ^{a}

(*) Measured in a 0-10 line scale (0=low and 10= high).
Average values within a column with different superscript are significantly different according to Tukey's test ($p \leq 0.05$).

2.3.3. Sample discrimination

In the case of sample set 2, assessors' ability to discriminate among wine samples with different concentration of added tannins in terms of maximum astringency during the time-intensity task was affected by palate cleanser. The highest discriminative ability was observed when yogurt was used as palate cleanser, as evidenced by the highest F-value for the sample effect in the ANOVA (Table 2.3). When yogurt was used as palate cleanser significant differences between every pair of samples were identified. For the rest of the palate cleansers three groups of samples were identified in terms of their maximum astringency, as differences between two of the samples were not significant (Table 2.3).

Ability to discriminate samples in terms of average astringency intensity at the end of the evaluation was also affected by palate cleanser. As shown in Table 2.4, the highest F-value for the sample effect in the ANOVA was found for yogurt.

In the case of time to maximum astringency, differences among samples were not significant for all the palate cleansers ($p>0.145$).

Table 2.3. F-value for the sample effect in the analysis of variance and average maximum astringency intensity during the time intensity task across assessors and sessions for wine samples with different concentration of added tannins (sample set 2), evaluated using different palate cleansers.

Palate cleanser	F-value for the sample effect	Sample			
		Wine	Wine + 0.1% tannins	Wine + 0.2% tannins	Wine + 0.3% tannins
Plain crackers	18.9	4.6 [a]	6.1 [b]	7.6 [c]	7.7 [c]
Pectin	14.9	5.1 [a]	6.7 [b]	7.4 [b]	8.7 [c]
Yogurt	33.6	3.8 [a]	5.5 [b]	7.5 [c]	8.5 [d]
Milk	8.1	5.3 [a]	5.9 [a]	7.1 [b]	8.1 [c]
Water	20.6	4.6 [a]	6.1 [b]	7.9 [c]	8.7 [c]

Average values within a row with different superscript are significantly different according to Tukey's test ($p\leq0.05$).

2.4. DISCUSSION

In this study astringency buildup during repeated assessment of wine samples was observed, in agreement with previous reports (Colonna et al., 2004; Guinard et al., 1986; Lee & Vickers, 2010; Ross et al., 2007). None of the palate cleansers was able to prevent the occurrence of astringency buildup (Table 2.1), in agreement with results reported by Lee and Vickers (2010). Other authors have reported that although some palate cleansers are able to reduce astringency build up, none has been reported to be able to fully prevent it (Breslin et al., 1993; Colonna et al., 2004; Ross et al., 2007). This

result indicates that the use of palate cleansers is not enough to reduce the influence of astringency buildup on astringency ratings and highlights the importance of limiting the number of wine samples evaluated in a single session and/or considering a pre-determined waiting time between samples. In this work maximum astringency intensity increased 1.0-1.2 points in the 0-10 line scale, from the first to the fourth sample (sets 2 and 1, respectively), whereas the increase in astringency intensity at the end of the time-intensity task ranged from 0.8 to 1.0 points (sets 2 and 1, respectively) (Figure 2.2). Therefore, it seems advisable not to evaluate more than 3-4 samples in a single session without a predetermined waiting time between samples. However, no consensus has been reached on the influence of waiting time on astringency buildup (Gillespie, 2000; Lee & Lawless, 1991), highlighting the importance of conducting further research on this topic.

Table 2.4. Average astringency intensity at the end of the time intensity task across assessors and sessions for wine samples with different concentration of added tannins (sample set 2), evaluated using different palate cleansers.

Palate cleanser	F-value for the sample effect	Sample			
		Wine	Wine + 0.1% tannins	Wine + 0.2% tannins	Wine + 0.3% tannins
Plain crackers	5.9	2.4 ^{a}	3.4^{a,b}	4.1 ^{b}	4.4 ^{b}
Pectin	5.7	2.9 ^{a}	4.5 $^{b, c}$	4.1 ^{b}	5.4 ^{c}
Yogurt	11.4	2.1 ^{a}	3.7 ^{b}	4.7 ^{c}	5.2 ^{c}
Milk	8.4	2.3 ^{a}	3.5 ^{a}	3.7 ^{b}	4.8 ^{c}
Water	6.8	2.9 ^{a}	3.9 ^{b}	4.6 ^{b,c}	5.0 ^{c}

Average values within a row with different superscript are significantly different according to Tukey's test ($p \leq 0.05$).

In the present work palate cleansers had a limited effect on astringency intensities (Tables 2.1 and 2.2). When assessors repeatedly evaluated the same wine sample the lowest intensity scores were obtained when crackers were used as palate cleansers, in agreement with results reported by Ross et al. (2007). According to these authors different phenomena can explain the lower intensity ratings obtained with plain crackers. Firstly, crackers can adsorb or physically entrap tannins, reducing astringent sensations, as hypothesized by Colonna et al. (2004). The buffering effect of bicarbonate can also contribute to counter the astringency sensations caused by wine acids. Besides, cracker residues that remains in the mouth can interact with astringent compounds of the next tasted sample, decreasing perceived astringency intensity. Another possible explanation for the lowest astringency ratings obtained using crackers is context effect (Lawless & Clark, 1992). Crackers might have felt drying in the mouth, inducing assessors to use lower intensity scores when evaluating wine samples (Ross et al., 2007). Further research is necessary to better understand the mechanisms that mediate

the reduction in astringency ratings due to the use of crackers, as reported by several authors.

No differences among palate cleansers were observed in maximum or minimum astringency intensities when assessors evaluated sample set 2, composed of wine samples with different concentration of added tannins. This result mirrors differences in the conclusions drawn by different authors when studying the influence of palate cleanser on astringency intensity.

Lee and Vickers (2010) reported that rinsing with crackers led to the lowest maximum astringency ratings compared to water, skimmed milk, carboxymethylcellulose and wax + lemons. Similarly, when comparing water, pectin, carboxymethylcellulose and crackers for repeated evaluation of wine samples, Ross et al. (2007) reported that the lowest astringency ratings were obtained with crackers, followed by pectin solutions. Brannan et al. (2001a) compared water and gum-based rinses, with and without oil, to alleviate astringent sensations caused by alum solutions and reported that the best results were obtained with 0.55% carboxymethylcellulose water solutions. Meanwhile, Colonna et al. (2004) reported that pectin solutions were more efficient in reducing astringency of red wine than water, carboxymethylcellulose, polyvinylpyrrolidone, gelatin or ovoalbumin.

The heterogeneity in the results reported in the literature suggest the nature of the astringent stimuli and experimental protocols for astringency evaluation may have a large impact on the efficacy of palate cleansers to reduce astringency sensations and prevent build up during repeated evaluations of astringent stimuli.

In this work the efficacy of palate cleansers was also determined in terms of discrimination among samples, following the recommendations of Lee and Vickers (2010). According to the F-value of the sample effect in the ANOVA yogurt showed the highest discriminative ability. This result can be explained considering different characteristics of yogurt. Firstly, milk proteins have been reported to bind with tannins (de Freitas & Mateus, 2001; Luck et al., 1994) and to reduce the astringency of polyphenolic extracts (Ares et al., 2009). However, in this work yogurt showed higher discriminative ability than milk. This difference can be explained considering that yogurt can also contribute to cleansing astringent sensations as a result of an increase in the lubrication of the oral mucosa due to an increase in salivation caused by acidity and sweetness (Froehlich et al., 1987). Also, its relatively high viscosity could also have contributed to alleviate astringent sensations, as previously hypothesized by Brannan et al. (2001a). Finally, anecdotal data indicated that assessors preferred yogurt to the other

palate cleansers, which has also been reported to be a relevant criterion when selecting palate cleansers for astringency evaluation (Lee & Vickers, 2010). Further research should study how easy yogurt can be removed with a water rinse after being used as a palate cleanser. In this sense, removing residuals of palate cleanser has been regarded as a key characteristic for assuring their discriminative ability (Colonna et al., 2004; Lee & Vickers, 2010).

Water showed higher discriminative ability than plain crackers, skimmed milk and pectin solutions, in agreement with results reported by Lee and Vickers (2010). These authors attributed these results to the fact that some palate cleansers, such as crackers and gum solutions, can mask astringency sensations during subsequent tastings due to the presence of residuals that can bind to tannins or stimulate saliva production. Results from this work stress the importance of considering discriminative ability when evaluating the performance of palate cleansers for the evaluation of different sensations.

2.5. CONCLUSIONS

Results from this work suggest that none of the evaluated palate cleansers (water, pectin, plain crackers, drinkable plain sweetened yogurt and skimmed milk) was efficient in preventing the occurrence of astringency buildup. However, among the five palate cleansers used in the study, drinkable plain sweetened yogurt, followed by a water rinse, provided the best results in terms of sample discrimination. This palate cleanser enhanced trained assessors' ability to identify astringency differences among samples, compared to other usual alternatives, such as water, pectin solutions or plain crackers.

CHAPTER 3

Sensory characterization of the astringency of commercial Uruguayan Tannat wines

ABSTRACT

Astringency is one of the most important characteristics that define the quality of red wine, and is of particular relevance for Tannat, Uruguayan emblematic red wine variety. Astringency is a time-dependent and complex sensory characteristic, related to several sensations, or sub-qualities, that can be simultaneously perceived. The aim of the present study was to obtain a sensory characterization of the astringency of commercial Uruguayan Tannat wines. Forty samples with different characteristics in terms of vintage, price segment and aging in oak barrels were assessed by a panel of 9 trained assessors. Total astringency intensity was evaluated using time-intensity (TI), while astringency sub-qualities were described using a check-all-that-apply (CATA) question composed of sixteen terms. TI and the CATA question provided different information on the astringency of Tannat wines. Regarding global astringency, samples mainly differed in intensity-related parameters rather than in the development of astringency over time, although the variability was moderate. A wide range of sub-qualities, from *silky* and *velvety* to *harsh* and *aggressive* were used to describe the astringency of the evaluated wines. Four groups of samples with different astringency characteristics were identified, but this sorting was not related to vintage, price segment or aging in oak barrels. Further research is necessary to better understand how astringency characteristics are influenced by production variables, and to understand their relationship to consumers' and experts' perceived quality of Tannat wines.

3.1. INTRODUCTION

Tannat is a red variety of *Vitis vinifera*, originally from the southwest of France, which is currently widely cultivated in Uruguay, representing 26% of the total national grape production (INAVI, 2017). In the last decades, the Uruguayan wine-making industry decided to develop Tannat as the national emblematic wine as a strategy to compete in the international varietal wine market (Carrau, 1997). This decision was based on Tannat's potential to produce high quality wines with a rich structure and good potential for ageing. These characteristics are mainly due to the total content and composition of phenolic compounds of Tannat wines, that differentiate it from other red varieties (Alcalde-Eon, Boido, Carrau, Dellacassa, & Rivas-Gonzalo, 2006; Boido et al., 2011; González-Neves, Gil, Favre, & Ferrer, 2012; González–Neves, Gómez-Cordovés, & Barreiro, 2001; Lloret et al., 2003;). Tannat wines have great tipicity, and in general present relatively intense colour and high astringency in comparison with other red varieties (Blanchard, 1999; Boidron et al., 1995). Uruguay is one of the few places in the world where Tannat is grown and research on the viticulture and enology of this variety is insufficient. In particular, although astringency is one of the differential attributes of Tannat wines, research on the astringency characteristics of this wine variety is still lacking. The evaluation of this complex sensory characteristic is relevant to better characterize this variety's wine quality potential, and to gain information to better communicate the sensory characteristics of Tannat wine to both national and international consumers.

Astringency has long been regarded as one of the most important sensory characteristics that define the quality, complexity and persistence of red wine (Cheynier & Sarni-Manchado, 2010; Peynaud, 1987). It can be defined as "*the complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins*" (ASTM, 2004). Red wine astringency has been mainly attributed to the presence of phenolic compounds, particularly proanthocyanidins (tannins) (Lesschaeve & Noble, 2005) However, the mechanisms of astringency perception are not completely known yet (Ferrer-Gallego, Hernández-Hierro, Rivas-Gonzalo, & Escribano-Bailón, 2014; Ma et al., 2014). The most accepted mechanism postulates that astringency sensation originates from the ability of some phenolic compounds to interact with salivary proteins and form insoluble complexes, leading to a decrease in the lubrication of the oral ephythelium (Breslin, Gilmore, Beauchamp, & Green, 1993; Kallithraka, Bakker, & Clifford, 1998; Lyman & Green, 1990; Thorngate & Noble, 1995) and an increase in the friction between mouth surfaces (Gawel, 1998). However, the interaction between tannins and salivary proteins does not

necessarily lead to the precipitation of the formed complexes; it depends on the colloidal state of the tannins (Cala et al., 2012). Besides, phenolic compounds which are incapable of precipitating proteins have been reported to induce astringency (Rossetti, Bongaerts, Wantling, Stokes, & Williamson, 2009). Furthermore, the precipitation of salivary proteins-tannins complexes does not fully explain all aspects of astringency (Ferrer-Gallego, Gonçalves, Rivas-Gonzalo, Escribano-Bailón, & de Freitas, 2012). It has been recently postulated that different components contribute to astringency sensation through different mechanisms that can occur simultaneously, including the reduction of lubrication in the oral cavity, disruption of the salivary film and the possible implication of receptors (Gibbins & Carpenter, 2013). Thus, it is not surprising that astringency has been shown to be a complex perceptual phenomenon, involving several sensations that are simultaneously perceived (Green, 1993; Lee & Lawless, 1991). Besides, several studies have shown that astringency is strongly time dependent (Guinard, Pangborn, & Lewis, 1986; Ishikawa & Noble, 1995; Lee & Lawless, 1991). Hence, accurate characterization of wine astringency is a big challenge.

Sensory analysis is the most common and direct method to evaluate wine astringency (Cheynier & Sarni-Manchado, 2010; Ma et al. 2014). This sensory characteristic is usually assessed as a single attribute by measuring its total intensity. However, due to its time dependency, static measurements can only capture either the maximum astringency intensity or the average astringency intensity perceived over a certain period of time (Ma et al., 2014). In order to fully characterize wine astringency, time-dependent methods are necessary (Ishikawa & Noble, 1995; Noble, 1995). Time intensity (TI) (Cadena, Vidal, Ares, & Varela, 2014; Lawless & Heymann, 2010), one of the most popular temporal methods, has been widely used to provide a detailed characterization of astringency development during consumption (Colonna, Adams, & Noble, 2004; Ishikawa & Noble, 1995; Lee & Lawless, 1991; Lee & Vickers, 2010; Noble, 1995; Ross, Hinken, & Weller, 2007; Valentová, Skrovánková, Panovská, & Pokorný, 2002).

However, the evaluation of total astringency intensity is usually insufficient to characterize all the sensations that are simultaneously experienced when consuming red wine (Bajec & Pickering, 2008). Wine astringency has been traditionally described by wine tasters and researchers with a wide range of subtle sensations such as: *drying, puckering, rough, sappy, harsh, woody* and *green* (Lawless, Corrigan, & Lee, 1994; Lee & Lawless, 1991; Peynaud, 1987). In line with this, Gawel, Oberholster, and Francis (2000) proposed a hierarquically structured vocabulary to precisely and comprehensively characterize the astringency of red wines, named "the Mouth-feel wheel". It includes 33

astringency descriptors grouped into 7 categories: *particulate, surface smoothness, complex, drying, dynamic, harsh,* and *unripe*. It is currently widely accepted that wine astringency quality is not only related with its total intensity, but also with its qualitative aspects. Consequently, in the last decades several authors have started to assess specific astringency sub-qualities in their research, using mainly descriptive analysis (Cáceres-Mella et al., 2014; del Barrio-Galán, Pérez-Magariño, & Ortega-Heras, 2011; Ferrer-Gallego et al., 2016; Ferrer-Gallego et al., 2014; Francis et al., 2002; Gawel, Iland, & Francis, 2001; Oberholster et al., 2015; Ortega-Heras, Pérez-Magariño, Cano-Mozo, & González-San José, 2010; Pickering & Robert, 2006; Vidal, Courcoux, et al., 2004; Vidal, Francis, Noble, et al., 2004).

Check-all-that-apply (CATA) questions (Adams, Williams, Lancaster, & Foley, 2007), a popular consumer-based sensory characterization method (Ares & Jaeger, 2015), has also been used with trained assessors for describing wine aroma (Campo, Ballester, Langlois, Dacremont, & Valentin, 2010). CATA questions have proven to be a simple task, which makes them an attractive approach to characterize astringency sub-qualities of a large sample set of wines.

In this context, the aim of the present study was to characterize the astringency of commercial Uruguayan Tannat wines, considering both the total astringency intensity and astringency sub-qualities. In spite of the relevance of astringency for Tannat wine, no study has been found reporting a complete sensory characterization of the astringency of this variety. Although Varela and Gámbaro (2006) conducted a sensory characterization of 10 Uruguayan Tannat wines using descriptive analysis, their assessment of astringency was static and they only considered global astringency.

3.2. MATERIALS AND METHODS

3.2.1. Wine samples

Forty commercial samples of Uruguayan varietal Tannat wine, available in the Uruguayan marketplace, were obtained directly from the wineries. Samples were selected to represent high quality Uruguayan Tannat wines (as opposed to "table wines" according to the wine classification in Uruguayan legislation) with different characteristics in terms of vintage, price segment and aging in oak barrels. Wines were bottled in 750 mL bottles and were conserved at 12°C until their analysis. A description of the wines included in the research is shown in Table 3.1.

Table 3.1. Characteristics of the 40 Uruguayan Tannat wines included in the research.

Characteristic	Number of wines	Percentage of wines
Aged in oak barrel		
No	12	30.0
Yes	28	70.0
Vintage		
2006	1	2.5
2007	1	2.5
2009	1	2.5
2010	1	2.5
2011	15	37.5
2012	9	22.5
2013	10	25.0
2014	2	5.0
Price range (USD)		
From 2.5 to 5	9	22.5
From 5 to 8	12	30
From 8 to 13	10	25
From 13 to 20	4	10
More than 20	5	12.5

3.2.2. Physicochemical characterization of the wines

The wines were characterized using a series of basic physicochemical parameters. Alcohol content (% v/v), total acidity (g/L expressed in tartaric acid) and pH were determined by FTIR-spectroscopy (FOSS WineScan™ FT 120, Denmark) accurately set in line with Vine and Wine International Office official methods. Total polyphenol index was determined according to Iland, Ewart, and Sitters (1993), by measuring the absorbance at 280 nm of 1:100 dilutions of the wines in water. For tannin concentration, the method proposed by Ribéreau-Gayon and Stonestreet (1966) was used. Wine samples were diluted 1:50 in water, and 4.0 mL of the dilution were placed in two tubes with 2.0 mL of water and 6.0 mL conc. HCl. One of the tubes was heated in boiling water for 30 min and then cooled protected from light. The other tube was maintained at room temperature. In each tube 1.0 mL of ethanol was added and absorbance was measured at 550 nm. The difference of absorbance between the heated and the unheated tubes was related to tannin concentration (g/L). For both analyses, absorbance measures were performed in a Spectronic Genesys 2 UV-Visible spectrophotometer (Spectronic Instruments, Rochester, NY). All samples were analysed in duplicate.

3.2.3. Trained assessor panel

The sensory panel consisted of nine assessors (7 females), ages ranging from 26 to 50 years old. Assessors were recruited among employees of the School of Chemistry from Universidad de la República (Uruguay) and selected according to the guidelines of the ISO 8586:2012 standard (ISO, 2012), as well as their availability to participate in the study. Six of the assessors had previous experience in sensory evaluation of other food of beverages, whereas the other three had extensive experience in wine tasting as part of their regular jobs. Assessors attended a total of 81 20-min training sessions over a period of 13 months prior to the study.

During training, assessors were introduced to astringency evaluation, intensity measurement and time-intensity methodology. In the first session, astringency was defined as the "tactile sensation felt in mouth and characterized by dryness and roughness", and an alum solution (5 g/L alum, McCormick, Hunt Valley, MD) was presented as reference standard. In subsequent sessions, assessors were trained to differentiate between astringency, bitterness and sourness by evaluating reference standards (5.0 g/L alum, 1.5 g/L citric acid and 0.8 g/L caffeine solutions, respectively). Paired comparisons of wine samples spiked with different concentrations of grape seed and skin tannins, citric acid and caffeine were also used for training.

Panel training also involved familiarizing assessors with the evaluation protocol and with astringency intensity measurement. Assessors were trained to quantify astringency using a line scale, anchored with the terms "low" and "high". Alum solutions of different concentration (0.5–5 g/L), commercial red wines with markedly different astringency level (as determined by two oenologists) and red wines with added grape seed and skin tannins (0.5–2.5 g/L) were used at this stage. The 5 g/L alum solution was considered as the reference for "high" astringency. Finally, assessors were introduced to the time-intensity methodology and to the software used for data collection.

Additionally, assessors were trained to describe astringency sub-qualities using check-all-that-apply questions involving a list of 16 terms. The selection of the list of terms was based on literature review and on results from a previous study on Uruguayan consumers' astringency vocabulary (Vidal, Giménez, Medina, Boido, & Ares, 2015). In order to obtain a description of Tannat wine's astringency that would be relevant for consumers, the terms *dry*, *rough*, *aggressive*, *sand paper*, *puckery*, *harsh*, *abrasive*, *hard*, *coarse grain*, *irritant* and *complex*, which were selected by at least 20% of consumers as applicable to describe red wine astringency (Vidal et al., 2015) were included. Also, by open discussion with the panel leader, trained assessors selected

additional terms that enabled them to describe a wide range of astringency-related sensations, such as *silky*, *fine emery*, *suede*, *mouthcoating*, and *velvety*. The complete list of terms for the check-all-that-apply question, together with their definition and references used during training are shown in Table 3.2. Twelve of the sixteen terms (highlighted in bold in Table 3.2), were included in the Mouth-feel wheel developed by Gawel et al. (2000).

3.2.4. Sample evaluation

The protocol for sample evaluation was based on the recommendations provided by Lee and Vickers (2010) and Colonna et al. (2004). Assessors were asked to click on the start button of the software and to simultaneously take a sip of palate cleanser in their mouth. Stirred plain yogurt was used as a palate cleanser considering results from previous studies (Vidal, Antúnez, Giménez, & Ares, 2016). No instructions were given regarding swallowing or expectoration. After 20 s, they had to take a sip of still mineral water. Then, after 40 s they had to take a sip of a sample (15 mL) and to start the time-intensity task. The evaluation protocol required assessors to swish the sample gently for 10 s while performing a standardized vertical tongue movement, without pressing the tongue against any mouth surface. After the 10 s, assessors were asked to spit the sample and to continue the evaluation for additional 30 s. A horizontal line scale anchored with the terms “low” and “high” was shown on the screen, and assessors used their finger to move the cursor along the line according to the intensity of perception. Intensity data were collected every second during the evaluation period. After a total evaluation time of 40 s, the time-intensity task ended. At that moment, assessors were presented with a CATA question with the 16 terms shown in Table 3.2. Assessors were asked to select all the terms that applied to describe the astringency-related sensations they felt during sample evaluation. The order of the terms was balanced between assessors, following a Williams’ Latin square design, as suggested by Meyners and Castura (2016).

The timeline for sample evaluation is shown in Fig. 3.1. Once assessors completed the evaluation protocol for one of the samples, they immediately started the evaluation of the next sample.

Table 3.2. Definition of the astringency terms considered in the check-all-that-apply (CATA) questions and references used during training.

Term	Definition	Reference
Dry	Lack of lubrication in the mouth	Black tea
Silky	Texture associated with silk	Silk cloth
Fine emery	Texture associated with emery paper	Emery paper
Suede	Texture associated with suede	Suede cloth
Rough	Irregularities or protuberances felt in mouth, not smooth	3 g/L grape skin and seed extract solution
Aggressive	Excessive astringency, characterized by excessive lack of lubrication	Commercial sample of Tannat wine
Sand paper	Texture associated with sand paper	Sand paper
Mouthcoating	Coating of film that adheres to mouth surfaces	Banana peel
Velvety	Texture associated with velvet	Velvet cloth
Puckery	Reflex action of mouth surfaces when being brought together	5 g/L alum solution
Harsh	Abrasive sensation due to particulate matter brushing against the surface of the mouth	Green banana
Abrasive	Excessive astringency of a strongly roughing nature	Commercial sample of Tannat wine
Hard	Combined effect of bitterness and astringency	Commercial sample of Tannat wine
Coarse grain	Texture associated with coarse grain matter	Sand paper
Irritant	Excessive astringency causing an irritant sensation	Commercial sample of Tannat wine
Complex	Term used to describe the astringency of wines that show several astringency sensations	-

Terms highlighted in bold are included in the Mouth-feel wheel (Gawel et al., 2000).

The 40 Tannat wine samples were evaluated in duplicate by each assessor. As astringency sensations exhibit a build-up upon repeated ingestions (Bajec & Pickering, 2008), assessors evaluated 4 samples in each session, meaning that a total of 20 sessions were needed to complete the study. Assessors attended two sessions per week during a period of 3 months. Samples were coded using 3-digit random numbers and presented following a Williams' Latin square design.

Testing took place in standard sensory booths in a sensory laboratory that was designed in accordance with ISO 8589 (ISO 2007), under artificial daylight and temperature control (22°C). Data collection was carried out using Compusense Cloud (Compusense Inc., Guelph, Ontario, Canada) on tablets.

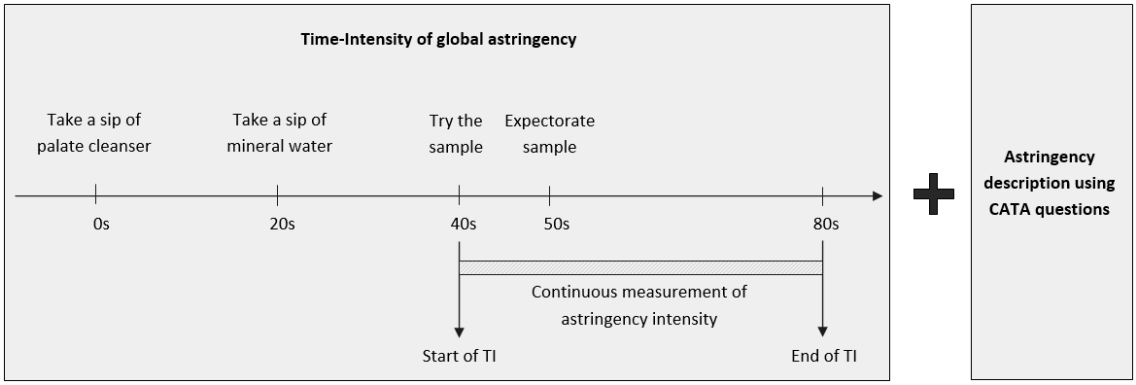

Fig. 3.1. Schematic representation of the evaluation protocol.

3.2.5. Data analysis

3.2.5.1. Analysis of time-intensity data

Time-intensity data should contain 720 curves of intensity vs. evaluation time (40 samples x 2 replicates x 9 assessors). However, as not all assessors were able to attend the 20 sessions, the data set was composed by 680 curves (12 - 18 curves per sample).

Assessor's performance was assessed by visually inspecting the time-intensity curves (Ovejero-López, Bro, & Bredie, 2005). For each sample and assessor combination, the time-intensity curves of both replicates were superimposed. Some variations in intensity and shape were observed within assessors, but in general there was good agreement between replicates. During this inspection, 9 curves were removed due to obvious technical problems with data collection (the intensity recorded was zero during the whole evaluation period). Also, a non-centered Principal Component Analysis (PCA) on a matrix containing the 671 time-intensity curves as columns was performed as a way of screening for outliers, but no curve was discarded at this point (Peyvieux & Dijksterhuis, 2001; Ovejero-López et al., 2005).

For each of the time-intensity curves, 10 parameters were extracted: maximum intensity (*Imax*), intensity at the end (*Iend*), starting time (*tstart*), time of maximum intensity (*tmax*), decline time (*tdec*), duration (*dur*), area under the curve (*auc*), increase area (*inc.auc*), decrease area (*dec.auc*) and area under the plateau (*plat.auc*). The definition of the parameters is shown in Table 3.3. The parameter finish time (*tend*), which corresponds to the end of perception (i.e. the time at which intensity returns to zero) was not extracted because only one of the curves returned to zero. Hence, the finish time corresponded to the task duration pre-determined in the evaluation protocol (80 s) in all cases except one. This was also the reason why the intensity at the end of the evaluation (*Iend*) was considered. The extracted parameters were analysed by Analysis of Variance (ANOVA). Linear mixed models were used, considering sample as fixed effect and assessor, replicate and all the two-way interactions as random effects. When significant differences were identified, Honestly Significant Difference test was used for post hoc mean comparisons of wine samples. A significance level of 5% was used for both statistical tests.

To better visualize differences among wine samples, PCA was performed on the correlation matrix of those time-intensity parameters that were significant for the sample effect.

Table 3.3. Definition of the parameters extracted from time-intensity curves.

Abbreviation	Parameter	Definition
Imax	Maximum intensity	Maximum observed intensity during the evaluation
Iend	Intensity at the end	Intensity observed at the end of the evaluation
Tstart	Starting time	First time at which intensity is > 0
Tmax	Time of maximum intensity	Time at which maximum intensity is first reached
Tdec	Decline time	Time at which the curve starts to decline from Imax; last time at which Imax is registered
Dur	Duration	Total time from tstart until the finish of the test
Auc	Area under the curve	Area under the curve
inc.auc	Increase area	Area under the ascending portion of the curve, from tstart to tmax.
dec.acu	Decrease area	Area under the descending portion of the curve, from tdec to the finish of the test.
plat.auc	Area under the plateau	Area under the plateau

3.2.5.2. Analysis of CATA data

The frequency of use of each astringency sub-quality was determined by counting the number of judgments (assessor x replicate) in which the term was used to describe each sample's astringency. Generalized linear models were carried out to identify significant differences among samples for the frequency of use of each of the terms. Analysis of deviance of each model was done using chi-squared test. When the sample effect was significant at a 5% significance level, pairwise comparisons were carried out using the sign test. Also, sample and term configurations were obtained by performing Correspondence Analysis (CA) on the frequency table.

3.2.5.3. Consensus representation of Time-intensity and CATA data

Multiple Factor Analysis (MFA) was used to obtain a consensus sample map based on both the temporal evolution of total astringency intensity and the description of astringency sub-qualities. The data matrices used for conducting the PCA on significant TI parameters (section 3.2.5.1) and the CA on the frequency table of astringency sub-qualities descriptors (section 3.2.5.2) were considered as two groups of variables describing the astringency of the 40 Tannat wine samples.

Hierarchical cluster analysis using Euclidean distance and Ward's agglomeration method was performed on the sample coordinates on the first two dimensions of the MFA to identify groups of wines with different astringency characteristics. Generalized linear models, followed by analysis of deviance using chi-squared test, were used to identify differences in the frequency of use of the different terms among the identified clusters. One-way analysis of variance was used to determine if clusters differed in their average time-intensity parameters and physicochemical parameters. Tukey's test was used for post hoc mean comparisons. Furthermore, Fisher's exact test was used to asses if samples' clustering according to the MFA was related to sample's characteristics in terms of vintage, price segment and whether they had been aged in oak barrels or not.

All statistical analyses were performed with R (R Core Team, 2017). Functions from `stats`, `FactoMineR` (Lê, Josse, & Husson, 2008), and `lmerTest` (Kuznetsova, Brockhoff, & Christensen, 2017) packages were used.

3.3. RESULTS

3.3.1. Physicochemical characterization of the wines

Basic compositional data of the wine samples was obtained and a summary is shown in Table 3.4. The range of ethanol content, total acidity, pH, total polyphenol index and tannin concentration were large. Thus, it was expected that differences in terms of global astringency and astringency sub-qualities existed in the sample set.

Table 3.4. Descriptive parameters (mean, standard deviation –SD-, minimum and maximum) of the oenological parameters determined in the 40 commercial Uruguayan Tannat wines included in the research.

Parameter	Mean ± SD	Minimum	Maximum
Ethanol (%)	13.3 ± 0.9	11.8	15.2
Total acidity (g/L)*	5.1 ± 0.4	4.2	6.1
pH	3.8 ± 0.2	3.5	4.2
Total polyphenol index (AU)	74.1 ± 14.8	50.8	117.4
Tannin concentration (g/L)	3.9 ± 0.9	2.4	6.6

***** Total acidity is expressed in g/L of tartaric acid.

3.3.2. Total astringency intensity of wine samples

A summary of the ANOVA results for the sample effect, together with descriptive statistics of the average TI parameters of wine samples are presented in Table 3.5. Seven of ten TI parameters were significantly affected by the sample effect: *Imax*, *Iend*, *tstart*, *dur*, *auc*, *inc.auc* and *plat.auc*. Wine samples significantly differed both in their maximum astringency intensity (*Imax*) and their intensity at the end of the evaluation (*Iend*). However, the variation of astringency intensity was not large, 50% of the wines presented *Imax* between 5.9 and 6.9, while *Iend* of half of the wines varied between 2.5 and 3.1 on a 0-10 scale. This result is further supported by the post-hoc mean comparisons. Only 31% and 23% of all possible pairwise comparisons between wine samples significantly differed in their *Imax* and *Iend,* respectively.

Similar results were found for the time-related parameters. Wine samples only differed significantly in their *tstart* and *dur*, but these differences were rather small. For these parameters, only 9% (*tstart*) and 10% (*dur*) of all possible pairwise comparisons were significantly different. Regarding the areas under the TI curves, the sample effect was significant for the total (*auc*), the increase (*inc.auc*) and the plateau areas (*plat.auc*), but not for the area under the descending portion of the curve (*dec.auc*; Table 3.5).

Table 3.5. Descriptive statistics (mean, standard deviation –SD-, minimum, 1st quartile, 3rd quartile and maximum) of the average time-intensity parameters of Tannat wine samples, and p-values from the Analysis of Variance results for the sample effect.

Parameter	Mean ± SD	Minimum	1st. Quartile	3rd. Quartile	Maximum	p-value*
Imax (0-10)	6.4 ± 0.7	5.1	5.9	6.9	8.1	**0.0000**
Iend (0-10)	2.9 ± 0.6	1.9	2.5	3.1	4.6	**0.0000**
tstart (s)	46.9 ± 0.9	45.7	46.4	47.3	50.7	**0.0057**
tmax (s)	56.4 ± 1.7	53.3	55.5	57.3	61.8	0.4655
tdec (s)	61.8 ± 1.8	59.6	60.5	62.7	65.7	0.3817
dur (s)	33.0 ± 0.9	29.3	32.7	33.5	34.3	**0.0134**
auc	146.2 ± 20.1	110.7	131.2	159.5	202.1	**0.0000**
inc.auc	43.3 ± 9.9	25.5	38.0	47.4	68.1	**0.0499**
dec.acu	67.6 ± 9.8	50.2	59.9	76.3	90.5	0.1330
plat.auc	35.3 ± 13.2	13.8	24.9	44.1	74.0	**0.0316**

Imax: maximum intensity; Iend: intensity at the end; tstart: starting time; tmax: time of maximum intensity; tdec: decline time; dur: duration; auc: area under the curve; inc.auc: increase area; dec.auc: decrease area; plat.auc: area under the plateau.
* Values in bold are significant at a significance level of 5%.

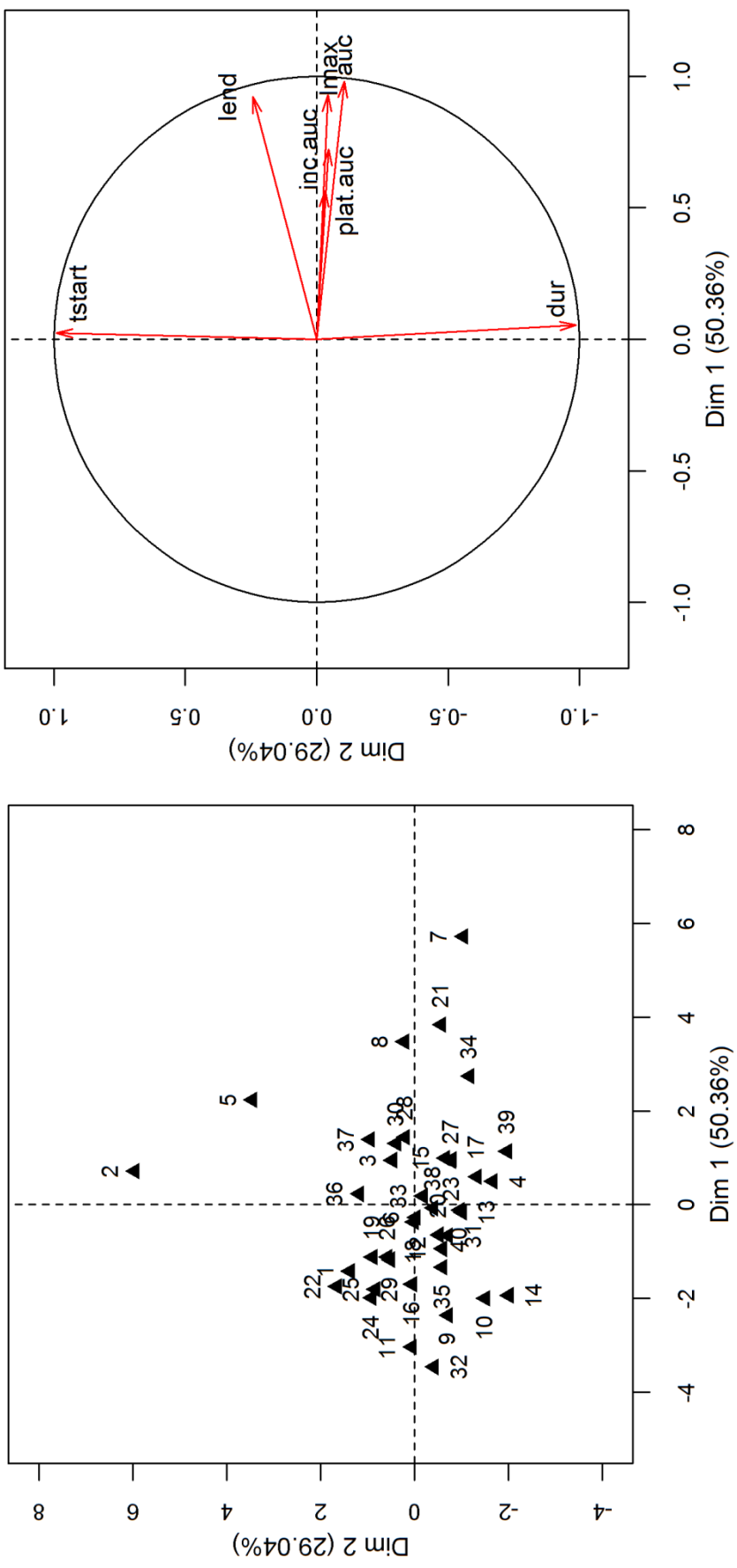

Fig. 3.2. Representation of wine samples (left) and time-intensity parameters (right) in the first two dimensions of the Principal Component Analysis performed on data from total astringency evaluation.

Results from the PCA performed on the significant TI parameters are shown in Fig. 3.2. The first two principal components accounted for 79.4% of the total variance. The TI parameters related to astringency intensity (*Imax* and *Iend*) and the area under the curve (*auc*, and *inc.auc*) were positively correlated to the first principal component, which explained 50.4% of the variance of the data. Meanwhile, time-related parameters, *tstart* and *dur*, were correlated to the second principal component. Not surprisingly, *dur* and *tstart* were negatively correlated.

Some of the samples were clearly separated from the rest. As shown in Fig. 3.2, samples 34, 8, 21 and 7 had higher values of *Imax*, *Iend* and *auc* compared to the rest of the samples, while samples 2 and 5 were distinct from the others in having a shorter duration of their astringency time-intensity curves.

3.3.3. Astringency sub-qualities of wine samples

From the 16 astringency sub-qualities descriptors included in the CATA question, the most frequently used by the trained panel to describe the astringency of Tannat wine samples were *dry*, *mouthcoating* and *rough* (Table 3.6). The terms *fine emery*, *suede* and *complex* were also frequently used to describe the samples, showing average percentage of use higher than 20%.

Table 3.6. Average, standard deviation (SD), maximum and minimum of the frequency of use (expressed in percentage) of each term included in the check-all-that-apply question to describe astringency sub-qualities of Tannat wine samples. p-Values from the analysis of deviance for the sample effect are also shown.

Term	Mean ± SD	Minimum	Maximum	p-value*
Dry	52.9 ± 13.5	18.8	75.0	0.1830
Silky	8.2 ± 11.2	0.0	43.8	**0.0000**
Fine emery	30.0 ± 11.4	8.3	50.0	0.3858
Suede	29.7 ± 14.7	6.3	62.5	**0.0042**
Rough	41.8 ± 16.3	0.0	66.7	**0.0004**
Aggressive	16.4 ± 11.6	0.0	43.8	**0.0007**
Sand paper	8.1 ± 8.8	0.0	31.3	**0.0009**
Mouthcoating	44.9 ± 15.0	17.6	81.3	**0.0169**
Velvety	17.3 ± 10.8	0.0	43.8	0.0735
Puckery	18.1 ± 10.7	0.0	42.9	0.0834
Harsh	13.5 ± 11.0	0.0	56.3	**0.0208**
Abrasive	8.5 ± 7.4	0.0	28.6	0.0789
Hard	17.2 ± 12.4	0.0	41.7	**0.0001**
Coarse grain	15.5 ± 10.0	0.0	43.8	0.0550
Irritant	14.0 ± 9.0	0.0	27.8	0.0737
Complex	23.0 ± 12.2	0.0	61.1	**0.0230**

* Values in bold are significant at a significance level of 5%.

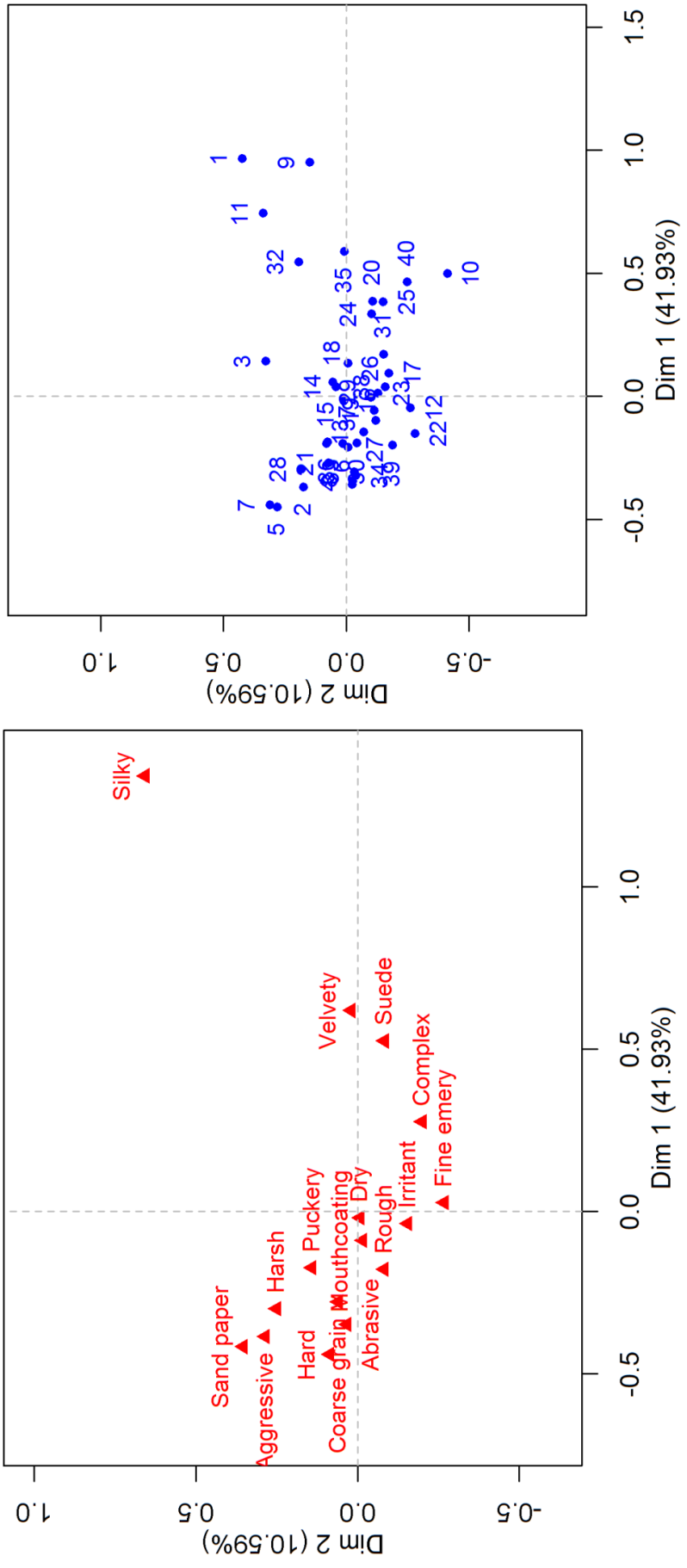

Fig. 3.3. Sample (right) and term (left) configurations in the first two dimensions of the Correspondence Analysis performed on the frequency table of the check-all-that-apply question describing astringency sub-qualities of 40 Tannat wines.

According to the generalized linear models, wine samples significantly differed in the frequency of use of the terms *silky*, *suede*, *rough*, *aggressive*, *sand paper*, *mouthcoating*, *harsh*, *hard* and *complex* (Table 3.6). Interestingly, according to the sign test, there were significant differences in the frequency of use of some astringency terms between samples that did not differ significantly in any of the TI parameters. For example, wine samples 23 and 17 both had an average maximum astringency intensity of 6.9, but differed in the frequency in which the trained panelists used the term *rough* to describe them (67% vs 17%). Similarly, samples 1 and 10 presented an average maximum astringency intensity of 5.7 but the term *silky* was more frequently used for the describing the astringency of sample 1 (44%) compared to sample 10 (6%).

Correspondence Analysis (CA) was performed on the frequency table of the CATA data to better visualize the relationships between samples and astringency sub-qualities descriptors. The first and second dimensions of the CA explained 41.9% and 10.6% of the data variance respectively (Fig. 3.3). A certain polarization of astringency related terms was observed, with terms describing soft textures such as *silky*, *velvety* and *suede* loading on positive values of the first dimension while terms related to rough textures and aggressiveness were located at negative values of the first dimension (e.g. *sand paper*, *aggressive*, *hard*, *harsh*, *coarse grain* and *abrasive*). Some of the samples were clearly discriminated from the rest. As shown in Fig. 3.3, samples 1, 9, 11 and 32 were associated with the terms *silky*, *velvety* and *suede*, whereas samples 2, 5, 7, 21 and 28 were associated with the terms *sand paper*, *aggressive* and *harsh*.

3.3.4. Consensus representation of wines based on total astringency intensity and astringency sub-qualities

MFA was performed considering the average TI parameters and the frequency table from the CATA question as two separate groups of variables in order to obtain a consensus representation of the 40 Tannat wine samples. The first two dimensions of the MFA explained 54.3% of the variance of the data. The consensus sample and variable configurations in the first two dimensions of the MFA are shown in Fig. 3.4. TI parameters related to astringency intensity and area under the curve were positively correlated with the first dimension of the MFA, which explained 40% of the variance of the data, while the parameters related to the duration of the sensation were correlated with the second dimension of the MFA. The terms from the CATA question for astringency description were distributed along the first dimension of the MFA, with terms describing soft textures (*silky*, *suede*, *velvety*) loading on negative values and terms related to rough textures and aggressiveness (*sand paper*, *harsh*, *hard*, *aggressive*, *coarse grain*, *abrasive*) loading on positive values of the first dimension. This suggests

a relationship between astringency intensity and astringency sub-qualities. Samples presenting low astringency intensity tended to be characterized by astringency sub-qualities related to soft textures, whereas those showing the highest astringency intensities tended to be characterized by rough and hard textures. The RV coefficient between the TI and the CATA data computed in the MFA was 0.45, which indicates that the two data matrices provide different information about the astringency of the evaluated wines.

A hierarchical cluster analysis was performed on the coordinates of the wine samples in the first two dimensions of the MFA for identifying groups of Tannat wines with different astringency characteristics. Four groups were identified in the analysis, and the average TI parameters and frequency of use of the astringency sub-qualities descriptors within each group are shown in Table 3.7.

The two larger groups of wines, Group 3 (n=16) and Group 4 (n=14), showed intermediate average values of *Imax*. On average, wine samples in Group 3 had higher astringency intensity than those from Group 4 (*Imax* 6.7 vs 6.0 and *Iend* 3.0 vs 2.5). The description of the astringency of both groups of samples was similar. As shown in Table 3.7, samples in both groups were mainly described as *dry*, *mouthcoating* and *rough,* although these descriptors were on average more frequently used for samples in Group 3. Also, wine samples in Group 4 were on average more frequently associated with astringency sub-qualities such as *suede* and *velvety*.

On the other hand, Groups 1 and 2 were smaller, and were composed of only 4 and 6 wines, respectively, with very distinct astringency characteristics. Wine samples in Group 1 showed a lower astringency intensity than the rest of the wines (*Imax* = 5.5 and *Iend* = 2.2), and were mainly described using the terms *suede*, *dry*, *silky*, *velvety* and *mouthcoating*. Astringency sub-qualities *dry* and *mouthcoating* were less frequently used for describing wines in Group 1 compared to the other three groups. Conversely, wine samples in Group 2 showed the highest astringency intensity (*Imax* = 7.3 and *Iend* = 3.9). Besides, the astringency of samples in this group was described more frequently as *dry, mouthcoating* and *rough* than samples in the other groups, while astringency sub-qualities indicating an excessive level of astringency, such as *hard*, *harsh*, and *aggressive* were only relevant for describing samples in Group 2.

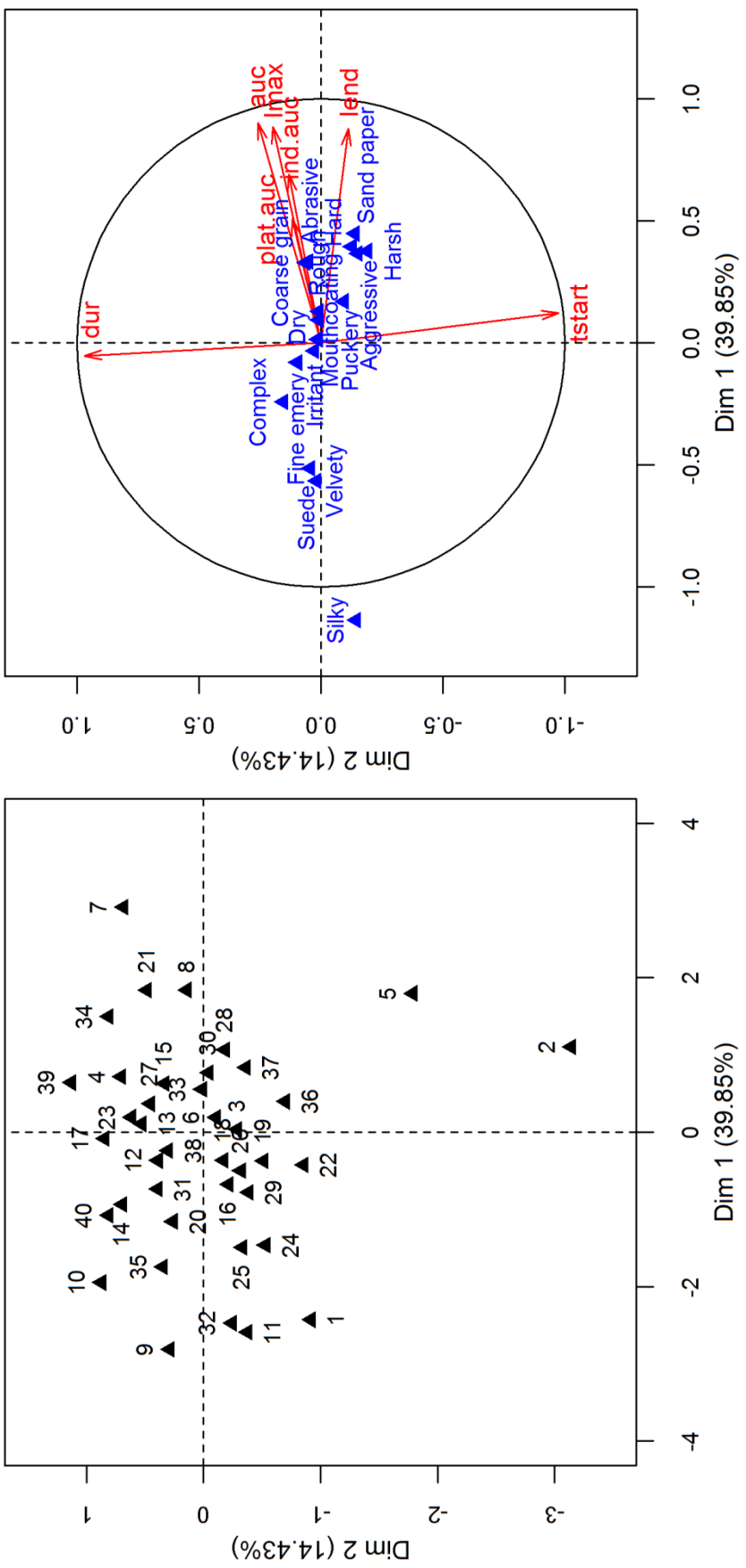

Fig. 3.4. Representation of samples (left) and variables (right) on the first two dimensions of the Multiple Factor Analysis performed on the data from time intensity and check-all-that-apply question tasks to describe the astringency of 40 Tannat wines.

Table 3.7. Average astringency time intensity parameters, average frequency of use (expressed in percentage) of the terms included in the check-all-that-apply (CATA) question to describe astringency sub-qualities and average physicochemical parameters for each of the four groups of Tannat wines identified using hierarchical cluster analysis. p-Values of Analysis of Variance and Fisher's tests used to identify differences among groups of wines are also shown for each variable.

Parameter	Group				p-value*
	1 (n=4)	2 (n=6)	3 (n=16)	4 (n=14)	
Imax (0-10)	5.5^{c}	7.3^{a}	6.7^{b}	6.0^{c}	**<0.0001**
Iend (0-10)	2.2^{c}	3.9^{a}	3.0^{b}	2.5^{c}	**<0.0001**
tstart (s)	47.0	47.7	46.7	46.8	0.1700
dur (s)	32.9	32.3	33.3	32.9	0.2190
Auc	118.9^{c}	176.4^{a}	153.2^{b}	133.0^{c}	**<0.0001**
inc.auc	34.4^{b}	56.2^{a}	45.1^{b}	38.3^{b}	**0.0001**
plat.auc	27.9^{b}	47.4^{a}	36.4^{a,b}	30.9^{b}	**0.0395**
Dry	37.9	59.5	60.1	46.2	**0.0011**
Silky	35.1	0.0	4.0	8.9	**<0.0001**
Fine emery	21.0	27.4	33.5	29.8	0.1859
Suede	47.1	15.2	23.0	38.6	**<0.0001**
Rough	14.9	52.4	47.3	38.6	**<0.0001**
Aggressive	7.5	30.2	19.5	9.4	**<0.0001**
Sand paper	1.6	13.7	10.0	5.3	**0.0063**
Mouthcoating	32.1	60.7	51.2	34.5	**<0.0001**
Velvety	32.3	8.3	12.1	22.7	**<0.0001**
Puckery	10.4	30.1	18.1	15.1	**0.0089**
Harsh	6.1	31.5	12.0	9.8	**<0.0001**
Abrasive	3.0	16.7	9.9	4.9	**0.0020**
Hard	3.0	32.9	19.8	11.5	**<0.0001**
Coarse grain	4.7	27.6	18.2	10.2	**<0.0001**
Irritant	7.1	14.4	14.6	15.1	0.3847
Complex	17.2	19.5	22.1	27.2	0.1763
Ethanol (%)	12.6	13.1	13.3	13.5	0.2730
Total acidity (g/L)	5.1	4.9	5.0	5.2	0.4800
pH	3.7	3.9	3.8	3.8	0.1130
Total polyphenol index (AU)	56.6^{c}	93.0^{a}	75.9^{b}	69.0^{b,c}	**<0.0001**
Tannin concentration (g/L)	3.0^{b}	5.0^{a}	3.9^{b}	3.7^{b}	**0.0005**

* Values in bold are significant at a significance level of 5%.
For time-intensity and physicochemical parameters, average values within a row with different superscripts are significantly different according to Tukey's test at a significance level of 5%.

Regarding the physicochemical parameters, significant differences among groups were found only for the total polyphenol index (p-value < 0.0001) and tannin content (p-value = 0.0005). Wines in Group 2, characterized by the highest astringency intensity and the highest frequency of use of the terms *hard, harsh* and *aggressive*, showed the highest average value of both parameters. On the other hand, Groups 1 and 4, characterized by the lowest astringency intensity, were the ones with the lowest total polyphenol index. However, Fisher's exact test showed that the four groups of wine samples did not significantly differ in terms of vintage, price segment nor in whether they had been aged in oak barrels or not ($p\text{-value}_{vintage} = 0.882$, $p\text{-value}_{price} = 0.454$, $p\text{-value}_{aged.in.oak} = 0.727$).

3.4. DISCUSSION

In the present work, the astringency of 40 commercial Tannat wines from Uruguay was characterized in terms of global astringency intensity and astringency sub-qualities, as perceived by a trained sensory panel. In terms of global astringency, the main differences among samples were related to the intensity of the sensation rather than to its development over time (Fig. 3.2). However, although the average maximum astringency intensity of Tannat wines ranged from 5.1 to 8.1 (on a 0-10 scale), the variation of this parameter can be regarded as moderate, as the *Imax* of half of the wines considered in the study varied between 5.9 and 6.9. Average astringency intensities reported in the present work cannot be compared with those reported for other wine varieties because of methodological differences such as the method used for the assessment of astringency intensity (descriptive analysis vs time-intensity), the specific protocols to evaluate the samples, the scales used and the reference of "high" astringency used during assessors' training (Boulet et al., 2016; Cáceres-Mella et al., 2014; Chira, Pacella, Jourdes, & Teissedre, 2011; Cliff, King, & Schlosser, 2007; Gonzalo-Diago, Dizy, & Fernández-Zurbano, 2014; Kallithraka, Kim, Tsakiris, Paraskevopoulos, & Soleas, 2011; Quijada-Morín et al., 2012; Sáenz-Navajas, Avizcuri, Ferreira, & Fernández-Zurbano, 2012; Sáenz-Navajas, Tao, Dizy, Ferreira, & Fernández-Zurbano, 2010).

CATA questions provided valuable information to describe astringency sub-qualities of a large sample set of Tannat wines, and to discriminate among samples. Compared to traditional descriptive analysis, CATA questions had the advantage of requiring less training, as assessors only had to be able to recognize astringency sub-qualities but not to quantify them using a scale. CATA questions had been successfully used by Campo et al. (2010) in the evaluation of the aroma of Pinot Noir wines by trained assessors, and recently, by Lezaeta, Bordeu, Næs, and Varela (2017) for the

assessment of Sauvignon Blanc wines' aroma by consumers. The frequency of use of some of the terms of the CATA question to describe astringency sub-qualities widely differed among samples (Table 3.6) and provided further discrimination than global intensity. Interestingly, although during the training sessions astringency was defined as the “tactile sensation felt in mouth and characterized by dryness and roughness”, the only terms that were relevant for describing the astringency of all samples were *dry* and *mouthcoating*, whereas the frequency of use of *rough* was zero for some samples.

Results from the present study highlight the complex nature of wine astringency, and the fact that a wide range of astringency sub-qualities are needed to fully characterize the astringency of Tannat wines. In addition, results confirm that total astringency intensity is not sufficient to fully characterize wine astringency, as previously stressed by Gawel et al. (2000), Gawel et al. (2001) and Vidal et al. (2015). In the present work, samples that did not differ in any of the parameters related to their astringency time-intensity curves differed in how their astringency sub-qualities were described.

Based on the characterization of the intensity and sub-qualities of astringency, it was possible to identify four groups of Tannat wines. These groups largely differed in their astringency profile, suggesting that large differences in Uruguayan commercial wines exist. Although the majority of the evaluated Tannat wines were characterized by intermediate astringency and were described as *dry, rough* and *mouthcoating*, some samples were clearly distinct by eliciting smooth astringency characteristics (described as *velvety, silky* and *suede*), whereas others were characterized by their strong astringency (described as *hard*, *harsh* and *aggressive*). This suggests that, although Tannat is usually characterized by its intense astringency (Carrau, 1997), widely different styles of Tannat wine exist in the Uruguayan marketplace. Further research is necessary to understand the relationship between astringency profile and quality perception of Tannat wine.

Sáenz-Navajas et al. (2015) found a negative correlation between astringency intensity assessed by a trained panel and consumers' quality scores of twelve commercial Spanish wines from different grape varieties. However, as astringency is one of the sensory cues that contributes to Tannat wine's tipicity, it is expected that both low and extremely high global astringency intensity would be perceived as indicators of low quality wines. In this sense, Varela and Gámbaro (2006) reported that Tannat wine astringency (assessed by a trained panel using descriptive analysis) positively contributed to wine's quality as perceived by a group of regular fine wine consumers. Yet, none of these studies considered astringency sub-qualities. Some astringency sub-qualities may be more desirable, such as *round/smooth* (Sáenz-Navajas et al., 2016) or

velvety (Ferrer-Gallego et al., 2014), while others, such as *aggressive* or *sand paper*, may be related to a bad quality assessment, even in the case of wines that are expected to be highly astringent. The identification of Tannat wine astringency characteristics that positively contribute to quality perception could contribute to achieve a better promotion and communication of the characteristics of this variety to both national and international wine consumers.

In the present work, groups of samples with different astringency profile did not significantly differ in their vintage, price range and aging in oak barrels. The sensory quality of wine has long been acknowledged to be determined by wine composition (Peynaud, 1987). In particular, its phenolic compounds are known to play a major role in mouthfeel sensations (Cheynier & Sarni-Manchado, 2010; Kennedy, Saucier, & Glories, 2006). The phenolic profile of a wine is affected by several factors, such as viticultural practices, winemaking procedures (e.g. maceration and ageing in oak barrels), as well as grape variety, vintage and region where the grapes are grown (Cliff et al., 2007; Garrido & Borges, 2013; Kennedy et al., 2006; Ma et al., 2014). All these factors lead to different categories of wines in terms of quality, which is supposed to be reflected in the price of the final product (Cáceres-Mella et al., 2012). The absence of a relationship between wines' astringency characteristics and vintage, price and ageing in oak barrels is probably linked to the sample set of wines selected for the study. The 40 samples corresponded to commercial Tannat wines, available in the Uruguayan marketplace, and according to Uruguayan legislation, commercial wines may present up to 15% of other varieties and still be considered monovarietals (González-Neves et al., 2001). Also, samples were purchased from different wineries, and thus a wide range of vineyards and winemaking techniques were represented in the sample set.

However, groups of wines with different astringency differed in their total polyphenol index and tannin content, supporting previous works that suggest that phenolic compounds contribute to astringency sensations (Arnold, Noble, & Singleton, 1980; Gonzalo-Diago et al., 2014; Hufnagel & Hofmann, 2008; Sáenz-Navajas et al., 2012; Sáenz-Navajas, Tao, et al., 2010). Further research should focus on the relationship between astringency intensity and astringency sub-qualities and the polyphenolic profile of wine (Kennedy et al., 2006). Considering that different mechanisms are involved in the interaction between polyphenols and salivary proteins (Bajec & Pickering, 2008), and that different compounds are likely to elicit astringency through different mechanisms (Gibbins & Carpenter, 2013), it could be hypothesized that different astringent sensations may be related to specific polyphenolic structures. Some advances have been made in this field, and relationships between specific compounds

and astringent sub-qualities have been reported (Ferrer-Gallego et al., 2016; Ferrer-Gallego et al., 2014; Gawell, Francis, & Waters, 2007; Hufnagel & Hofmann, 2008; Vidal et al., 2003; Vidal, Francis, Williams, et al., 2004). However, studies involving commercial wine samples are still scarce (Sáenz-Navajas et al., 2012; Sáenz-Navajas, Tao, et al., 2010), and are largely encouraged, as the effect of individual compounds on astringency has been shown to be highly matrix-dependent (Sáenz-Navajas et al., 2012). The study of Tannat wine astringency and its relationship to wine phenolic composition would be of great interest to the Uruguayan industry, as it could enable the selection of vineyard managing and winemaking practices to obtain Tannat wines with specific astringency characteristics.

3.5. CONCLUSIONS

The astringency of commercial Uruguayan Tannat wines was assessed by a trained panel, considering both the total astringency intensity and its development over the evaluation period, and the description of astringency sub-qualities. Samples differed in their average maximum intensity, although in general the variability was moderate. Commercial wines were described using a wide range of astringency sub-qualities, from soft related textures such as *silky*, *velvety* and *suede*, to those related to excessive astringency, such as *harsh*, *hard* and *aggressive*. Further research is necessary to better understand how astringency characteristics are influenced by production variables, and to understand their relationship to consumers' and experts' perceived quality of Tannat wines.

CHAPTER 4

Relationship between astringency and phenolic composition of commercial Uruguayan Tannat wines: Application of boosted regression trees

ABSTRACT

Phenolic compounds play a major role in the intensity and characteristics of wine astringency. However, studies involving commercial wine samples are still scarce. The aim of the present work was to study the relationship between astringency and phenolic composition of commercial Uruguayan Tannat wines using boosted regression trees (BRT), a novel predictive method. Forty commercial Tannat wines were evaluated by a trained sensory panel (9 members), who assessed their total astringency intensity using time-intensity (TI) and described their astringency sub-qualities using a check-all-that-apply (CATA) question composed of sixteen terms. The polyphenolic profiles of the wines were determined by HPLC-MS and conventional oenological parameters were also obtained. Fifty BRT models with different partitions of the data in training and test sets were built for astringency maximum intensity (Imax) and for the frequency of use of the 16 astringency sub-qualities considered in the CATA question. As predictor variables, 84 phenolic compounds and oenological parameters were considered for all BRT models. Both strong and weak predictive models were obtained for each response variable. Predictive accuracy was much higher for astringency intensity than for the frequency of mention of astringency sub-qualities. Still, the BRT models allowed to point out to some compositional variables most likely involved in wine astringency perception. Total tannin concentration (chemically determined) was the most relevant explanatory variable for sensory astringency, while flavan-3-ols were the individual phenolic compounds with the highest contribution to astringency, particularly some dimers, trimers and the sum of non-galloylated tetramers. However, the effect of these predictors differed according to the astringency sub-quality considered as response. As expected, non-linear relationships between phenolic compounds and astringency were found. These results contribute to the understanding of the influence of phenolic composition on wine astringency and stress the potential of BRT models for identifying the compounds responsible for this complex sensory characteristic.

4.1. INTRODUCTION

Red wine astringency has been reported to have a strong influence on wine's quality, complexity and persistence, which has placed it as one of the most relevant sensory characteristics of this product (Cheynier & Sarni-Manchado, 2010; Gawel, 1998; Peynaud, 1987). This sensory characteristic comprises a complex set of sensations related to drying, roughing and puckering of the mouth epithelium (ASTM, 2004).

Wine astringency has been mainly related to the presence of non-volatile phenolic compounds (Cheynier & Sarni-Manchado, 2010; Kennedy, Saucier, & Glories, 2006) and their ability to interact with salivary proteins. However, the mechanisms of astringency perception have not been fully unveiled yet (Ferrer-Gallego, Hernández-Hierro, Rivas-Gonzalo, & Escribano-Bailón, 2014; Ma et al., 2014). It is currently thought that different components might elicit astringency through different mechanisms that may occur simultaneously, such as the reduction of lubrication in the oral cavity and disruption of the salivary film as a consequence of interactions with salivary proteins, or the direct implication of chemosensory and mechanosensory receptors (Ferrer-Gallego et al., 2016; Gibbins & Carpenter, 2013; Schöbel et al., 2014).

Wine phenolics comprise a huge and heterogeneous family of compounds such as anthocyanins, phenolic acids, flavonols, flavanols and tannins, as well as a vast number of compounds derived from them through different chemical reactions (Garrido & Borges, 2013). For this reason, one of the foremost challenges of identifying the key individual or groups of compounds that contribute to astringency is the wide diversity of chemical structures (Ferrer-Gallego et al., 2014). Phenolic compounds have at least one aromatic ring with one or more hydroxyl group attached, and range from simple low-molecular-weight compounds to large polymers with diverse substituents (Crozier, Jaganath, & Clifford, 2006). They can be classified based on their number and arrangement of carbon atoms into flavonoids and non-flavonoids (Crozier et al., 2006; Garrido & Borges, 2013). Non-flavonoid phenolic compounds found in grapes and wine are mainly phenolic acids, which can be divided into hydroxybenzoic acids (C6-C1; e.g. gallic acid) and hydroxycinnamic acids (C6-C3; e.g. *p*-coumaric acid) (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006). Flavonoids, on the other hand, share a basic structure of 15 carbon atoms in which two aromatic rings are bound through a 3 carbon chain (C6-C3-C6). Differences in the arrangement, degree of oxidation and substitution to this carbon skeleton gives rise to a wide diversity of compounds, which can be further divided into several classes. Relevant families of flavonoids for grape and wine are flavonols (e.g. quercetin), flavan-3-ols (e.g. (+)-catechin) and anthocyanins (e.g.

malvidin-3-O-glucoside), among others (Garrido & Borges, 2013). Flavan-3-ols are the most complex family of flavonoids, with compounds ranging from simple monomers to large and complex polymers (Crozier et al., 2006). Tannins are by definition substances capable of binding with proteins and other polymers, and correspond to polymers of simpler monomeric phenolic compounds (Ribéreau-Gayon et al., 2006). Usually, they are classified into hydrolyzable and non-hydrolyzable or condensed tannins. Hydrolyzable tannins are polymers of gallic acid and hexahydroxydiphenoyl acid, and as their name suggest, they can be degraded though pH changes and through enzymatic or non-enzymatic hydrolysis into smaller fragments (Crozier et al., 2006; Garrido & Borges, 2013). On the other hand, condensed tannins are oligomers and polymers of flavan-3-ols, and are also known as proanthocyanidins because when they are heated in acidic medium they give rise to anthocyanidins, mainly cyanidin (Ribéreau-Gayon et al., 2006). Tannins that are naturally present in grapes and wine are predominantly of the condensed type (Garrido & Borges, 2013).

Proanthocyanidins have been pointed out by several authors as the major contributors of astringency intensity (Broussaud, Cheynier, & Noble, 2001; Lesschaeve & Noble, 2005). Besides its total concentration, individual characteristics of proanthocyanidins, such as their average and mean degree of polymerization and their subunit composition and distribution, have been reported to largely influence astringency perception (Chira, Jourdes, & Teissedre, 2012; Chira, Pacella, Jourdes, & Teissedre, 2011; Preys et al., 2006; Quijada-Morín et al., 2012; Vidal et al., 2003). However, recent research has demonstrated that non-volatile low molecular weight molecules, including flavan-3-ol and flavonol monomers and dimers, hydroxycinnamic and hydroxybenzoic acids, are also implied with red wine astringency (Ferrer-Gallego et al., 2016; Ferrer-Gallego et al., 2014; Gonzalo-Diago, Dizy, & Fernández-Zurbano, 2014; Hufnagel & Hofmann, 2008; Sáenz-Navajas, Avizcuri, Ferreira, & Fernández-Zurbano, 2012; Sáenz-Navajas, Tao, Dizy, Ferreira & Fernández-Zurbano, 2010).

In addition, it could be also hypothesized that different astringent sensations may be related to specific polyphenolic structures that elicit astringency through different mechanisms. Although most studies on the relationship between phenolic composition and wine astringency have focused on its global intensity (Boulet et al., 2016; Gonzalo-Diago et al., 2014; Kallithraka, Kim, Tsakiris, Paraskevopoulos, & Soleas, 2011; Preys et al., 2006; Quijada-Morín et al., 2012; Quijada-Morín, Williams, Rivas-Gonzalo, Doco, & Escribano-Bailón, 2014), some advances have been recently made in identifying relationships between specific compounds and astringency sub-qualities (Ferrer-Gallego et al., 2016; Ferrer-Gallego et al., 2014; Gawell, Francis, & Waters, 2007; Hufnagel &

Hofmann, 2008; Vidal et al., 2003; Vidal, Francis, Williams, et al., 2004). For example, Hufnagel & Hofmann (2008) have reported that certain flavon-3-ol and dihydroflavon-3-ol glycosides elicit velvety astringency. However, there are still too few studies involving commercial wine samples (Sáenz-Navajas et al., 2015; Sáenz-Navajas et al., 2012; Sáenz-Navajas, Tao, et al., 2010).

A vast number of regression methods are available both for prediction and to extract information about the mechanisms that associate response variables to a set of exploratory variables (Elith, Leathwick, & Hastie, 2008). Most research attempting to establish relationships between sensory characteristics and wine composition have relied on simple correlation tests and simple or multiple linear least square regressions (Boulet et al., 2016; Gonzalo-Diago et al., 2014; Kallithraka et al., 2011; Quijada-Morín et al., 2012; Sáenz-Navajas et al., 2015). Others have used more sophisticated multivariate statistical methods, such as Principal Component Analysis (PCA), Partial Least Square Regression (PLSR) and Common Components and Specific Weights Analysis (CCSWA) (Bindon et al., 2014; Preys et al., 2006; Sáenz-Navajas et al., 2012, Sáenz-Navajas, Tao, et al., 2010). These approaches rely on statistical data modelling and therefore they assume that the mechanism that generated the data can be described by an appropriate stochastic model. For this reason, they may not be appropriate to study complex phenomena, such as wine astringency.

Algorithmic modelling assumes that the observed data is generated by an unknown and complex process, and relies on an algorithm to learn patterns in the data and predict the response variable from the independent ones (Breiman, 2001). A vast number of these algorithmic methods, also known as machine learning methods, have been developed and popularized in the last decades (Hastie, Tibshirani, & Friedman, 2009). In the present work, a relatively new method called Boosted Regression Trees (BRT) was applied to build a predictive model for astringency intensity and astringency sub-qualities based on the polyphenolic profile of the wines.

BRT takes advantage of both statistics and machine learning techniques: it combines a large amount of simple regression trees to build a single model that optimizes the predictive performance (Elith et al., 2008). This approach has several advantages compared to other methods, such as the ability to include both categorical and continuous variables and to handle missing data, a high tolerance to outliers and invariance under transformations of the predictors (thus, scaling is not an issue). They also perform internal feature selection, so they are not affected by the inclusion of many irrelevant predictor variables (Hastie et al., 2009). Besides, they can model nonlinear responses, which are likely to be relevant for astringency perception. The application of

BRT in food science is still scarce although some recent applications can be found in the literature, including predictive models for wine age (Rendall, Pereira, & Reis, 2017) and for antioxidant capacity of soluble coffee (Podio et al., 2015) and wheat (Podio, Baroni, & Wunderlin, 2017) based on their phenolic profile.

In this context, the aim of the present work was to study the relationship between astringency and phenolic composition of commercial Uruguayan Tannat wines using boosted regression trees. Tannat is a red variety of *Vitis vinifera* that has become Uruguay's emblematic wine variety (Carrau, 1997). Tannat wine is characterized by its high total content and differential composition of phenolic compounds, which are responsible for its intense colour and high astringency compared with other red wines (Alcalde-Eon, Boido, Carrau, Dellacassa, & Rivas-Gonzalo, 2006; Blanchard, 1999; Boidron et al., 1995; González-Neves, Gómez-Cordovés, & Barriero, 2001). Research on the astringency characteristics of Tannat wine and its relationship with phenolic composition is relevant for the Uruguayan wine industry, as it could enable winemakers to modify their vineyard managing and winemaking practices to obtain high quality Tannat wines with specific astringency characteristics. The present work focused on 40 commercial Tannat wines, which have been previously characterized by a sensory trained panel (Vidal et al., 2017).

4.2. MATERIALS AND METHODS

4.2.1. Wine samples

Forty commercial samples of Uruguayan varietal Tannat wine were obtained directly from the wineries. Samples were selected to represent high quality Uruguayan Tannat wines but providing different astringency characteristics. Samples from different wineries, vintages (2006 to 2014) and price segments were selected. Furthermore, 28 wines had been aged in oak barrels while the rest had not. Wines were bottled in 750 mL bottles and were conserved at 12°C until their analysis.

4.2.2. Physicochemical characterization of the wines

4.2.2.1. Oenological parameters

Basic physicochemical parameters (alcohol content (% v/v), total and volatile acidity (g/L expressed in tartaric acid) and pH) were determined for each sample using FTIR-spectroscopy (FOSS WineScan™ FT 120, Denmark) accurately set in line with Vine and Wine International Office official methods. Total polyphenol index was determined according to Iland, Ewart, and Sitters (1993), while tannin concentration was measured using the method proposed by Ribéreau-Gayon and Stonestreet (1966). This

assay is based on the property of condensed tannins of releasing carbocations that are partially converted into cyanidin when they are heated in an acid medium (Ribéreau-Gayon et al., 2006). Thus, this assay gives an approximation of the condensed tannin (or proanthocyanidin) concentration, which are the main tannins found in wine (Garrido & Borges, 2013). In the rest of the chapter, tannin concentration will refer to the chemically determined concentration of proanthocyanidins using this assay. Absorbance measures were performed in a Spectronic Genesys 2 UV-Visible spectrophotometer (Spectronic Instruments, Rochester, NY). All samples were analysed in duplicate.

4.2.2.2. Phenolic profile of the wines using HPLC-MS analyses.

Wine samples were diluted (1:5) with ultrapure water (MilliQ), and filtered through 0.45 µm Millex® syringe driven filter units, before being analysed by HPLC-DAD-MS. All samples were analysed in triplicate.

The HPLC-DAD-MS analysis was carried out in an HPLC instrument (Agilent 1200, Agilent Technologies, Palo Alto, CA, USA) equipped with a vacuum degasser, an auto sampler, a diode-array detector (DAD), a binary pump and a thermostated column oven, coupled to an ion trap mass spectrometer (Esquire 6000, Bruker Daltonik GmbH, Bremen, Germany).

Samples were analysed using a reversed-phase C18 analytical column (Zorbax Eclipse Plus 18, Agilent) with 100 mm length, 3 mm diameter and 3.5 µm of particle size, maintained at 30°C, with a flow rate of 0.3 mL/min and 2µL as injection volume. Mobile phases A and B were respectively, 0.1% formic acid in water and acetonitrile. The following gradient was used to achieve the chromatographic separation: hold at 5%B for 10 min, increased to 40%B over 20 min and hold for 5 min, then returned to initial conditions over 3 min and re-equilibrated for 7 min.

Mass detection was performed in full scan mode in positive and negative alternating polarity mode. The electrospray source conditions were as follows: endplate off set voltage -500 V, capillary voltage -4000 V, nebulizer 40 psi, dry gas flow 9.0 L/min, and dry gas temperature 365°C. Nitrogen was used as drying and nebulizing gas. Acquisition and data analysis were performed with Compass 1.3 for Esquire/HCT series software (version 6.2, Buker Daltonik GmbH, Bremen, Germany). MS/MS analysis was performed in SmartFrag mode, with a fragmentation amplitude of 0.7 V.

Quantification was performed by HPLC-MS using calibration curves of commercial standards: (+)-catechin for flavan-3-ols, quercetin for flavonols, gallic acid for phenolic acids, and malvidin-3-O-glucoside for anthocyanidin-3-O-glucosides and

their derivates. Calibration curves of (+)-catechin and malvidin-3-O-glucoside were obtained using 10 concentration levels from 0.5 to 150 mg/L, while for quercetin and gallic acid 12 concentration levels from 0.1 to 50 mg/L were used. All standards were purchased from Sigma Aldrich (Steinheim, Germany).

The identification of (+)-catechin, quercetin, gallic acid, peonidin-3-O-glucoside and malvidin-3-O-glucoside was made by comparison of the retention time and mass spectra with their respective standards. Some of the compounds were identified based on their main molecular ion, and the comparison of relative retention times with published data (Boido et al., 2011; Ivanova et al., 2011; Kelebek, 2016). These identifications are tentative, and some inaccuracies are likely to occur due to stereochemical differences (Sarnoski, Johnson, Reed, Tanko, & O'Keefe, 2012). For some of the compounds, MS/MS analysis was performed in positive and negative mode, and their identification was based in the comparison of MS/MS fragments with published data (Boido et al., 2011, Ivanova et al., 2011; Liang et al., 2014; Sarnoski et al., 2012).

The concentration of individual phenolic compounds was expressed in mg/L of the corresponding standard. The total content of the different groups of phenolic compounds analysed was calculated as the sum of the concentrations obtained for each individual compound, expressed in mg/L.

4.2.3. Sensory characterization of Tannat wine astringency

The astringency of the forty commercial samples of Tannat wine was characterized by a trained panel using time-intensity and check-all-that-apply (CATA) questions (Vidal et al., 2017). A brief description of the experimental procedure is included here, but further details can be found in Vidal et al. (2017).

The sensory panel was composed of nine assessors (7 females), ages ranging from 26 to 50 years old. Assessors were employees of the School of Chemistry from Universidad de la República (Uruguay), selected according to the guidelines of the ISO 8586:2012 standard (ISO, 2012) and their availability to participate in the study. They had been thoroughly trained over a period of 13 month, in astringency intensity evaluation using time-intensity, and astringency sub-qualities description using CATA questions. A list of 16 terms was considered for the CATA question: *dry*, *silky*, *fine emery*, *suede*, *rough*, *aggressive*, *sand paper*, *mouthcoating*, *velvety, puckery*, *harsh*, *abrasive*, *hard*, *coarse grain*, *irritant*, and *complex*.

Assessors evaluated astringency intensity of the wine samples following a standardized protocol. The timeline for the evaluation was as follows: i) at the beginning they were asked to click on the start button of the software and simultaneously take a sip

of palate cleanser (stirred plain yogurt; Vidal, Antúnez, Giménez, & Ares, 2016); .ii) at 20 s, they had to take a sip of still mineral water; iii) at 40 s they had to take a sip of a sample (15 mL) and start assessing astringency intensity over time; iv) at 50 s assessors had to spit the sample and continue the evaluation for additional 30 s. To indicate the intensity of astringency perception, assessors used their finger to move the cursor along a horizontal line scale anchored with the terms “low” and “high”, which was shown on the screen. The time-intensity task had a total duration of 40 s, and intensity data were collected every second during the evaluation period. After the time-intensity task ended, the CATA question with the 16 astringency sub-qualities was shown on the screen. Assessors were asked to select all the terms that applied to describe the astringency of the wine sample they had just tasted. A Williams’ Latin square design was used to balance the order of the terms between assessors, as suggested by Meyners and Castura (2016).

The 40 Tannat wine samples were evaluated in duplicate by each assessor and 4 samples were evaluated in each session. Samples were coded using 3-digit random numbers and presented following a Williams' Latin square design.

Testing took place in standard sensory booths in a sensory laboratory, designed in accordance with ISO 8589 (ISO, 2007), under artificial daylight and temperature control (22°C). Data collection was carried out using Compusense Cloud (Compusense Inc., Guelph, Ontario, Canada) on tablets.

4.2.4. Data analysis

4.2.4.1. Analysis of physicochemical data

Basic compositional data of the wines (section 4.2.2.1) was averaged for each sample, and descriptive statistics for the whole sample set were computed. Descriptive statistics were also computed for the individual phenolic compounds quantified by HPLC-MS and for the calculated sums of each family of compounds (section 4.2.2.2).

Analysis of variance (ANOVA) considering the sample as fixed effect was performed for each individual compound concentration, and for each of the calculated sums of compounds. Only those compounds (or sums of compounds) that were significantly different among samples for a 5% significance level were considered as predictor variables in the boosted regression trees models.

4.2.4.2. Analysis of time-intensity and CATA data

Time-intensity data was composed by 671 curves of astringency intensity versus evaluation time (for details see Vidal et al., 2017). For each of the time-intensity curves, 10 parameters were extracted but only maximum intensity (*Imax*) was considered to build a predictive model based on the sample's phenolic profile. This decision was based on the fact that the wine samples mainly differed in intensity-related parameters and these parameters were highly correlated (Vidal et al., 2017).

Regarding CATA data, the frequency of use of each astringency sub-quality to describe each sample's astringency was calculated and expressed as percentage.

4.2.4.3. Boosted regression trees

Boosted regression trees (BRT) models were built to predict *Imax* and each of the astringency sub-qualities considered in the CATA question. A separate model was built for each of these response variables. From the phenolic profile of the wines determined by HPLC-MS, only those variables that significantly differed among samples were considered as predictors. The basic oenological parameters were also included as predictor variables, as they have been shown to affect astringency perception (Fontoin, Saucier, Teissedre, & Glories, 2008; Obreque-Slier, Peña-Neira, & López-Solís, 2010a; Vidal, Courcoux, et al., 2004).

BRT models are based on the combination of two algorithms: decision trees and boosting. Decision trees algorithms use a series of rules to split the predictor space into rectangles, identifying regions with most homogeneous responses to predictors. Then, a constant is assigned to each region, for example the mean response for observations in the region in the case of regression trees (Elith et al., 2008). Although the method has several advantages, its main drawbacks are its lower accuracy compared to other methods, and its high dependence on the sample data: small perturbations in the data can result in very different models (Hastie et al., 2009). Hence, the boosting algorithm was introduced as a way to improve stability and accuracy of regression trees. Boosting is based on the idea that the combination of a large number of weak predictive models can give rise to a single very strong predictive model. A set of hyper parameters need to be established in order to run a BRT algorithm, and usually they are adjusted to optimize the model's performance. The *learning rate* (lr), or shrinkage parameter, determines the contribution of each tree to the model that is being built. The *tree complexity* (tc) controls the complexity of the model in term of interactions between predictors: if tc is 1 only main effects are modeled, if tc is 2, up to two-way interactions are modeled, and so on. These two parameters control the *number of trees* (nt) that are necessary to optimize predictive

accuracy. As lr decreases nt increases, which increases the computational cost, but still, smaller lr (and larger nt) tend to give better results (Elith et al., 2008). Different values of lr (0.001, 0.005 and 0.01) and tc (1, 2 and 5) were explored, but there were no significant differences in terms of model performance, probably because of the sample size considered in this work (40 wine samples). As shown by Elith et al. (2008), the modification of these parameters has little impact on model performance when dealing with small sample sizes. Thus, tc complexity was set to 2, lr was set to 0.001 and nt was determined by cross-validation using the function `gbm.step` from the `dismo` package (Hijmans, Phillips, Leathwick, & Elith, 2017) for R software (R Core Team, 2017). The other parameter that needs to be established for BRT modeling is the *bag fraction*, i.e. the proportion of data that are drawn at random (without replacement) at each iteration during the process of model building. Such introduction of some randomness into the boosted model has the objective of improving accuracy and speed while reducing overfitting (Elith et al., 2008). In this work, a bag fraction of 0.8 was used.

Once a BRT model is built, the relative importance of each predictor variable can be obtained. It measures the contribution of each predictor to the model, averaged across all trees, and it is scaled to sum up to 100. Thus, a higher relative importance indicates a stronger influence of the variable on the response (Elith et al., 2008). Another useful tool for interpretation of the BRT models are partial dependence plots, which show the effect of a predictor on the response after accounting for the average effects of the rest of the variables in the model (Elith et al., 2008).

Model validation in algorithmic modeling is measured by predictive accuracy (Breiman, 2001). To do so, data needs to be randomly divided into a training set and a test set. The training set is used to fit the model, and the test set is used to assess the model's prediction accuracy. A usual split was used in this study: 80% of the data was used for training and 20% for testing (Rendall et al., 2017). Also, a framework described by Rendall et al. (2017) was applied. These authors proposed a comparison framework based on Monte Carlo simulations and cross-validation as a sound and robust way to select the best regression method to predict wine age from different datasets, including wines' polyphenols composition. They proposed to use a cycle of 50 runs of Monte Carlo simulations, and to randomly divide the dataset into a training set (80%) and a test set (20%) for each iteration. Then, regressions models were built using the training set, and their predictive performance was evaluated on the test set. The rest of the framework proposed by Rendall et al. (2017) was focused on computing a performance index in order to compare the different regression methods they were testing. Although in the present work only one regression method was used (BRT), the Monte Carlo cycle

proposed by Rendall et al. (2017) is a nice approach to obtain more robust information on the relative importance of the predictor variables, especially when considering that the number of samples is relatively small (40) compared to other applications of machine learning algorithms.

In summary, the procedure to build and assess the BRT models for each response variable was:

i. The dataset was randomly split into a training set (80%) and a test set (20%) 50 times.
ii. For each iteration, a BRT model was built (lr=0.001, tc=2, bag.fraction=0.8, nt determined by 10-fold cross-validation).
iii. Model performance was assessed by computing the root mean square error of prediction (RMSEP)[1], the relative RMSEP[2] (%), and Pearson's correlation coefficient between the observed and the predicted response, using the test dataset.
iv. The relative importance of each predictor variable was stored for each model.

BTR models can be fitted for many type of response variables by specifying the type of distribution to be used in the model, which controls the loss function to be minimized. In the case of *Imax*, a continuous variable, the Gaussian distribution was used. In the case of astringency sub-qualities, response variables are percentages (or proportions). Thus, the most correct approach would be to use a zero-inflated beta distribution (Ospina & Ferrari, 2010). However, this distribution is not implemented in the packages available for BRT (`dismo` and `gbm`), so these responses were treated as continuous data by using the Gaussian distribution as well.

All statistical analyses were performed with R (R Core Team, 2017). Functions from `stats`, `dismo` (Hijmans et al., 2017) and `gbm` (Ridgeway, 2017) packages were used.

[1] $RMSEP = \sqrt{\frac{\sum_{i=1}^{n}(y_{pred}-y_{obs})^2}{n}}$

[2] $Relative\ RMSEP = 100 \times \sqrt{\frac{\sum_{i=1}^{n}(y_{pred}-y_{obs})^2}{\sum_{i=1}^{n} {y_{obs}}^2}}$

4.3. RESULTS

4.3.1. Physicochemical characterization of the wines

A summary of the basic compositional data of the wine samples is shown in Table 4.1. Large differences in ethanol content, total acidity, volatile acidity, pH, total polyphenol index and tannin concentration were found among samples.

Table 4.1. Descriptive parameters (mean, standard deviation –SD-, minimum and maximum) of the oenological parameters determined in the 40 commercial Uruguayan Tannat wines included in the research.

Parameter	Mean ± SD	Minimum	Maximum
Ethanol (%)	13.3 ± 0.9	11.8	15.2
Total acidity (g/L)*	5.1 ± 0.4	4.2	6.1
Volatile acidity (g/L) *	1.1 ± 0.2	0.8	1.6
pH	3.8 ± 0.2	3.5	4.2
Total polyphenol index (AU)	74.1 ± 14.8	50.8	117.4
Tannin concentration (g/L)	3.9 ± 0.9	2.4	6.6

***** Total and volatile acidity are expressed in g/L of tartaric acid.

A total of 79 individual compounds were (tentatively) identified and quantified by HPLC-MS in the Tannat wine samples: 44 flavan-3-ols, 11 phenolic acids, 15 anthocyanins and 9 flavonols (see Table A1 in the Appendix). A summary with descriptive information on some individual compounds and groups of compounds is presented in Table 4.2.

ANOVA on the content of phenolic compounds was performed for each of the 79 quantified individual compounds, as well as for the 20 calculated sums of compounds, considering the wine sample as fixed effect. Significant differences among samples were established for 79 compounds or sums of compounds (p values < 0.036), which were considered as predictors in the BRT models. The complete list of the predictor variables considered, including phenolic compounds and oenological parameters, with their corresponding p values for the sample effect can be found in Table A2 in the Appendix.

Table 4.2. Minimum, maximum, average and standard deviation (SD) of the content of phenolic compounds of Tannat wines.

	Minimum	Maximum	Mean ± SD	
Falvan-3-ols (mg/L)				
(+)-gallocatechin	1.9	5.3	3.2 ± 0.8	
(-)-epigallocatechin	0.7	2.6	1.3 ± 0.4	
(+)-catechin	22.0	89.3	48.8 ± 13.7	
(+)-epicatechin	11.6	79.7	36.3 ± 14.9	
(+)-epicatechin gallate	1.8	11.8	4.7 ± 2.4	
∑ Dimer non-galloylated	63.5	625.7	245.5 ± 100.5	
∑ Trimer non-galloylated	37.0	731.3	255.0 ± 144.6	
∑ Tetramer non-galloylated	5.3	129.4	32.6 ± 23.0	
∑ Dimer galloylated	0.0	16.2	4.5 ± 4.3	
∑ non-galloylated	105.8	1486.4	533.1 ± 255.1	*(99.1%)*
∑ galloylated	0.0	16.2	4.5 ± 4.3	*(0.9%)*
∑ procyanidin	63.6	1301.6	358.0 ± 266.4	*(66.6%)*
∑ prodelphinidin	42.2	322.1	179.7 ± 69.8	*(33.4%)*
∑ monomer	36.6	174.0	93.0 ± 27.7	*(14.8%)*
∑ dimer	63.5	638.3	250 ± 102.1	*(39.6%)*
∑ oligomer	42.3	860.7	287.6 ± 166.5	*(45.6%)*
∑ **flavan-3-ol**	**142.4**	**1673.0**	**630.6 ± 280.5**	
Phenolic acids (mg/L)				
Gallic acid	31.5	176.7	84.1 ± 38.9	
Protocatechuic acid	13.0	40.9	26.2 ± 6.4	
Methyl gallate	4.5	7.6	5.8 ± 1.1	
cis-caftaric acid	28.2	203.4	110.0 ± 45.0	
trans-fertaric acid	6.3	33.7	20.6 ± 7.9	
trans-caftaric acid	3.3	223.9	49.2 ± 70.4	
p-coumaric acid	29.1	169.5	92.0 ± 32.2	
p-coumaroyl hexose	6.3	83.2	27.4 ± 21.5	
trans-caffeic acid	16.2	86.5	36.3 ± 17.6	
p-coumaroyl hexose (2)	4.3	55.1	20.9 ± 14.6	
p-coumaroyl hexose (3)	2.0	58.7	23.5 ± 19.4	
∑ hydroxybenzoic acid	53.7	203.6	110.4 ± 41.9	*(24.6%)*
∑ hydroxycinnamic acid	220.2	518.3	339.0 ± 81.3	*(75.4%)*
∑ **phenolic acid**	**278.5**	**719.9**	**449.4 ± 91.7**	

Table 4.2 *(Continued)* Minimum, maximum, average and standard deviation (SD) of the content of phenolic compounds of Tannat wines.

	Minimum	**Maximum**	**Mean ± SD**	
Anthocyanins (mg/L)				
Delphinidin-3-O-glucoside	0.3	18.9	5.3 ± 4.4	
Cyanidin-3-O-glucoside	0.1	3.5	0.9 ± 0.8	
Petunidin-3-O-glucoside	0.6	46.2	13.7 ± 10.9	
Peonidin-3-O-glucoside	0.2	18.2	5.4 ± 4.4	
Malvidin-3-O-glucoside	4.5	188.4	70.7 ± 48.5	
Delphinidin-3-O-acetylglucoside	0.1	7.6	1.7 ± 1.7	
Cyanidin-3-O-acetylglucoside	0.1	2.8	0.6 ± 0.6	
Petunidin-3-O-acetylglucoside	0.2	20.0	3.9 ± 4.4	
Peonidin-3-O-acetylglucoside	0.1	9.4	2.3 ± 2.2	
Malvidin-3-O-acetylglucoside	0.6	67.7	19.5 ± 17.7	
Delphinidin-3-O-p-coumaroylglucoside	0.5	4.4	1.5 ± 1.0	
Petunidin-3-O-p-coumaroylglucoside	1.5	24.6	5.1 ± 4.7	
Cyanidin-3-O-p-coumaroylglucoside	0.6	4.5	1.5 ± 0.9	
Peonidin-3-O-p-coumaroylglucoside	1.1	17.7	5.5 ± 3.3	
Malvidin-3-O-p-coumaroylglucoside	0.5	48.5	14.5 ± 11.5	
∑ glucoside	5.7	274.9	95.9 ± 66.9	*(64.4%)*
∑ acetylglucoside	0.9	107.5	27.3 ± 26.2	*(18.1%)*
∑ coumaroylglucoside	6.4	80.4	28.0 ± 19.5	*(18.5%)*
∑ **anthocyanin**	**18.2**	**461.8**	**151.2 ± 103.9**	
Flavonols (mg/L)				
Quercetin-3-O-glucuronide	0.4	7.5	1.9 ± 2.0	
Myricetin-3-O-glucoside	2.4	95.6	13.6 ± 16.2	
Quercetin-3-O-glucoside	0.9	22.6	5.2 ± 4.9	
Isorhamnetin-3-O-glucoside	0.4	5.7	2.7 ± 1.9	
Laricetrin-3-O-glucoside	2.3	25.2	6.8 ± 4.8	
Siringetin-3-O-glucoside	0.2	12.3	3.0 ± 2.8	
Myricetin	0.3	14.7	5.0 ± 3.3	
Quercetin	0.3	9.3	3.0 ± 2.1	
Laricetrin	0.2	2.1	0.7 ± 0.4	
∑ **flavonol**	**11.3**	**159.1**	**35.5 ± 25.6**	

4.3.2. Sensory characterization of Tannat wine astringency

A summary of results from the sensory characterization of Tannat wine astringency is shown in Table 4.3. Interested readers are referred to Vidal et al. (2017) for further details. The average maximum astringency intensity of Tannat wines ranged from 5.1 to 8.1 (on a 0-10 scale), and presented a moderate variation. Regarding astringency sub-qualities, the most frequently used descriptors to describe the astringency of the wine samples were *dry*, *mouthcoating* and *rough*, which showed an average frequency of use of 52.9%, 44.9% and 41.8%, respectively (Table 4.3). *Fine emery*, *suede* and *complex* were also frequently used to describe the samples, showing average frequency of use of 30.0%, 29.7% and 23.0%, respectively.

Table 4.3. Average, standard deviation (SD), maximum and minimum of astringency maximum intensity determined by time-intensity method and of the frequency of use (expressed in percentage) of each term included in the check-all-that-apply question to describe astringency sub-qualities of Tannat wine samples.

Term	Mean ± SD	Minimum	Maximum
Imax	6.4 ± 0.7	5.1	8.1
Dry	52.9 ± 13.5	18.8	75.0
Silky	8.2 ± 11.2	0.0	43.8
Fine emery	30.0 ± 11.4	8.3	50.0
Suede	29.7 ± 14.7	6.3	62.5
Rough	41.8 ± 16.3	0.0	66.7
Aggressive	16.4 ± 11.6	0.0	43.8
Sand paper	8.1 ± 8.8	0.0	31.3
Mouthcoating	44.9 ± 15.0	17.6	81.3
Velvety	17.3 ± 10.8	0.0	43.8
Puckery	18.1 ± 10.7	0.0	42.9
Harsh	13.5 ± 11.0	0.0	56.3
Abrasive	8.5 ± 7.4	0.0	28.6
Hard	17.2 ± 12.4	0.0	41.7
Coarse grain	15.5 ± 10.0	0.0	43.8
Irritant	14.0 ± 9.0	0.0	27.8
Complex	23.0 ± 12.2	0.0	61.1

4.3.4. Predictive models for maximum astringency intensity and astringency sub-qualities based on Boosted Regression Trees.

Fifty BRT models with different partitions of the data in training and test sets were built for each response variable (maximum astringency intensity and frequency of use of the 16 astringency sub-qualities considered in the CATA question). The training sets were used to build the BRT models, and the tests sets were used to assess the models' predictive accuracies by computing the RMSEP, relative RMSEP and Pearson's correlation coefficient (Section 4.2.4.3). The median and range of these measures for each response variable are shown in Table 4.4. For all response variables, the range of the three measures of predictive accuracy were large, suggesting that both strong and weak predictive models were built in the Monte Carlo iterations.

Predictive accuracy largely differed for the different response variables. In the case of Imax, predictive accuracy was good in general, as the median of the relative RMSEP was lower than 10%, while for half of the models Pearson's correlation coefficient between the predicted and the observed responses in the test datasets was higher than 0.58. On the contrary, when the frequency of use of the different astringency sub-qualities in the CATA question were considered as response variables, the predictive accuracy of the BRT models was much worse. Although the median of Pearson's correlation coefficients was acceptable for a few terms (0.53 for *Silky*, 0.66 for *Aggressive*, 0.50 for *Harsh*), in all cases the median relative RMSEP was higher than 25%, reaching values as high as 119% for the term *Silky*. This result might be a consequence of the distribution family chosen to build the BRT models. As mentioned in Section 4.2.4.3, the frequency of use of each astringency sub-quality was modeled as if it was continuous data, by selecting the Gaussian distribution family. However, the correct approach would have been to use zero-inflated beta distribution (Ospina & Ferrari, 2010), which is not supported by the packages available to build BRT models. In the case of the attribute *Dry*, which ranged between 19% and 75%, and was on average the most used attribute to describe the astringency of wine samples (Table 4.3), the median relative RMSEP was the lowest (25.4%). However, those attributes that had a very low frequency of use on average, because they applied only to some samples, such as *Silky* and *Sand Paper* (Table 4.3) had the highest median relative RMSEP (Table 4.4).

Table 4.4. Median and range (between brackets) of root mean squared error of prediction (RMSEP), relative RMSEP and Pearson's correlation coefficient of the 50 Boosted Regression Trees models that were built for each response variable: astringency maximum intensity determined by time-intensity method and frequency of use of each term included in the check-all-that-apply question to describe astringency sub-qualities of Tannat wine samples.

Response	RMSEP	Relative RMSEP (%)	Pearson's corr
Imax	5.54 (3.18 – 9.17)	8.63 (4.96 – 14.26)	0.58 (-0.06 – 0.89)
Dry	12.92 (8.15 – 21.31)	25.38 (15.96 – 44.11)	0.06 (-0.70 – 0.90)
Silky	9.64 (4.37 – 16.32)	119.37 (52.38 – 265.06)	0.53 (-0.45 – 0.89)
Fine emery	11.19 (5.07 – 15.45)	37.90 (16.69 – 50.88)	-0.07 (-0.80 – 0.61)
Suede	13.66 (5.80 – 20.59)	45.13 (19.21 – 75.23)	0.43 (-0.28 – 0.87)
Rough	14.07 (9.27 – 23.01)	33.81 (18.42 – 58.04)	0.46 (-0.63 – 0.92)
Aggressive	9.41 (5.04 – 13.11)	55.77 (31.37 – 91.59)	0.66 (0.18 – 0.92)
Sand paper	8.36 (4.92 – 13.63)	102.08 (62.06 – 184.73)	0.15 (-0.80 – 0.82)
Mouthcoating	14.53 (4.18 – 22.85)	32.73 (8.94 – 53.57)	0.34 (-0.68 – 0.83)
Velvety	10.29 (2.89 – 17.52)	57.77 (19.88 – 98.28)	0.47 (-0.45 – 0.90)
Puckery	11.30 (6.71 – 16.76)	62.36 (32.90 – 114.64)	-0.03 (-0.60 – 0.73)
Harsh	9.03 (4.71 – 17.87)	65.05 (33.69 – 160.88)	0.50 (-0.55 -0.95)
Abrasive	6.53 (3.36 – 12.02)	80.96 (38.11 – 224.83)	0.40 (-0.80 – 0.73)
Hard	12.40 (7.94 – 16.18)	68.19 (43.27 – 114.72)	0.30 (-0.50 – 0.75)
Coarse grain	9.84 (4.30 – 16.04)	62.93 (24.76 – 120.29)	0.48 (-0.19 – 0.89)
Irritant	8.75 (3.74 – 12.02)	59.84 (27.84 – 133.57)	0.45 (-0.61 – 0.96)
Complex	10.53 (4.85 – 14.89)	49.89 (22.74 – 72.58)	-0.07 (-0.60 – 0.53)

Although most of the models built to predict astringency sub-qualities based on wine's composition and phenolic profile were bad predictors, the approach of using 50 Monte Carlo iterations allowed to find some models with high predictive accuracy. Besides, the aggregate information provided by the 50 models could be useful to identify predictors that most likely contribute to each response variable. Following the approach presented by Rendall et al. (2017), if certain variable consistently presents a high relative importance for the majority of the models, it is likely that it is indeed relevant to explain the response.

Table 4.5 presents the median and the range of relative importance of the five predictors with the highest relative importance for each response variable. For completeness, an extension of this information is shown in Table A3 of the Appendix. The variables that consistently presented higher relative importance than the others were different for each response variable, although some variables were among the five most relevant predictors for various responses. For example, tannin concentration (chemically determined by the Ribéreau-Gayon and Stonestreet (1966) method) was in the top five of *Imax*, *silky*, *fine emery*, *suede*, *rough*, *aggressive*, *sand paper*, *mouthcoating*, *puckery*, *abrasive* and *coarse grain*, with median relative importance ranging from 5 to 35%. Quercetin was also relevant for 9 different response variables, with median relative importance ranging from 5 to 21%. On the contrary, one of the procyanidin trimers (procyanidin trimer (3)) was relevant only to predict *irritant* (median relative importance = 18%). These results are also illustrated in Fig. 4.1, which depicts the relative importance of each of the 84 predictor variables (Table A2 of the Appendix) for the 50 BRT models superimposed, for three exemplar responses: Imax, *aggressive* and *velvety*.

For some responses, there appears to be strong evidence of the relative importance of certain predictor variables. For example, for *velvety*, procyanidin trimer (7) presented a relative importance higher than 54% in half of the BRT models. Similarly, the median relative importance of tannin concentration was 31% for *aggressive*, 22% for *suede* and 35% for *silky* (35). However, for some astringency sub-qualities, none of the considered predictor variables presented a relatively high median relative importance. In the case of *hard* and *complex*, the highest median relative importance was 9%, while for *puckery* the median relative importance of Laricetrin-3-O-glucoside was 12%, but for the rest of the predictors it was equal or lower than 5% (Table 4.5).

Table 4.5. Median and range (between brackets) of the predictor's relative importance (%) of the 50 Boosted Regression Trees models that were built for each response variable: astringency maximum intensity (Imax) determined by time-intensity method and frequency of use of each term included in the check-all-that-apply question to describe astringency sub-qualities of Tannat wine samples. For each response variable, only the five predictors with highest median relative importance are shown.

Predictor	Response	Predictor	Response
	Imax		***Dry***
Prodelphinidin trimer (7)	18 (0-39)	Procyanidin dimer B-type (2)	18 (1-61)
Tannin concentration	17 (4-37)	Myricetin	5 (0-20)
Prodelphinidin trimer (9)	12 (0-36)	Cyanidin-3-O-acetylglucoside	4 (0-35)
Quercetin	12 (2-29)	Cyanidin-3-O-glucoside	4 (0-23)
Σhydroxybenzoic acid	4 (0-25)	Volatile acidity	4 (0-40)
	Silky		***Fine emery***
Tannin concentration	35 (11-74)	Prodelphinidin trimer (8)	12 (0-47)
Quercetin	12 (3-26)	Tannin concentration	10 (0-41)
Myricetin	8 (0-20)	Cyanidin-3-O-glucoside	7 (0-49)
Prodelphinidin trimer (7)	2 (0-15)	Prodelphinidin dimer (4)	4 (0-30)
Procyanidin trimer (7)	2 (0-23)	Σphenolic acid	3 (0-26)
	Suede		***Rough***
Tannin concentration	22 (3-66)	Tannin concentration	13 (1-48)
Quercetin	21 (4-58)	Myricetin	9 (0-37)
Laricetrin	7 (0-34)	Σphenolic acid	7 (1-22)
trans-caffeic acid	4 (0-22)	Laricetrin	6 (0-37)
Procyanidin dimer B-type (2)	3 (0-17)	Prodelphinidin trimer (10)	6 (0-23)
	Aggressive		***Sand paper***
Tannin concentration	31 (4-57)	Σphenolic acid	19 (0-47)
Σphenolic acid	19 (6-55)	Procyanidin tetramer (3)	14 (1-58)
Gallic acid	8 (0-36)	Tannin concentration	6 (0-40)
Myricetin	6 (1-17)	Σflavonol	4 (0-25)
ΣTetramer non-galloylated	4 (0-22)	Procyanidin trimer (7)	2 (0-21)
	Mouthcoating		***Velvety***
Quercetin	13 (1-28)	Procyanidin trimer (7)	54 (0-79)
Volatile acidity	10 (0-55)	Total acidity	6 (0-35)
Tannin concentration	6 (0-23)	Prodelphinidin trimer (8)	4 (0-26)
Laricetrin	5 (0-12)	Procyanidin tetramer (3)	3 (0-55)
Procyanidin dimer B-type (2)	3 (1-51)	ΣTetramer non-galloylated	2 (0-26)

Table 4.5 *(Continued)* Median and range (between brackets) of the predictor's relative importance (%) of the 50 Boosted Regression Trees models that were built for each response variable: astringency maximum intensity (Imax) determined by time-intensity method and frequency of use of each term included in the check-all-that-apply question to describe astringency sub-qualities of Tannat wine samples. For each response variable, only the five predictors with highest median relative importance are shown.

Predictor	Response	Predictor	Response
	Puckery		***Harsh***
Laricetrin-3-O-glucoside	12 (1-42)	Procyanidin dimer B-type (2)	26 (5-56)
Tannin concentration	5 (0-30)	ΣTetramer non-galloylated	17 (0-43)
Quercetin	5 (0-23)	Malvidin-3-O-acetylglucoside	5 (0-23)
(-)-epigallocatechin	5 (0-25)	Quercetin	3 (0-20)
Prodelphinidin trimer (9)	4 (0-22)	*trans*-caffeic acid	3 (0-13)
	Abrasive		***Hard***
Total acidity	24 (6-52)	Quercetin	9 (0-28)
pH	9 (0-38)	ΣTetramer non-galloylated	6 (0-28)
Quercetin	8 (0-29)	Procyanidin dimer B-type (3)	6 (0-26)
Laricetrin	8 (0-36)	*trans*-caffeic acid	6 (0-29)
Tannin concentration	8 (0-32)	Σmonomer	5 (0-34)
	Coarse grain		***Irritant***
Tannin concentration	13 (3-61)	Procyanidin trimer (3)	18 (2-70)
Procyanidin trimer (7)	8 (0-42)	Prodelphinidin dimer (4)	9 (0-32)
Procyanidin tetramer (3)	6 (0-28)	Petunidin-3-O-(p-cumaroyl)glucoside	8 (0-29)
Σphenolic acid	5 (0-21)	Prodelphinidin trimer (7)	5 (0-33)
Procyanidin tetramer (4)	4 (0-23)	pH	3 (0-22)
	Complex		
Σmonomer	9 (0-34)		
Ethanol	7 (0-40)		
(-)-epicatechin	6 (0-29)		
cis-caftaric acid	6 (0-20)		
Quercetin	5 (0-30)		

Among the oenological variables considered as predictors, tannin concentration was by far the most relevant predictor for different aspects of astringency perception. On the contrary, total acidity was among the five most relevant predictors only for two astringency sub-qualities, but it was the most important predictor for *abrasive*, showing a median relative importance of 24%.

Flavan-3-ols were in general the family of compounds with higher relative importance for the prediction of Tannat wine astringency. Among these compounds, procyanidin dimer B-type (2), procyanidin trimer (3), procyanidin trimer (7), prodelphinidin trimer (7), prodelphinidin trimer (8), prodelphinidin trimer (9), procyanidin tetramer (3) and the sum of non-galloylated tetramers had median relative importance higher than 10% for some response variables. The sum of phenolic acids had a median relative importance of 19% for *aggressive* and *sand paper*, but individual phenolic acids were not consistently regarded as important contributors to any astringency characteristic. The same happened with anthocyanins; none of the quantified compounds from this family had median relative importance higher than 10%. Regarding flavonols, the median relative importance of quercetin was 12% for Imax, 12% for *silky*, 21% for *suede* and 13% for *mouthcoating*, while laricetrin-3-O-glucoside had a median relative importance of 12% for *puckery*.

Another important tool for the interpretation of BRT models are partial dependence plots. In these plots, the fitted response is represented as a function of one predictor variable, after having accounted for the influence of the rest of the predictors. Fig. 4.2 shows the influence of tannin concentration on Imax, and the astringency sub-qualities *aggressive*, *silky* and *suede*, while Fig. 4.3 shows the influence of quercetin on Imax, *mouthcoating*, *silky* and *suede*. It can be observed that the relationship between the response variables and the predictors was not linear and that it was different for the different responses. Imax and frequency of use of *aggresive* increased with tannin concentration, whereas the frequency of use of *silky* and *suede* had an inverse relationship with this predictor (Fig. 4.2). Similarly, both *Imax* and frequency of use of *mouthcoating* increased with quercitin concentration, while the opposite pattern was observed for the frequency of use of the terms *silky* and *suede* (Fig, 4.3).

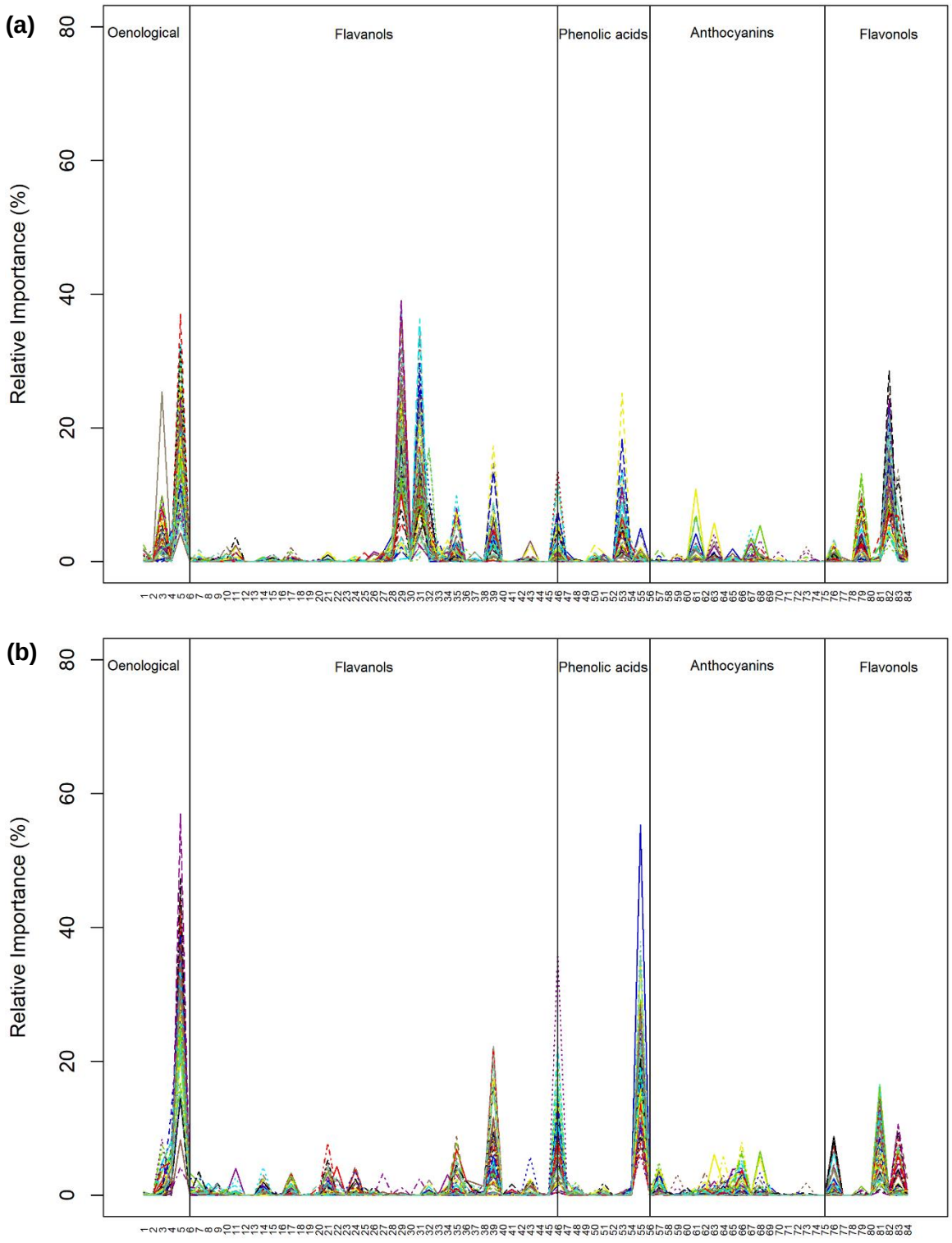

Fig. 4.1. Predictor variables' relative importance (%) for the 50 Boosted Regression Trees models built for the responses maximum astringency intensity (Imax) (a), *aggressive* (b) and *velvety* (c). The numbers of predictor variables correspond to those from Table A2 from the Appendix.

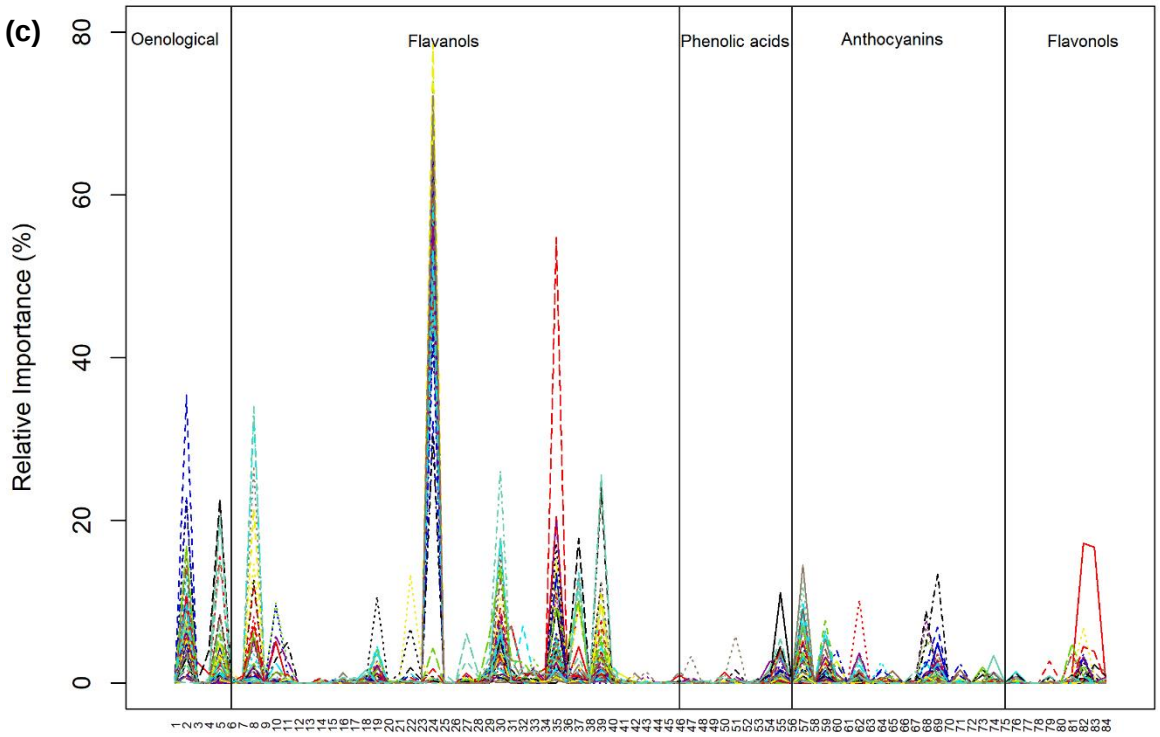

Fig. 4.1. *(Continued)* Predictor variables' relative importance (%) for the 50 Boosted Regression Trees models built for the responses maximum astringency intensity (Imax) (a), *aggressive* (b) and *velvety* (c). The numbers of predictor variables correspond to those from Table A2 from the Appendix.

4.4. DISCUSSION

Research on the relationship between astringency and phenolic composition of commercial red wines is still scarce, particularly when dealing with Tannat wines. The present research aimed at filling this gap by studying the relationship between phenolic composition and astringency of 40 commercial Uruguayan red wines.

Flavan-3-ols were the most abundant family of phenolic compounds in the commercial Tannat wines (Table 4.2). On average, dimers and oligomers were the most abundant, while monomers represented 14.8% of the quantified flavan-3-ols. Regarding anthocyanins, malvidins were the most abundant class, followed by petunidins. In the phenolic acids family, the proportion of hydroxycinnamic acids was, on average, larger than the proportion of hydroxybenzoic acids. These results are in line with previous studies involving the characterization of the phenolic composition of Tannat wines (Alcalde-Eon et al., 2006; Boido et al., 2011; Favre et al., 2014; González-Nevez, Ferrer, & Gil, 2012). On the contrary, the average content of flavonols was lower than that

reported by Boido et al. (2011) and higher than that reported by Favre et al. (2014). However, it should be taken into account that in the study conducted by Boido et al. (2011) only two young Tannat wines were analysed (in duplicate), while in the study conducted by Favre et al. (2014) four wines produced by different winemaking procedures were assessed. In the present study 40 commercial samples of very different characteristics were considered. Thus, it is not surprising that results differed from those reported in literature, nor that the dispersion of the data was much larger.

A novel predictive method, Boosted Regression Trees, was used to model the relationship between astringency intensity and sub-qualities and the phenolic composition of Tannat wine. A framework proposed by Rendall et al. (2017), based on Monte Carlo iterations to randomly split the dataset into training and test sets was used. Results showed that both strong and weak predictive models were obtained in the different Monte Carlo simulations (Table 4.4), which highlights the importance of using this approach to obtain more robust results and conclusions. As predictive models are built using the training dataset, they tend to highly depend on these data. Although the number of samples included in the present work (n=40) was higher than that used in many studies involving commercial wines (Boulet et al., 2016; Gonzalo-Diago et al., 2014; Quijada-Morín et al., 2012; Quijada-Morín et al., 2014; Rendall et al., 2017; Sáenz-Navajas et al., 2012; Sáenz-Navajas, Tao, et al., 2010), it is still low compared to many data-mining applications in which algorithmic modelling is more often used. The influence of the specific training dataset used to build the model is expected to be larger when dealing with relatively small sample sets, such as the one used in the present work.

One of the main reasons for choosing BRT was that they allow to model non-linear responses, as the influence of individual phenolic compounds on astringency was expected to be complex. Astringency is expected to depend more on the balance and interaction of different phenolic compounds among them and with other constituents of the wine matrix, than on the concentration of an individual compound (Holt, Francis, Field, Herderich, & Iland, 2008). Besides, a saturation effect is likely to occur, as the increase in the concentration of a certain compound is expected to increase astringency perception only up to a certain threshold (Lawless & Heymann, 2010). Results from the present work showed the relationship between astringency total intensity or astringency sub-qualities, and oenological parameters and phenolic compounds was indeed not linear (Fig. 4.2 and 4.3).

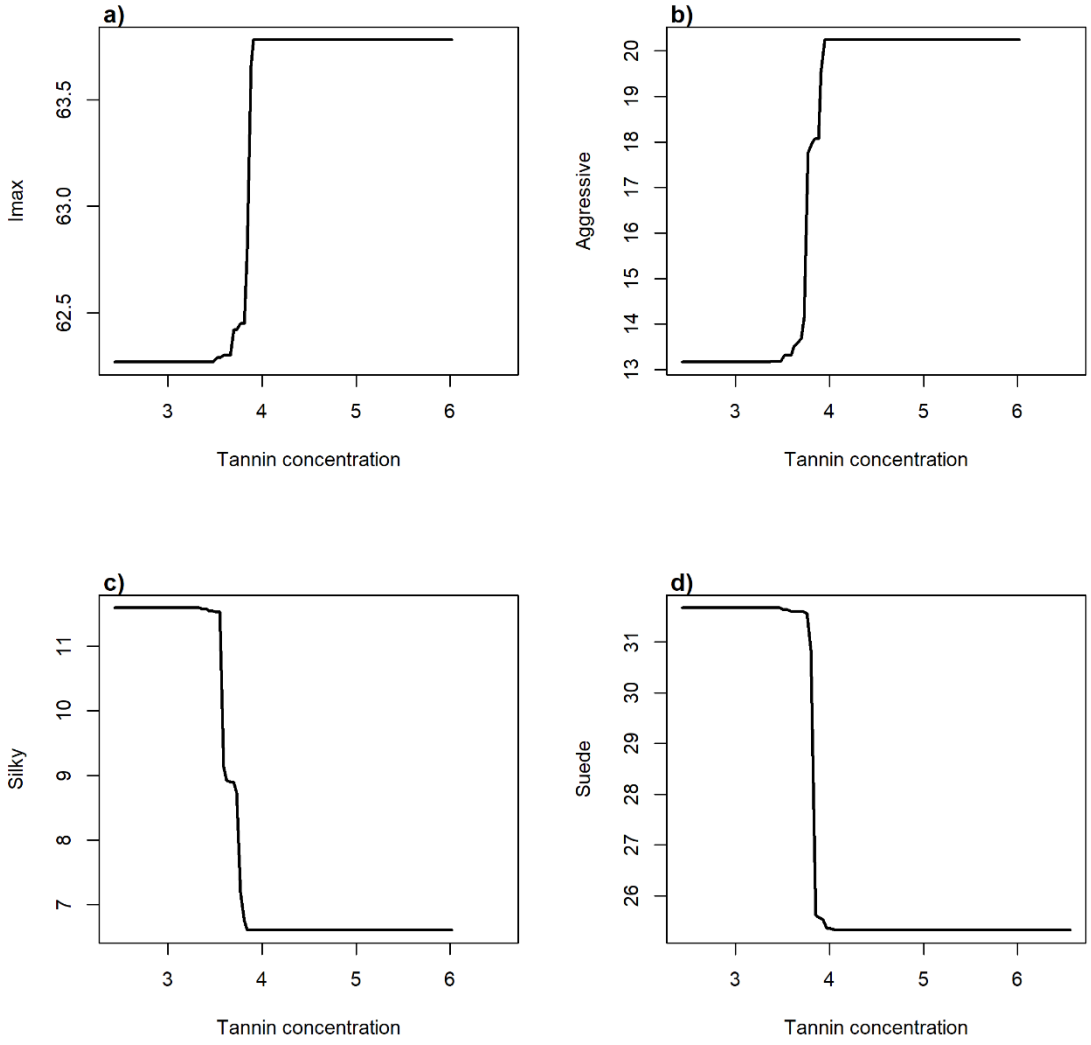

Fig. 4.2. Partial dependence plots for tannin concentration in the Boosted Regression Trees (BRT) models of: a) maximum astringency intensity (Imax); and the frequency of use of b) *aggressive*, c) *silky* and d) *suede*. Each response variable was fitted using the BRT model with higher predictive accuracy.

Another reason for selecting BRT models for this work was that they are not affected by the inclusion of irrelevant predictor variables, so there is no need to perform a priori variable selection. Fig. 4.1 shows clearly that from the 84 predictor variables that were considered to build the models, only a few were consistently selected as having an important contribution to sensory astringency. A high degree of data sparsity can raise problems with many predictive methodologies (Rendall et al., 2017), which explains that in general a priori variable selection is made. For example, Boulet et al. (2016) built a vast number of multiple linear regression models with all possible combinations of up to 4 variables (selected from 233 spectroscopic and compositional parameters) to identify the most suitable ones to predict red wine astringency intensity. Also, other studies have only included the compounds that were found in concentration higher than their corresponding sensory threshold (Sáenz-Navajas et al., 2012; Sáenz-Navajas, Tao, et

al., 2010). Although this is a valid approach, it should be taken into account that the influence of specific phenolic compounds on astringency perception has been reported to be highly matrix dependent (Sáenz-Navajas et al., 2012), and thus, sensory thresholds in aqueous solutions probably differ from those in wine matrices. In this sense, the fact of being able to use all the available data to build a predictive model is a clear advantage.

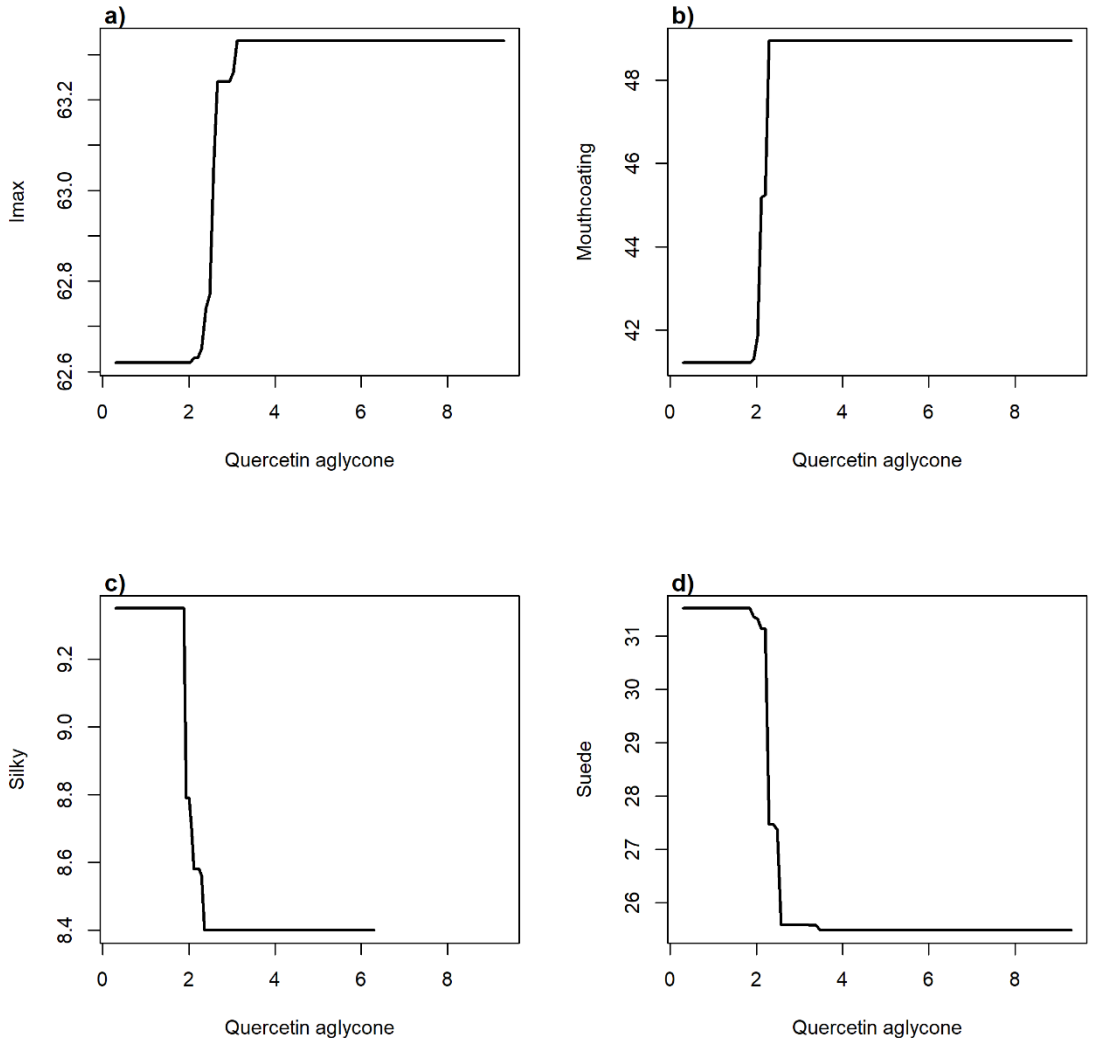

Fig. 4.3. Partial dependence plots for quercetin concentration in the Boosted Regression Trees (BRT) models of: a) maximum astringency intensity (Imax); and the frequency of use of b) *mouthcoating*, c) *silky* and d) *suede*. Each response variable was fitted using the BRT model with higher predictive accuracy.

Although the predictive accuracy of BRT models was moderate, and both weak and strong predictive models were obtained (Table 4.4), the approach based on 50 Monte Carlo simulations allowed us to identify predictor variables that consistently presented a high contribution to each response variable. Among the oenological parameters considered in the present work, tannin concentration was by far the most relevant predictor of maximum astringency intensity and various astringency sub-qualities. This result is in line with several previous studies, which report a positive

relationship of proanthocyanidin (i.e. tannin) concentration with astringency intensity (Bindon et al., 2014; Kallithraka et al., 2011; Monteleone, Condelli, Dinnella, & Bertuccioli, 2004; Preys et al., 2006; Robichaud & Noble, 1990; Vidal, Courcoux, et al., 2004). However, it should be noted that the effect of tannin concentration on astringency depended on the astringency characteristic considered. As shown in Fig. 4.2, an increase in tannin concentration resulted in higher Imax or a higher frequency of use of *aggressive*, while it tended to reduce the frequency of mention of *suede* and *silky*. Similarly, Vidal, Courcoux, et al. (2004) reported that an increase in grape seed procyanidin concentration significantly increased overall astringency intensity and some astringency sub-qualities such as dry, pucker and chalk, while it decreased fine surface smoothness, a sub-quality related to silky astringency. Gawel et al. (2007) also found a negative correlation between in-mouth chamois sensation and tannin concentration in Shiraz wines.

Flavan-3-ols were the family of phenolic compounds with the highest contribution to astringency intensity and astringency sub-qualities, in contrast to Hufnagel and Hoffman (2008) and Sáenz-Navajas et al. (2012), who suggested that flavanols are not the main compounds involved in wine astringency. In this study, some dimers, trimers and the sum of non-galloylated tetramers were consistently selected as relevant contributors to some astringency sub-qualities (Table 4.5), while monomeric flavanols were not, in agreement with previous studies (Quijada-Morín et al., 2012). Kallithraka et al. (2011) also reported a positive relationship between total flavanols and perceived astringency, but neither monomeric nor dimers and trimers were correlated to astringency intensity in their study. Conversely, Sáenz-Navajas et al. (2017) reported that oligomers and polymers of flavanols would be responsible of coarse, grainy and dry astringency sensations in wine fractions.

The constant contradiction found in literature about which are the chemical structures responsible for astringency perception (Hufnagel & Hoffman, 2008) is probably a consequence of the high diversity of chemical structures of phenolic compounds. For example, Kallithraka et al. (2011) only quantified two dimers (procyanidins B1 and B2) and one trimer (procyanidin C1). In this study, 8 procyanidin dimers, 5 prodelphinidin dimers, 7 procyanidin trimers, 11 prodelphinidin trimers and 8 procyanidin tetramers were quantified (Table A1 from the Appendix), but only a few of them were identified as relevant for astringency perception.

In the present study, anthocyanins were not consistently regarded as important contributors to astringency intensity or sub-qualities by the BRT models, in agreement with Kallithraka et al. (2011) and Gawel et al. (2007). However, Gonzalo-Diago et al.

(2014) found correlations between four anthocyanins and perceived astringency. On the other hand, individual phenolic acids did not show high median relative importance in the BRT models either, although the sum of phenolic acids appears to be a relevant contributor to astringency sub-qualities *aggressive* and *sand paper* (Table 4.5). Certain phenolic acids, such as *trans*-caffeic, *trans*-coutaric, protocatechuic acids, and the non-phenolic *cis*-aconitic acid, have been pointed out as relevant contributors to astringency in recent studies (Ferrer-Gallego et al., 2014; Hufnagel & Hoffman, 2008; Preys et al., 2006; Sáenz-Navajas et al., 2012; Sáenz-Navajas, Tao, et al., 2010), while other authors have found no relationship of phenolic acids with astringency (Kallithraka et al., 2011).

Flavonols have also been reported to contribute to astringency perception (Cliff, King, & Schlosser, 2007; Ferrer-Gallego et al., 2016; Hufnagel & Hoffman, 2008), and in this study quercetin was the most relevant amongst the quantified flavonols. In contrast to Hufnagel and Hoffman (2008) who reported that several flavonols were described as having a velvety astringency, in the present study none of the quantified flavonols consistently presented high relative importance for the response *velvety* (Table 4.5). Moreover, responses related to soft textures, such as *silky* and *suede* decreased when quecetin concentration increased (Fig. 4.3). Ferrer-Gallego et al. (2014) reported that velvety texture significantly decreased with the addition of the flavonol quercetin-3-O-glucoside to white wines, while in red wines this addition increased the perception of sub-qualities such as harshness and dryness.

This lack of consistency in results reported in literature might also be a consequence of the methodological variability among studies, with some working with model solutions, others with additions to base wines, commercial wines or wine fractions. Methodological differences in sensory assessments also might be involved in the differences in correlations between chemical and sensory data. Beyond the selection of specific methods for sensory evaluation of astringency (descriptive analysis, time-intensity, check-all-that-apply questions), the lack of reference materials that clearly illustrate specific mouthfeel sensations which can be used for panel training is probably involved in the misalignment of vocabulary interpretation among sensory panels from different research groups, as suggested by Sáenz-Navajas et al. (2017).

Moreover, a great diversity of statistical approaches have been used in the different studies reported in literature. This study is the first to report the use of Boosted Regression Trees to predict wine astringency based on wine's phenolic composition. Predictive accuracy was good in general when Imax was considered as response variable, but it was much worse when astringency sub-qualities assessed through a CATA question were modeled (Table 4.4). From a sensory point of view, CATA questions

provided valuable information to describe astringency sub-qualities of a large sample set of Tannat wine samples, with the advantage of requiring less training than traditional descriptive analysis (Vidal et al., 2017). However, if these data are to be used as a response in predictive models, CATA questions do not seem to be the best choice, as the accurate distribution family to model these data is not easily available in many softwares. Although the percentage of assessors selecting a term is usually correlated with attribute intensity (Ares & Jaeger, 2015), CATA questions do not provide a direct measurement of the intensity of sensory attributes. In future studies, alternative methods which provide data that can be modeled as continuous should be used. In this sense, Rate-all-that-apply (RATA) questions seem to be a promising alternative (Ares et al., 2014; Reinbach, Giacalone, Ribeiro, Bredie, & Frøst, 2014). Sáenz-Navajas et al. (2017) have recently used RATA questions to describe astringency sub-qualities of wines and wine fractions.

Another limitation of the present study is that only some common oenological parameters and the phenolic composition (mainly based on low-molecular weight compounds) were considered as predictors of astringency sensation. Several studies have shown that other wine constituents such as polysaccharides and oligosaccharides (Quijada-Morín et al., 2014; Vidal, Courcoux, et al., 2004; Vidal, Francis, Williams, et al., 2004), as well as the degree of polymerization and subunit composition of large polymers of proanthocyanidins (Chira et al., 2012; Chira et al., 2011; Preys et al., 2006; Quijada-Morín et al., 2012; Vidal et al., 2003) also modulate astringency perception. Thus, the inclusion of additional physicochemical variables as predictors in BRT models might significantly increase their predictive accuracy.

4.5. CONCLUSIONS

Boosted Regression Trees models were used to predict the astringency of commercial Uruguayan Tannat wines based on their phenolic profile. The method showed higher predictive accuracy for astringency intensity, while the prediction of the frequency of mention of astringency sub-qualities was much worse. Still, results pointed out to some compositional variables that are most likely involved in wine astringency perception.

CHAPTER 5

Dynamic characterization of red wine astringency: Case study with Uruguayan Tannat wines

ABSTRACT

Astringency is one of the most important sensory characteristics of red wine. It involves several mouthfeel sensations, which have been commonly used to describe red wines. However, the dynamics of astringency sensations have not been previously studied. In this context, the aim of the present work was to obtain a dynamic description of the astringency of red wines. Seven commercial Uruguayan Tannat wines were evaluated in triplicate by a panel of 9 trained assessors. They were asked to describe the astringency of the wines during 40 s in a Temporal Dominance of Sensations (TDS) task comprising a list of 8 terms: 'dry', 'fine emery', 'harsh', 'mouthcoating', 'puckery', 'rough', 'silky', and 'velvety'. After completing the TDS task they were asked to rate global astringency intensity using an unstructured scale. The wines significantly differed in their average global astringency intensity. Between two and three terms were significantly dominant to describe the astringency of each of the seven wines and enabled to discriminate samples with different astringency characteristics. Samples differed in the dominance of the terms and the time elapsed until they became dominant. Wines which did not significantly differ in their average astringency rating showed different dynamic astringency profiles, which evidenced that the dynamics of astringency characteristics was not related to global astringency intensity. TDS seems to be an interesting methodological choice to characterize the dynamics of wine astringency and opens new possibilities to better understand this complex sensory characteristic.

5. 1. INTRODUCTION

Astringency is one of the most important sensory characteristics that define the complexity and quality of red wine (Peynaud, 1987). It is a tactile sensation, caused by the interaction of polyphenolic compounds and salivary proteins, which leads to a decrease in the lubrication of the oral ephythelium (Lyman & Green, 1990). Astringency can be basically defined as "*the complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins*" (ASTM, 2004).

Unlike taste sensations, astringency perception is strongly time-dependant (Guinard, Pangborn, & Lewis, 1986). Perceived astringency intensity has been reported to increase after ingestion (Ishikawa & Noble, 1995), and can last up to six minutes after expectoration or swallowing (Lee & Lawless, 1991). For this reason, time-dependent methods are necessary to fully characterize the astringency of red wine (Ishikawa & Noble, 1995; Noble, 1995).

One of the most popular methods for astringency evaluation is time intensity (TI), which relies on continuous measurement of astringency intensity over a period of time (Colonna, Adams, & Noble, 2004; Lee & Vickers, 2010; Ross, Hinken, & Weller, 2007; Valentová, Skrovánková, Panovská, & Pokorný, 2001). This method provides a detailed characterization of astringency development during consumption (Cadena, Vidal, Ares, & Varela, 2014; Robichaud & Noble, 1990). However, astringency intensity is usually insufficient to characterize all the sensations that are simultaneously experienced when consuming red wine (Bajec & Pickering, 2008).

Astringency has been reported to be a complex perceptual phenomenon that involves several sensations that are simultaneously perceived (Green, 1993). A wide range of subtle sensations have been traditionally used by wine tasters and researchers to describe wine astringency, including: 'drying', 'puckering', 'rough', 'sappy', 'harsh', 'woody' and 'green' (Lawless, Corrigan, & Lee, 1994; Lee & Lawless, 1991; Peynaud, 1987). Gawel, Oberholster, and Francis (2000) proposed a Mouth-feel wheel to precisely and comprehensively characterize the astringency of red wines. This wheel includes 33 astringency descriptors grouped into 7 categories ('particulate', 'surface smoothness', 'complex', 'drying', 'dynamic', 'harsh', 'unripe'). Several authors have used this list to describe the astringent sensations of red wine (Francis et al., 2002; Gawel, Iland, & Francis, 2001; Pickering & Robert, 2006).

Astringency description has been performed using static methods, i.e. a single astringency description was obtained by averaging the sensations perceived during

consumption. Lee and Lawless (1991) presented evidence of the time dependency of the sub-qualities of astringency. These authors reported that the temporal evolution of total astringency and drying, puckering and roughing sensations differed. However, the dynamics of astringency sensations have not been fully studied yet. For this reason, dynamic methods could contribute to a more comprehensive description of the astringency of red wines during consumption.

Temporal Dominance of Sensations (TDS) is a novel temporal method which enables assessment of the temporal sensory profile of products by simultaneously evaluating all the sensations perceived (Pineau et al., 2009; Meillon, Urbano, & Schlich, 2009; Cadena et al., 2014). The method consists of presenting a list of attributes to assessors, who are asked to select which attribute is perceived as dominant at each moment of the evaluation, i.e. the attribute that catches the attention at a given time, not necessarily the most intense (Pineau et al., 2009). Along the evaluation, each time the dominant attribute changes the panelists have to select the new dominant sensation. This methodology has already been used for dynamic sensory characterization of wine (Meillon et al., 2009; Sokolowsky & Fischer, 2012), which makes it a good methodological choice for dynamic characterization of astringency.

In this context, the aim of the present work was to obtain a dynamic description of the astringency of Tannat red wines using Temporal Dominance of Sensations.

Tannat is a red cultivar of *Vitis vinifera* which has become the emblematic wine of the Uruguayan wine-making industry (Carrau, 1997). It is one of the varieties with the highest content of anthocyanins and other polyphenolic compounds (Alcalde-Eon, Boido, Carrau, Dellacassa, & Rivas-Gonzalo, 2006; Boido et al., 2011), which makes astringency one of its differential characteristics. Considering that Uruguay is one of the few places in the world where Tannat is grown, research on the viticulture and enology of this variety is still necessary to better characterize its wine quality potential.

5.2. MATERIALS AND METHODS

5.2.1. Samples

Seven commercial samples of Uruguayan varietal Tannat wine, sold in the Uruguayan market, were selected for the study and obtained directly from the wineries. Samples were selected to represent high quality Uruguayan Tannat wines with different characteristics in terms of vintage, price segment and aging in oak barrels. Wines were bottled in 750 mL bottles and were conserved under 15 °C until their analysis. A description of the wines is shown in Table 5.1.

Table 5.1. Characteristics of the Uruguayan Tannat wine samples considered in the study.

Sample	Vintage	Aged in oak barrel	Price (US$)	Ethanol (%)	Total acidity (g/L)	Total polyphenol index	Tannins (g/L)
1	2014	No	7	$12.2^{b,c}$	4.97^{b}	50.8^{a}	2.43^{a}
2	2012	No	6	12.3^{c}	4.85^{a}	66.2^{b}	3.85^{b}
3	2013	No	7	11.8^{a}	5.13^{c}	57.8^{a}	2.74^{a}
4	2012	No	14	14.4^{e}	4.97^{b}	98.7^{d}	5.06^{c}
5	2006	Yes	43	12.9^{d}	5.52^{e}	81.6^{c}	5.05^{c}
6	2012	No	17	12.9^{d}	4.96^{b}	52.3^{a}	2.88^{a}
7	2013	Yes	13	$11.9^{a,b}$	5.31^{d}	117.4^{e}	6.56^{d}

Average values within a column with different superscripts are significantly different according to Tukey's test ($p<0.05$).

Alcohol content (% v/v) and total acidity (g/L expressed in tartaric acid) were determined by FTIR-spectroscopy (FOSS WineScan™ FT 120, Denmark) accurately set in line with Vine and Wine International Office official methods. Total polyphenol index was determined according to Iland, Ewart, and Sitters (1993), by measuring the absorbance at 280 nm of 1:100 dilutions of the wines in water. For tannin concentration the method proposed by Ribéreau-Gayon and Stonestreet (1966) was used. Wine samples were diluted 1:50 in water, and 4.0 mL of the dilution were placed in two tubes with 2.0 mL of water and 6.0 mL conc. HCl. One of the tubes was heated in boiling water for 30 min and then cooled protected from light. The other tube was maintained at room temperature. In each tube 1.0 mL of ethanol was added and absorbance was measured at 550 nm. The difference of absorbance between the heated and the unheated tubes was related to tannin concentration (g/L). For both analyses, absorbance measures were performed in a Spectronic Genesys 2 UV-Visible spectrophotometer (Spectronic Instruments, Rochester, NY).

5.2.2. Trained assessor panel

The sensory panel consisted of nine assessors (7 females), ages ranging from 26 to 50 years old. Assessors were selected according to the guidelines of the ISO 8586:2012 standard (ISO, 2012).

Assessors had 18 months experience in astringency evaluation using unstructured line scales and time intensity methodology. Astringency was defined as the "tactile sensation felt in mouth and characterized by dryness and roughness". Assessors had been trained to differentiate between astringency, bitterness and sourness by evaluating reference standards (5.0 g/L alum, 1.5 g/L citric acid and 0.8 g/L caffeine solutions, respectively). The 5.0 g/L alum solution was considered as the reference for "high" astringency. The evaluation protocol required assessors to take a sip (15 mL) in

their mouth, to swish the sample gently for 10s while performing a standardized vertical tongue movement. Then, assessors were asked to spit the sample.

Assessors were also trained to describe astringency using check-all-that-apply questions involving a list of 16 terms, of which 12 were included in the Mouth-feel wheel (Gawel et al., 2000), during a total of twelve 15-minutes sessions. Six additional 15 minutes training sessions were considered to introduce assessors to the notion of Temporal Dominance of Sensations, as well as to allow familiarization with the software used for data collection.

5.2.3. Experimental procedure

The protocol for sample evaluation was based on the recommendations provided by Lee and Vickers (2010). Assessors were asked to click on the start button of the software and to simultaneously take a sip of palate cleanser in their mouth. After 20 s they had to take a sip of still mineral water. Then, after 40 s they had to take a sip of sample (15 mL) and to start the TDS task. The evaluation protocol required assessors to take a sip (15 mL) in their mouth, to swish the sample gently for 10 s while performing a standardized vertical tongue movement. Then, assessors were asked to spit the sample and to continue the evaluation for additional 30 s. The timeline for sample evaluation is shown in Fig. 5.1.

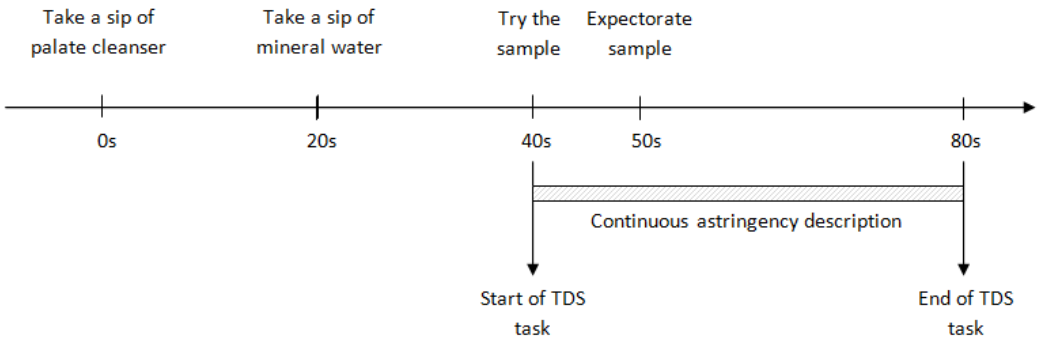

Figure 5.1. Schematic representation of the evaluation protocol.

During the TDS task, which lasted a total of 40 s, assessors had to continuously select the dominant astringency characteristic at each moment of the evaluation from a list of 8 terms. A dominant characteristic was defined as the one that caught most the attention at a given moment, not necessarily being the most intense. The eight terms included in the list were: *dry*, *fine emery*, *harsh*, *mouthcoating*, *puckery*, *rough*, *silky*, and *velvety*. To avoid list order bias, the order of the attributes was different for each

assessor, following Williams' Latin square design. The terms of the list were selected by open discussion with the panel leader, in a session in which the assessors were presented with 10 different samples of Tannat which had been previously evaluated by the panel using static methods (check-all-that-apply questions). The definition of the terms and the references used during training are shown in Table 5.2.

Table 5.2. Definition of the astringency terms considered in the Temporal Dominance of Sensations task and references used during training.

Term	Definition	Reference
Dry	Lack of lubrication in the mouth	Black tea
Fine emery	Texture associated with fine emery paper	Emery paper
Harsh	Abrasive sensation due to particulate matter brushing against the surface of the mouth	Green banana
Mouthcoating	Coating of film that adheres to mouth surfaces	Banana peel
Puckery	Reflex action of mouth surfaces when being brought together	0.5% alum solution
Rough	Irregularities or protuberances felt in mouth, not smooth	0.3% grape skin and seed extract solution
Silky	Texture associated with silk	Silk cloth
Velvety	Texture associated with velvet	Velvet cloth

After the TDS task, assessors were asked to rate the maximum astringency intensity perceived during the evaluation using a line scale, ranging from 0=nil to 10= high.

Stirred plain yogurt was considered as palate cleanser considering results from previous studies (Vidal, Antúnez, Giménez, & Ares, 2016). Three replications of each sample were evaluated by each assessor. Astringency sensations exhibit a build-up upon repeated ingestions (Bajec & Pickering, 2008), which limits the amount of samples that can be evaluated in a single session. Thus, in this study sample evaluations were divided in five sessions which were held along three weeks. Assessors evaluated four samples in the first four sessions and five samples in the last one.

Data collection was carried out using Compusense-at-hand (Compusense Inc., Guelph, Ontario, Canada). Testing took place in a sensory laboratory in standard sensory booths designed in accordance with ISO 8589 (ISO, 2007), under artificial daylight and temperature control (22ºC). Samples were presented in white plastic cups labelled with random 3-digit codes following a Williams' Latin square design.

5.2.4. Data analysis

5.2.4.1. Characterization of wine samples

Analysis of variance (ANOVA) was used to determine and assess differences among samples in ethanol content, total acidity, total polyphenol index and tannin concentration. Honestly significant differences were calculated using Tukey's test for a confidence level of 95%.

5.2.4.2. Astringency intensity

Astringency intensity was evaluated using analysis of variance considering 'sample' as a fixed source of variation, while 'session' and 'assessor' were considered as random effects. Two-way interactions were also considered in the model. Performance of the trained panel was considered adequate since interactions of assessor * session, assessor * sample and sample * session were not significant. A 5% significance level was considered in the analyses. When the effects were significant, honestly significant differences were calculated using Tukey's test for a confidence level of 95%.

5.2.4.3. Temporal Dominance of Sensations

For each assessor, the astringency characteristic regarded as dominant at each time of the evaluation was recorded. Dominance rates for each characteristic at a given time (every 1 s) were determined as the proportion of judgments (assessors x replicates) for which the given characteristic was selected as dominant. Since astringency perception is extremely subject-dependent, the time elapsed since assessors tried the wine samples and the first dominant characteristic was selected differed among assessors. To take this into account, data from each assessor was standardized according to the individual TDS evaluation duration (Lenfant, Loret, Pineau, Hartmann, & Martin, 2009).

For each characteristic, dominance rates were smoothed using a spline type polynomial with the pspline package of R language (R Core Team, 2017) and plotted against standardized time for each sample to obtain TDS curves. Significance level (P_s) was calculated using a binomial test, as recommended by Pineau et al. (2009) when dealing with few evaluations, and represented on the TDS curves.

TDS difference curves for specific comparisons of couple of samples were constructed by subtracting their TDS curves at each standardized time of the evaluation. Dominance rate differences were considered significant when they were significantly

different from 0 according to a classical test of comparison of binomial proportions (Pineau et al., 2009).

The area under the TDS curves and above significance was calculated for each attribute, to simultaneously take into account the dominance and duration of each sensation (Bruzzone, Ares, & Giménez, 2013). Principal Component Analysis (PCA) was carried out to obtain a bi-dimensional representation of samples. Average astringency intensity was considered as supplementary variable.

Correlation between the area under the TDS curves and above significance of each of the TDS terms and astringency intensity, total polyphenol index and tannin concentration was evaluated using Pearson's correlation coefficient.

All statistical analyses were performed with R (R Core Team, 2017).

5.2. RESULTS

5.3.1. Total astringency intensity

Significant differences among the wines were found in their total astringency intensity ($p<0.001$). Average astringency intensity ranged from 4.5 to 8.3, as shown in Table 5.3. Samples 4 and 7 showed the highest astringency intensity, whereas samples 1 and 3 showed the lowest intensities.

Table 5.3. Average astringency intensity of the seven Tannat wines.

Sample	Average astringency intensity (0-10)
1	4.5^{a}
2	5.8^{b}
3	$5.2^{a, b}$
4	7.7^{c}
5	6.0^{b}
6	6.0^{b}
7	8.3^{c}

Average values with different superscripts are significantly different according to Tukey's test ($p<0.05$)

As expected, wines with the highest total polyphenol indexes and tannin concentrations showed the highest astringency intensities (c.f. Tables 5.1 and 5.3).

5.3.2. Dynamic astringency characterization

Fig. 5.2 shows smoothed TDS curves for the seven Tannat wines. Significance level is represented on the curves. The astringency temporal profile of the wines was characterized by the terms *velvety*, *rough*, *fine emery*, *puckery*, *moathcoating*, *dry* and *silky*, which showed different dominance depending on the sample and the evaluation time. The term *harsh* was the only one that was not significantly dominant throughout the evaluation for all the evaluated wines.

The wines differed in the characteristics that were dominant for describing their astringency, their dominance rates, as well as in the time elapsed until the characteristics became dominant.

For example, sample 1 was characterized by the dominance of the terms *silky*, *dry* and *velvety*, whereas for sample 3 only *dry* and *velvety* were dominant (Fig. 5.2a and c). Besides, dominance rates of *velvety* tended to be higher in sample 1 than in sample 3, while dominance rates of *dry* were higher in sample 3.

Samples 1 and 3 also differed in the period of time during which the term *dry* was dominant. As shown in Fig. 5.2a and c, sample 1 was characterized by dryness dominance between 33 and 38 standardized seconds, whereas in sample 3 dominance was reached between 21 and 81 standardized seconds.

Sample 6 showed a differential dynamic profile, as shown in Fig. 5.2f. The astringency of this sample was characterized by the dominance of *fine emery* at the beginning of the evaluation, followed by the dominance of *puckery* and finally *dry*.

Meanwhile, the dynamic profile of sample 4, which showed one of the highest average astringency intensity scores, was characterized by the dominance of *rough* at the beginning of the evaluation, followed by the dominance of *dry* and *mouthcoating*, while at the end of the evaluation *dry* was the only dominant characteristic (Fig. 5.2d).

Wines which did not significantly differ in their average astringency intensity showed different temporal astringency profiles. For example, samples 4 and 7 did not significantly differ in their astringency scores and were characterized for showing the highest scores (Table 5.3). However, compared to sample 4, sample 7 had significantly higher dominance rates for *dry* in the period of time elapsed between 40 and 48 standardized seconds, whereas sample 4 had significantly higher dominance rates for *mouthcoating* at the beginning and between 30 and 44 standardized seconds of the evaluation (Fig. 5.3a).

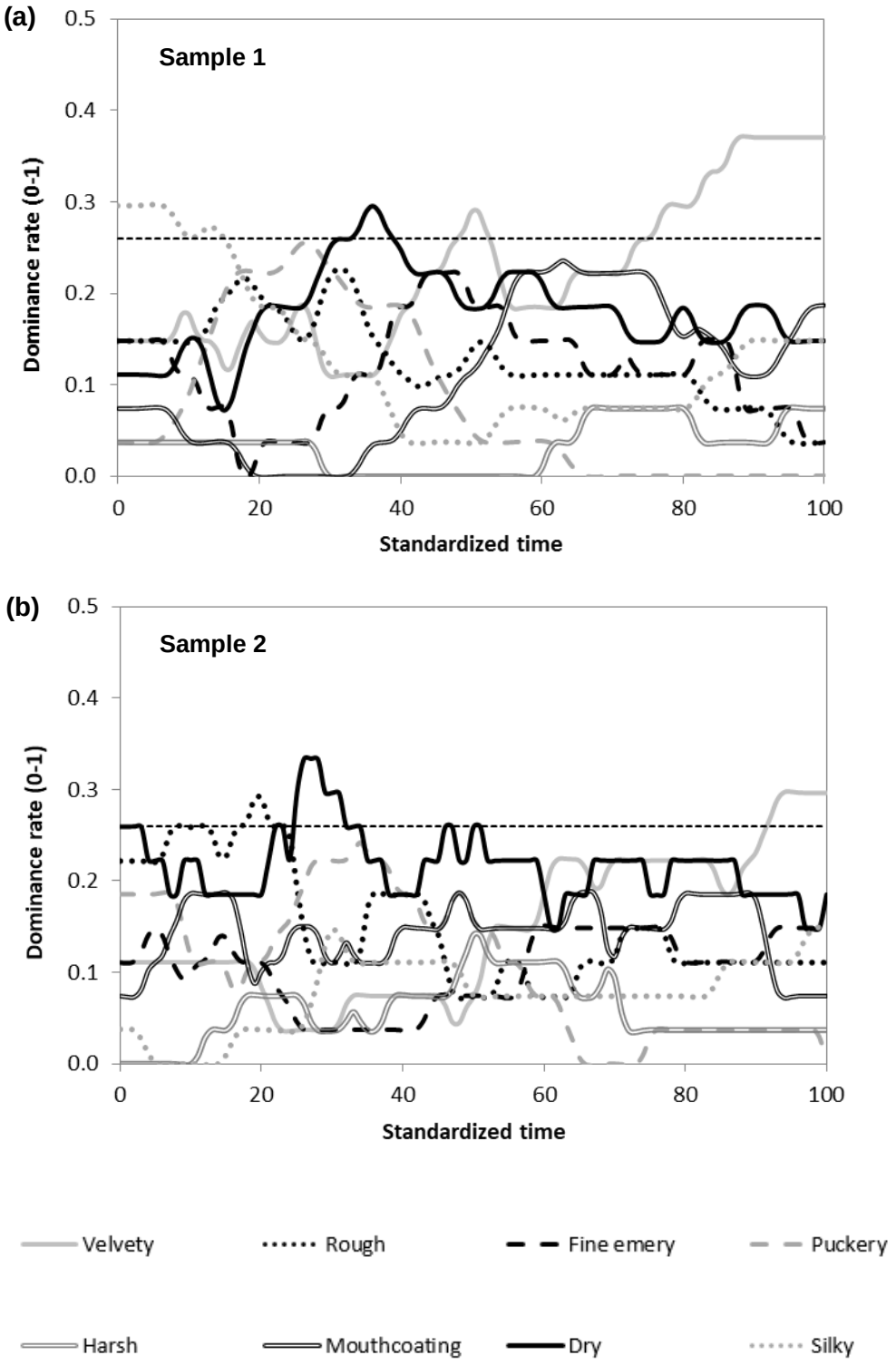

Fig. 5.2. Temporal dominance of sensations curves for astringency description of seven Tannat wines (1-7). Significance level (p=0.05) is indicated with a dotted horizontal line.

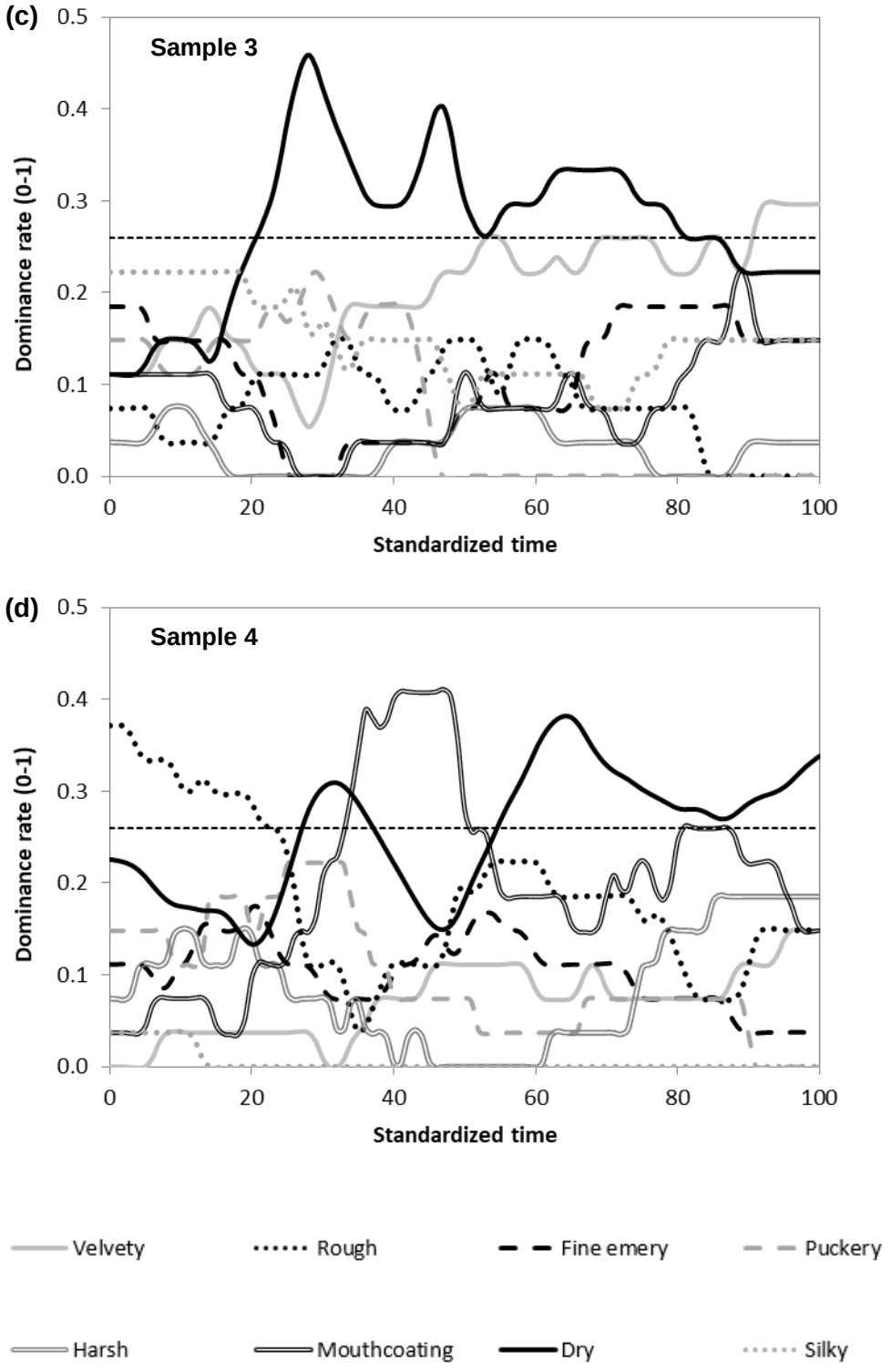

Fig. 5.2. *(Continued)* Temporal dominance of sensations curves for astringency description of seven Tannat wines (1-7). Significance level (p=0.05) is indicated with a dotted horizontal line.

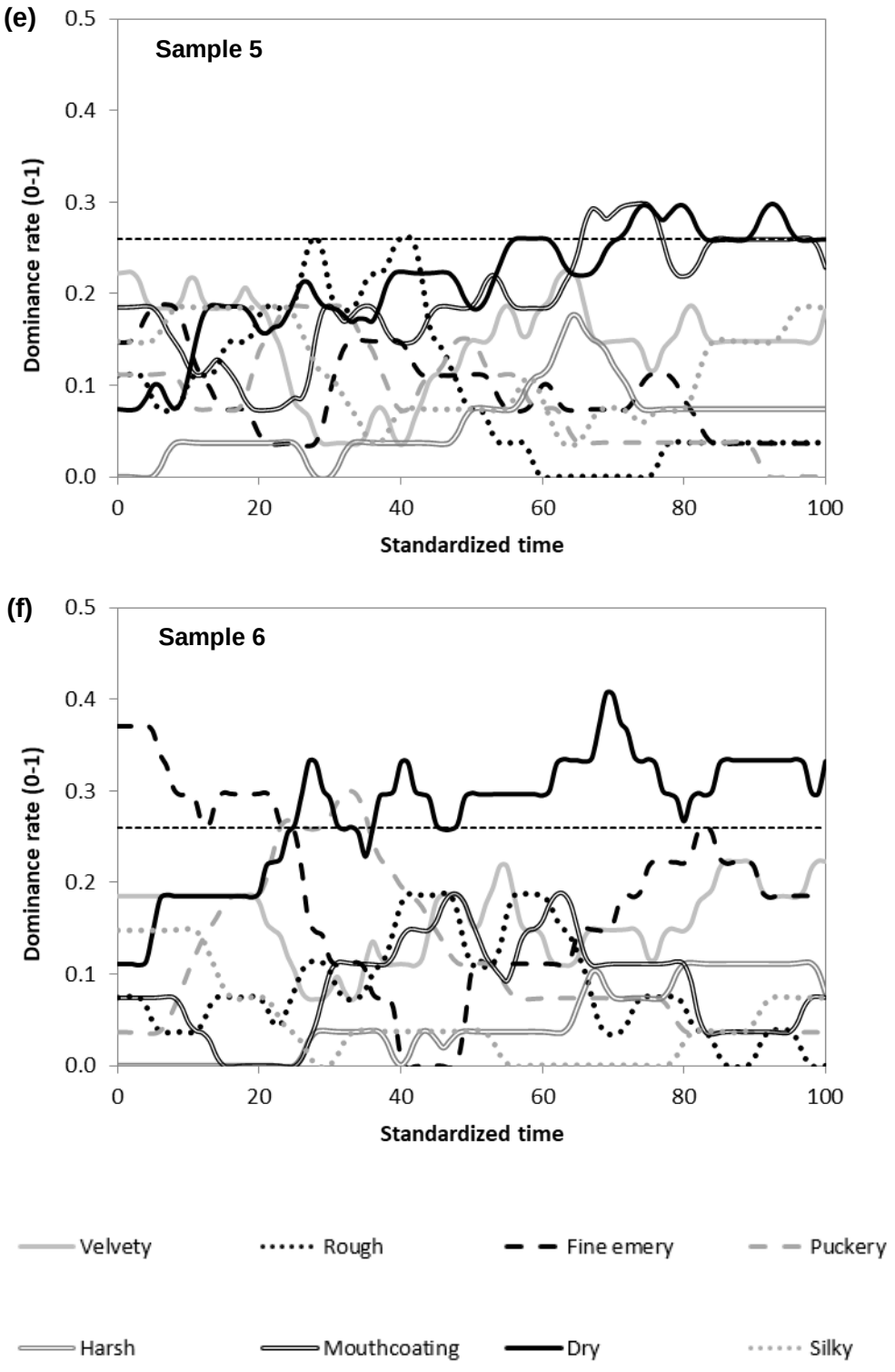

Fig. 5.2. *(Continued)* Temporal dominance of sensations curves for astringency description of seven Tannat wines (1-7). Significance level (p=0.05) is indicated with a dotted horizontal line.

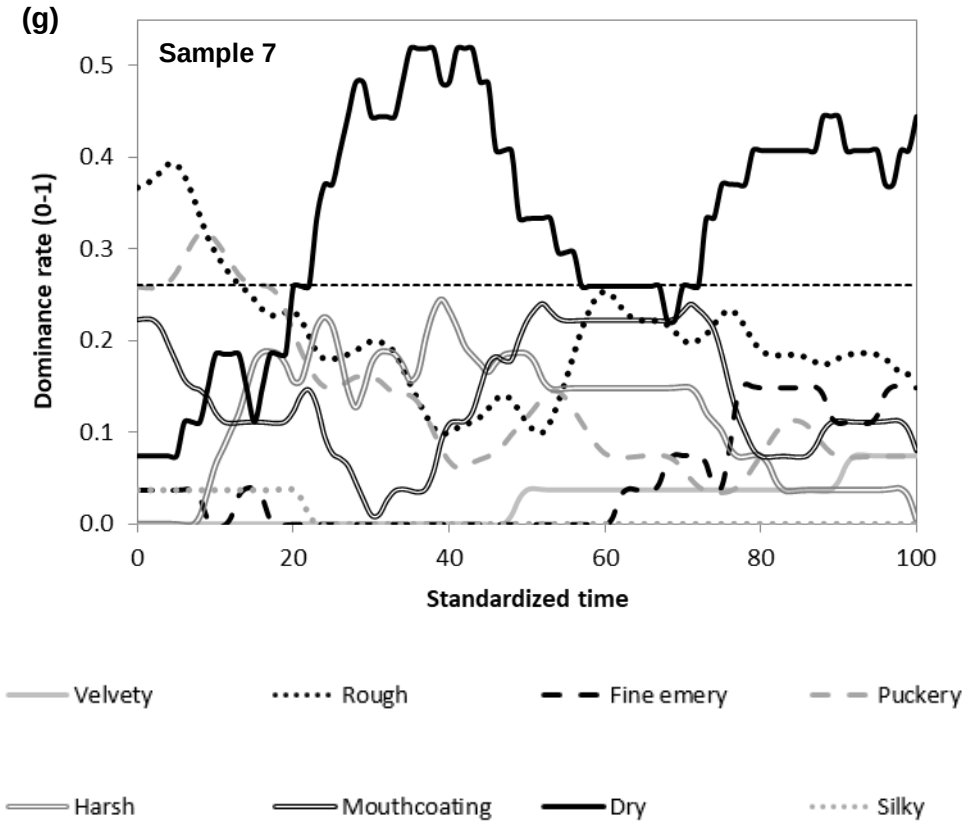

Fig. 5.2. *(Continued)* Temporal dominance of sensations curves for astringency description of seven Tannat wines (1-7). Significance level (p=0.05) is indicated with a dotted horizontal line.

Something similar was observed for samples 5 and 6 which received average intensity scores of 6.0 (Table 5.3) but differed in their temporal profile (c.f. Table 5.1 and Fig. 5.3b). Sample 6 was characterized by higher dominance rates of *fine emery* and lower dominance rate of *moathcoating* at different moments of the evaluation.

Principal Component Analysis (PCA) was used to evaluate similarities and differences among the evaluated wines when considering the significant area under the TDS curves of the eight astringency characteristics. The first and second dimensions of the PCA explained 44.32% and 29.98% of the variance of the experimental data, respectively.

As shown in Fig. 5.4b, samples were widely distributed along the first two dimensions of the PCA. Sample 6 was sorted apart from the rest, as it was located at positive values of the first two dimensions. This sample was mainly characterized by the dominance of *dry*, *puckery* and *fine emery* (c.f. Fig. 5.4a and b). Sample 1 was also located in a distinct position of the bidimensional space, being characterized by the dominance of *velvety*.

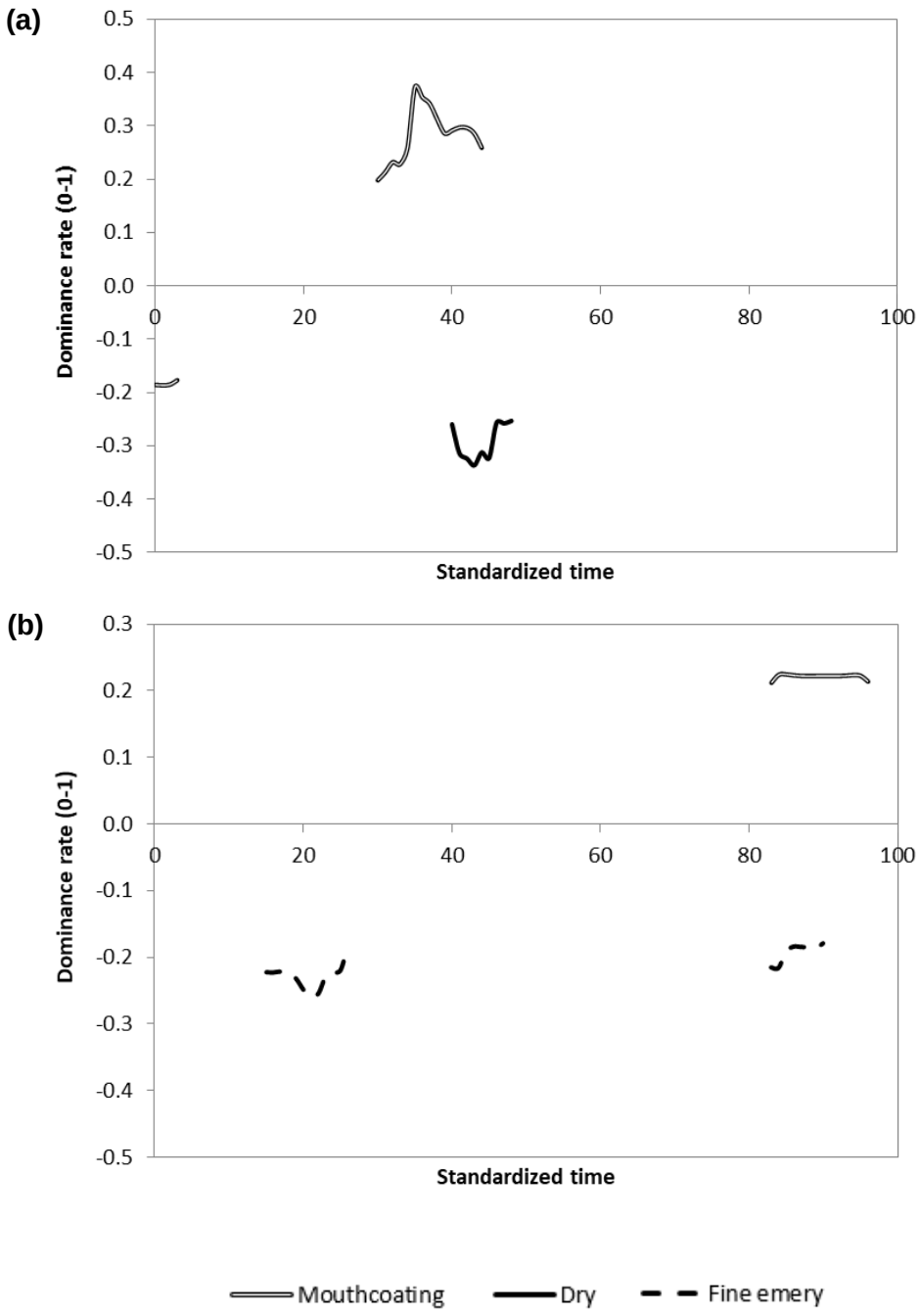

Fig. 5.3. Difference curves between temporal dominance of sensations curves of selected pairs of Tannat wine: **(a)** Sample 4 - Sample 7, **(b)** Sample 5 - Sample 6.

Samples 4, and 5 were located at negative values of the second dimension. These samples were mainly associated to the dominance of *mouthcoating*, and *rough*. Sample 7 was located at positive values of the first dimension and was mainly characterized by the dominance of *dry*. Meanwhile, samples 2 and 3 were located in an intermediate position of the map, showing intermediate astringency characteristics from those of sample 1 and samples 4, 5, and 7.

Fig. 5.4a shows that astringency was negatively correlated to velvety (r=-0.76, p=0.049), suggesting that this astringency characteristic was associated with wines with the lowest astringency intensity. The correlation between astringency intensity and the dominance of the rest of the astringency characteristics was not significant (p>0.97), suggesting that both measures provided different information for characterizing the astringency of the evaluated Tannat wines.

5.4. DISCUSSION

Astringency is a complex sensory characteristic that involves several different sensations that are simultaneously perceived (Green, 1993). Although several authors have characterized red wines in terms of astringency sub-qualities (Ferrer-Gallego, Hernández-Hierro, Rivas-Gonzalo & Escribano-Bailón, 2014; Francis et al., 2002; Gawel et al., 2001; Pickering & Robert, 2006), the dynamics of these sensations has received little attention in the literature. In this context, the present work contributes to bridging this gap in knowledge by applying Temporal Dominance of Sensations (TDS) for characterizing the astringency of seven Uruguayan Tannat wines.

As shown in Fig. 5.2, different astringency terms caught assessors' attention during different moments of the evaluation, which indicates that astringency sensations evolve during consumption. Astringency intensity has been reported to be time-dependent, increasing linearly during the first 13 to 15 seconds after ingestion and then slowly decreasing until baseline conditions are re-established (Guinard et al., 1986; Ishikawa & Noble, 1995; Ross et al., 2007; Valentová et al., 2001). Results from the present work shows that qualitative aspects of astringency are also time-dependent, i.e. different sensations are perceived at different moments during the evaluation. Similar results have been reported by Lee and Lawless (1991) when evaluating the time course of intensity of astringent sensations (astringency, mouth drying, puckery feeling, mouth roughing) during consumption of three chemical substances (alum, tannic acid and tartaric acid).

The dynamics of astringent sensations differed among the evaluated Tannat wine samples. As shown in Fig. 5.2, TDS was able to identify differences between samples in the dominance rate of the terms *velvety*, *rough*, *fine emery*, *puckery*, *moathcoating*, *silky* and *dry*. Interestingly, samples which did not differ in their average astringency ratings showed different dynamic profiles in the TDS task (c.f. Table 5.3 and Fig. 5.3).

Average astringency intensity was significantly and negatively correlated to the dominance of the term *velvety* throughout the evaluation, which occurred in wines characterized by low astringency. The dominance of the rest of the astringency terms

was not significantly correlated to average intensity, suggesting that the dominance of astringency sub-qualities cannot be accurately predicted by astringency intensity. Interestingly, although astringency was defined as the "tactile sensation felt in mouth and characterized by dryness and roughness", average measurements across time were not significantly correlated to the dominance of the terms *dry* and *rough* throughout the evaluation. In this sense, it is important to highlight that previous studies have shown that static intensity measurements of sensory characteristics do not provide the same information than dominance rates during consumption (Bruzzone et al., 2013; Labbe, Schlich, Pineau, Gilbert, & Martin, 2009; Meillon et al., 2009).

The results discussed above suggest that average astringency ratings might not be enough to fully characterize astringency. Therefore, qualitative aspects of astringency should be taken into account when characterizing red wines, as previously stressed by Gawell et al. (2000; 2001).

Samples did not only differ in the dominance of the astringency sub-qualities but also in the period of time during which they were dominant. Samples with similar dominance rates of a term differed in the moment of the evaluation during which that term was dominant, as exemplified in Fig. 5.2a and c for the term *dry* in the case of samples 1 and 3. This suggests that static evaluations of astringent sub-qualities may not capture subtle differences among samples in the moment at which different sensations are experienced during consumption.

Some relationships between the dynamic astringency profile of the evaluated Tannat wines and their characteristics were identified. Firstly, the cheapest samples (1-3) tended to be characterized by their low astringency intensity and the dominance of the term *velvety* throughout the evaluation (Fig. 5.4). This suggests that high-quality Tannat wines do not tend to be characterized by the dominance of the term 'velvety'. Besides, the two samples which had been aged in oak barrel were characterized by the dominance of the terms *mouthcoating* and *rough*. Further research is necessary to characterize the dynamics of astringency sensations of commercial Uruguayan Tannat wines and to better understand how they are affected by production variables. Besides, comparison of the dynamics of astringency sensations also deserves exploration.

Although results from the present study allowed identifying differences among wine samples in the dynamics of astringency sensations, only two or three attributes were significantly dominant for each sample, and the dominance rates were in general low (Fig. 5.2). This could be due to the nature of TDS methodology, in which assessors must focus exclusively on the dominant attribute. As astringency involves several

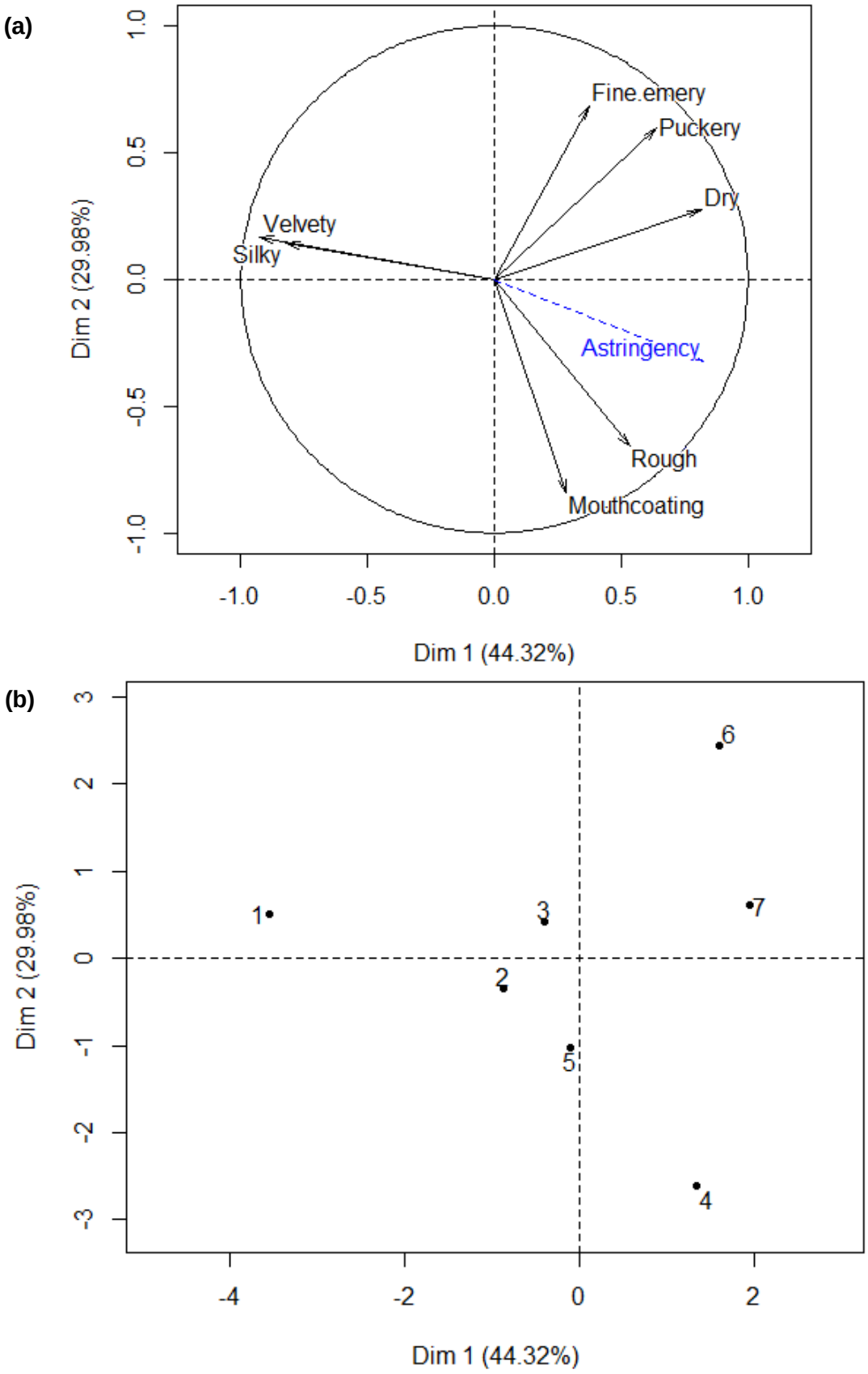

Fig. 5.4. Results from the principal component analysis performed on the areas below the Temporal Dominance of Sensations curves and above significance for the seven Tannat wines: **(a)** representation of the variables, **(b)** representation of the samples. Astringency intensity was considered as supplementary variable.

characteristics that can be simultaneously perceived, some of this characteristics may not be captured by TDS method (Ares et al., 2015). In this sense, the recently introduced Temporal-Check-All-That-Apply (TCATA) method (Castura, Antúnez, Giménez, & Ares, 2016) may be an alternative to obtain a more detailed characterization of the dynamics of red wine astringency.

In summary, results from the present work suggest that the dynamics of astringency sensations of red wine during consumption deserve further research, particularly in the case of Tannat wine, which is characterized by its intense astringency (Carrau, 1997). One of the main challenging areas of research is further understanding the relationship between the dynamics of astringency sub-qualities and the polyphenolic profile of wine. Considering that different mechanisms are involved in the interaction between polyphenols and salivary proteins (Bajec & Pickering, 2008), it could be hypothesized that the dynamics of the dominance of astringent sensations may be related to specific polyphenolic structures. This type of study could extend the work of other authors who have found relationships between specific compounds and astringent sub-qualities, evaluated using static methods which average sensations perceived during consumption (Gawell, Francis, & Waters, 2007; Vidal et al., 2003; Vidal, Francis, Williams, et al., 2004). Another interesting avenue for further research is the relationship between the dynamics of astringency sensations and consumers' perceived quality of different wine varieties.

5.5. CONCLUSIONS

The dynamics of wine astringency were characterized using Temporal Dominance of Sensations (TDS). This methodology enabled to identify significant differences of Tannat wines, which were not identified using static methods. Astringency intensity was not significantly correlated to the dominance of astringency terms and wines which did not significantly differ in their average astringency intensity showed different dynamic astringency profiles. These results suggest that dynamics of astringency characteristics seems to be not related to global astringency intensity, suggesting the need to further investigate the dynamics of astringency sensations. In this sense, it seems that the use of TDS, or other temporal methodologies such as TCATA, opens new possibilities to better understand this complex sensory characteristic.

CHAPTER 6

Astringency evaluation of Tannat wines: Comparison of assessments from trained assessors and experts

ABSTRACT

Astringency is one of the differential characteristics of Tannat wine, the emblematic wine of the Uruguayan wine-making industry. The aim of this study was twofold: first, to compare wine astringency characterization of experts and trained assessors, and second, to identify which astringency characteristics influence experts' perception of astringency quality. Six commercial Uruguayan Tannat wines were evaluated in triplicate by a trained assessors panel (n=9). Assessors rated the global astringency intensity using a time-intensity task, and described astringency sub-qualities using a Check-All-That-Apply (CATA) question composed of 16 terms. Samples were also assessed by 30 experts who rated astringency intensity and quality using 9-point structured scales. In addition, experts described astringency sub-qualities of samples and of their ideal high quality Tannat wine using a CATA question. Significant differences among samples were found in astringency intensity for both panels and expert's perceived quality. Agreement between trained assessors and experts in astringency intensity evaluations was observed, but differences in their description of astringency sub-qualities were found. Astringency quality as perceived by experts was not related only to astringency global intensity; astringency sub-qualities *complex* and *velvety* were the main positive drivers of astringency quality, while *puckery*, *sand paper* and *irritant* significantly lowered quality scores.

PRACTICAL APPLICATIONS

Wine astringency characterization is a very complex task that should ideally be performed by a group of trained assessors following standardized protocols. However, most wineries still rely on the judgment of wine experts for decision making. Results from the present work suggest that although trained assessors and experts evaluated wine astringency similarly, their description of astringency sub-qualities strongly differed.

6.1. INTRODUCTION

Red wine is a product mainly consumed for hedonic pleasure (Charters & Pettigrew, 2007), appreciated for the wide spectrum of sensations that consumers' experience in a drinking situation (Jackson, 2014). This suggests that the sensory characteristics of wine, such as colour, aroma, and mouthfeel sensations, are closely linked to wine quality perception, which is a complex and multidimensional construct hard to define (Charters & Pettigrew, 2007; Hopfer & Heymann, 2014).

Astringency, one of the most complex mouthfeel sensations, has been identified as a major contributor to the perceived quality, complexity and persistence of red wine (Cheynier & Sarni-Manchado, 2010; Gawel, 1998; Peynaud, 1987). The relevance of astringency has been widely acknowledged, and although it has been studied since decades ago (Arnold, Noble, & Singleton, 1980; Bate-Smith, 1954; Joslyn & Goldstein, 1964), it continues to be a relevant and challenging area of research (Laguna, Bartolomé, & Moreno-Arribas, 2017; Ma et al., 2014; Scollary, Pásti, Kállay, Blackman, & Clark, 2012).

Astringency can be defined as a complex set of sensations related to drying, roughing and puckering of the mouth epithelium (ASTM, 2004). Although the mechanisms of astringency perception are not fully understood (Gibbins & Carpenter, 2013), it is widely accepted that red wine phenolic compounds and their interaction with salivary proteins are key determinants of astringency perception (Ma et al., 2014; McRae & Kennedy, 2011). Thus, several instrumental methods have been developed in order to objectively estimate wine astringency (Laguna, Bartolomé, et al., 2017), but up to now none of them is able to predict all aspects of astringency perception. For that reason, sensory analysis continues to be a useful tool, and the most direct method to evaluate wine astringency (Cheynier & Sarni-Manchado, 2010; Ma et al., 2014).

There are several challenges to overcome for the accurate sensory characterization of red wine astringency. First, it is a strongly time-dependent attribute (Guinard, Pangborn, & Lewis, 1986; Ishikawa & Noble, 1995) which exhibits buildup effect upon repeated ingestions (Colonna, Adams, & Noble, 2004; Courregelongue, Schlich, & Noble, 1999; Lee & Vickers, 2008, 2010; Noble, 2002; Ross, Hinken, & Weller, 2007). Second, the evaluation of total astringency intensity is usually insufficient to characterize all the subtle sensations that can be concurrently experienced when consuming red wine (Bajec & Pickering, 2008; Gawel, Iland, & Francis, 2001; Gawel, Oberholster, & Francis, 2000; Vidal et al., 2017).

Wine sensory analysis should ideally be carried out by a trained sensory panel, in order to obtain accurate and reliable measurements. However, most wineries still rely on the judgment of wine experts for decision making (Lesschaeve & Noble, 2010). Experts' assessments of the sensory characteristics and quality of wine are also a valuable source of information for consumers' purchase decisions. Average wine consumers frequently rely on wine experts' recommendations and other trusted sources to obtain guidance for purchasing wine (Gawel & Godden, 2008; Hopfer & Heymann, 2014).

Experts are known to have better abilities to describe and evaluate the sensory characteristics of complex products such as wine (Gawel, 1997; Lawless, 1984), and to act more analytically than consumers when assessing wine quality (D'Alessandro & Pecotich, 2013; Hopfer & Heymann, 2014). In addition, experts are usually able to identify wine defects and evaluate if the wine typifies the variety, region or style it is aimed to (Gawel & Godden, 2008). However, although experts have an aligned conceptualization of which sensory characteristics contribute positively or negatively to wine quality (Sáenz-Navajas et al., 2016), high variability in wine quality assessments by experts have been reported (Gawel & Godden, 2008; Hodgson, 2008). Given the relevance of wine experts' assessments for marketability, it is critical to evaluate their ability to objectively measure wine sensory characteristics and to understand the sensory drivers of their quality perception (Sáenz-Navajas et al., 2016).

Astringency intensity has been shown to influence wine quality assessments, being the relationship dependent on the type of wine and the level of expertise of the assessors (Lattey, Bramley, & Francis, 2010; Sáenz-Navajas et al., 2015; Sáenz-Navaja*s,* Ballester, Pêcher, Peyron, & Valentin, 2013; Varela & Gámbaro, 2006). For example, Sáenz-Navajas et al. (2015) found a negative correlation between astringency intensity assessed by a trained panel and consumers' quality scores of twelve commercial Spanish wines from different grape varieties. In a different study involving Spanish and French commercial wines, astringency intensity was positively correlated with quality assessments by experts but negatively correlated with quality assessments by consumers (Sáenz-Navajas et al., 2013). On the contrary, Varela and Gámbaro (2006) reported that Tannat wine astringency (assessed by a trained panel using descriptive analysis) positively contributed to wine's quality as perceived by a group of regular fine wine consumers.

Tannat is the most extensively cultivated red *Vitis vinífera* in Uruguay (INAVI, 2017) and is established as the national emblematic wine variety (Carrau, 1997). Red wines elaborated with Tannat grapes have a very distinct phenolic composition (Alcalde-

Eon, Boido, Carrau, Dellacassa, & Rivas-Gonzalo, 2006; Boido et al., 2011; González-Neves, Gil, Favre, & Ferrer, 2012; González-Neves, Gómez-Cordovés, & Barreiro, 2001; Lloret et al., 2003), which confers them a great tipicity characterized by intense colour and high astringency compared to other red varieties (Blanchard, 1999; Boidron et al., 1995). As the name suggest, Tannat wines are very tannic, which makes astringency one of its differential sensory attributes. Therefore, it is expected that both low and extremely high global astringency intensity would be perceived as indicators of low quality Tannat wines.

The specific astringency sub-qualities perceived in red wine also contribute to astringency quality. Some astringency sub-qualities are regarded as more desirable, such as *round/smooth* (Sáenz-Navajas et al., 2016) or *velvety* (Ferrer-Gallego, Hernández-Hierro, Rivas-Gonzalo, & Escribano-Bailón, 2014), while others, such as *aggressive* or *sand paper*, are usually related to a bad quality assessment, even in the case of wines that are expected to be highly astringent. In the specific case of Tannat wine, the identification of the astringency characteristics that positively contribute to quality perception could contribute to achieve a better promotion and communication of the characteristics of this variety to both national and international wine consumers. Recently, the astringency of 40 commercial Tannat wines was characterized by a trained sensory panel, and different styles of Tannat wines were identified (Vidal et al., 2017). However, the relationship of these characteristics to astringency quality perception was not considered.

In this context, the aim of this study was twofold: first, to compare wine astringency characterization of experts and trained assessors, and second, to identify which astringency characteristics influence experts' perception of the quality of Tannat wine astringency.

6.2. MATERIALS AND METHODS

6.2.1. Wine samples

Six commercial samples of Uruguayan varietal Tannat wine, available in the Uruguayan marketplace, were obtained directly from the wineries. All samples were high quality Uruguayan Tannat wines with different characteristics in terms of vintage, price segment and aging in oak barrels. Wines were bottled in 750 mL bottles and were kept at 12°C until their analysis. The six samples were selected to represent different styles of Tannat wine, according to their astringency intensity and sub-qualities, as determined by a trained sensory panel in a previous study (Vidal et al., 2017). Samples 1 and 2 were characterized by their low astringency intensity and for eliciting smooth texture attributes,

samples 3-5 were among samples with intermediate astringency intensity, while sample 6 was included in the group of samples which showed the highest astringency intensity and were described as aggressive (Vidal et al., 2017).

The wines were characterized using a series of basic physicochemical parameters. Alcohol content (% v/v), total acidity (g/L expressed in tartaric acid) and pH were determined by FTIR-spectroscopy (FOSS WineScan™ FT 120, Denmark) accurately set in line with Vine and Wine International Office official methods. Total polyphenol index was determined according to Iland, Ewart, and Sitters (1993) and tannin concentration following the method proposed by Ribéreau-Gayon and Stonestreet (1966). Absorbance measures were performed in a Spectronic Genesys 2 UV-Visible spectrophotometer (Spectronic Instruments, Rochester, NY). All samples were analysed in duplicate. A description of the wines included in the research is shown in Table 6.1.

Table 6.1. Characteristics of the Uruguayan Tannat wine samples considered in the study.

Sample	Vintage	Aged in oak barrel	Price (US$)	Ethanol (%)	Total acidity (g/L)*	Total polyphenol index	Tannins (mg/L)
1	2014	No	6	12.2	4.97	50.8	2.43
2	2013	No	6	11.8	5.13	57.8	2.74
3	2013	No	5	13.4	4.47	64.5	3.26
4	2012	No	6	12.3	4.85	66.2	3.85
5	2006	Yes	40	12.9	5.52	81.6	5.05
6	2012	No	14	14.4	4.97	98.7	5.06

***** Total acidity is expressed in g/L of tartaric acid.

6.2.2. Astringency evaluation by a panel of trained assessors

A sensory panel of nine assessors (7 females), ages ranging from 26 to 50 years old participated in the study. Assessors had been selected according to the guidelines of the ISO 8586:2012 standard (ISO, 2012). They had been trained to evaluate astringency intensity using time-intensity methodology and to describe astringency sub-qualities using check-all-that-apply questions, as reported elsewhere (Vidal et al., 2017).

Assessors evaluated astringency intensity of the wine samples following a standardized protocol. At the beginning of the evaluation, they had to click on the start button of the software and simultaneously take a sip of stirred plain yogurt as palate cleanser (Vidal, Antúnez, Giménez, & Ares, 2016). After 20 s, they had to take a sip of still mineral water. Then, after 40 s they had to take a sip of a sample (15 mL) and to start the time-intensity task. Ten seconds later, assessors were asked to spit the sample and to continue the evaluation for additional 30 s. A horizontal line scale anchored with the terms “low” and “high” was shown on the screen, and assessors used their finger to

move the cursor along the line according to the intensity of perception. Intensity data were collected every second during the evaluation period. The total duration of the time-intensity task was 40 s. Once it ended, assessors were presented with a CATA question involving a list of 16 astringency sub-qualities: *dry*, *silky*, *fine emery*, *suede*, *rough*, *aggressive*, *sand paper*, *mouthcoating*, *velvety*, *puckery*, *harsh*, *abrasive*, *hard*, *coarse grain*, *irritant* and *complex*. Assessors were asked to select all the terms that applied to describe the astringency-related sensations they felt during sample evaluation. The terms were presented in balanced order between assessors, following a Williams' Latin square design, as suggested by Meyners and Castura (2016). Once assessors completed the evaluation protocol for one of the samples, they immediately started the evaluation of the next sample. Samples were evaluated in duplicate, and 4 samples were assessed per session.

6.2.3. Astringency evaluation by a panel of wine experts

A group of 30 wine professionals (15 females, ages ranging from 19 to 54 years old) participated in the study. Participants were sommeliers, oenologists, oenology students or professors, with 1 to 20 years of wine tasting experience (mean=5.2 years). They all signed an informed consent agreement and received a small gift for their participation.

Experts were instructed to evaluate the wine samples focusing only on the perceived astringency. In order to minimize the bias in expert responses, sample evaluation was done as close as possible to the conditions they are familiar with. Thus, the evaluation protocol proposed was different from the one used by trained assessors. Samples (30 mL) were presented at room temperature (20 °C) in clear 190 mL standard glasses (ISO, 1977). Experts were asked to take a sip of the sample and to swish the sample gently for 10 s while performing up and down tongue movement without pressing the tongue against any mouth surface. After the 10 s they had to expectorate the sample and rate its total astringency intensity using a 9-point structured scale anchored with the terms "low" and "high". Then, experts were presented with the same CATA question with 16 astringency sub-qualities used by the trained assessors panel, and were asked to select all the terms that applied to describe the sample's astringency. The terms were presented in balanced order between assessors, following a Williams' Latin square design. Finally, they were asked to assess how was the astringency of the sample they evaluated with respect to their expectations of the astringency of a high quality Tannat wine. To answer the question, they were provided with a 9-point structured scale anchored with the terms "very different" and "exactly the same". The question was formulated in this way to prevent experts from giving an overall quality score, as they

were asked to focus solely on astringency quality. Still mineral water was provided for palate cleansing. No instructions were given to experts regarding waiting time between samples. Replications were not considered for experts' evaluations.

Once assessors completed the evaluation protocol for the six samples, they were asked to rate the total astringency intensity they expected in a high quality Tannat wine. Additionally, they had to respond the same CATA question to indicate which astringency sub-qualities should a high quality Tannat wine have.

All testing took place in standard sensory booths in a sensory laboratory that was designed in accordance with ISO 8589 (ISO, 2007), under artificial daylight and temperature control (22°C). Samples were coded using 3-digit random numbers and presented following a Williams' Latin square design. Data were collected on tablets using Compusense Cloud (Compusense Inc., Guelph, Ontario, Canada).

6.2.4. Data analysis

All statistical analyses were performed with R (R Core Team, 2017). Functions from `stats`, `FactoMineR` (Lê, Josse, & Husson, 2008), and `lmerTest` (Kuznetsova, Brockhoff, & Christensen, 2017) packages were used.

6.2.4.1. Astringency intensity and astringency quality

Astringency intensity data for trained assessors corresponded to time-intensity curves for each assessor, sample and replicate. For each curve, 4 parameters were extracted: maximum intensity (*Imax*: maximum observed intensity during the evaluation), intensity at the end (*Iend*: intensity observed at the end of the evaluation), starting time (*tstart*: first time at which intensity is greater than zero) and area under the curve (*auc*). The extracted parameters were analysed by Analysis of Variance (ANOVA) considering linear mixed models with sample as fixed effect and assessor, replicate and all the two-way interactions as random effects.

Astringency intensity and quality ratings from the expert panel were also analysed with linear mixed models and ANOVA, considering sample as fixed effect and assessor as random. When significant differences were identified (α=0.05), Honestly Significant Difference test was used for post-hoc comparisons of means between wine samples.

The correlations between astringency intensity and astringency quality rated by experts, and between experts' quality scores and the four time-intensity parameters obtained from the trained assessors' evaluations were assessed using Pearson's correlation coefficient. Correlations of these variables with total polyphenol index and tannin concentration of the samples were also assessed.

6.2.4.2. CATA questions

Regarding CATA data, for each panel the frequency of use of each astringency sub-quality to describe each sample's astringency was determined by counting the number of judgments (assessor x replicate) in which the term was checked. Fisher's exact test (Fisher, 1954) was used to determine significant differences between panels in the total number of terms used to describe whole sample set, and differences in the frequency of use of each astringency sub-quality. Generalized linear models were carried out to identify significant differences among samples in the frequency of use of each of the terms. Analysis of deviance of each model was done using chi-squared test. When the sample effect was significant (α=0.05) pairwise comparisons were carried out using the sign test.

6.2.4.3. Penalty-lift analysis

Penalty-lift analysis was used to identify the main drivers of astringency quality perceived by experts. Average quality scores were calculated considering participants and samples for which the attribute was not selected and considering those for which the attribute was selected (Meyners, Castura, & Carr, 2013). Differences between the two average quality values were calculated and their significance was evaluated through unpaired t-test assuming equal variance.

6.3. RESULTS

6.3.1. Astringency intensity

For both panels, significant differences among samples were found in their astringency intensity scores. Table 6.2 shows the average time-intensity parameters extracted from the time-intensity data from trained assessors, as well as the average intensity scored provided by the expert panel.

Table 6.2. Average time-intensity parameters (Imax, Iend, tstart, auc) for trained assessors, and average astringency intensity and astringency quality evaluated by experts, for the six Tannat wine samples.

Sample	Trained assessors				Experts	
	Imax	Iend	Tstart	Auc	Astringency intensity	Astringency quality
1	5.1^{b}	1.9^{b}	$46.6^{a,b}$	110.7^{b}	3.7^{e}	$4.4^{b,c}$
2	$5.7^{a,b}$	$2.7^{a,b}$	$47.7^{a,b}$	$125.9^{a,b}$	$3.9^{e,d}$	4.0^{c}
3	$5.7^{a,b}$	2.2^{b}	$46.1^{a,b}$	$126.6^{a,b}$	$5.2^{b,c}$	$5.2^{a,b}$
4	$5.9^{a,b}$	$2.3^{a,b}$	$47.5^{a,b}$	$127.2^{a,b}$	$4.6^{c,d}$	$4.9^{a,b,c}$
5	6.4^{a}	$2.6^{a,b}$	46.0^{b}	$146.3^{a,b}$	$5.5^{a,b}$	5.6^{a}
6	7.0^{a}	3.9^{a}	49.1^{a}	161.0^{a}	6.2^{a}	$5.3^{a,b}$

Imax: maximum intensity; Iend: intensity at the end; tstart: starting time; auc: area under the curve.
Average values within a column with different superscripts are significantly different according to Tukey's test at a significance level of 5%.

Sample discrimination according to astringency intensity was larger according to experts' scores than to the trained assessors' parameters from time-intensity. However, both panels agreed on the samples with the highest and lowest astringency intensities. Samples 1 and 2 were perceived by the experts as the samples with lowest astringency intensity, scoring on average 3.7 and 3.9 on a 9 point scale. These samples were also ranked with the lowest Imax for trained assessors, although only Sample 1 was significantly different from samples 5 and 6. On the contrary, Sample 6 was given the highest intensity ratings by both panels.

6.3.2. Description of astringency sub-qualities

Fig. 6.1 shows the total frequency of use of the sixteen astringency sub-qualities to describe the six samples of Tannat wine, for both panels. According to Fisher's exact test, no significant differences were found between panels in the total average use of the terms included in the CATA question (16.1% vs. 14.5%, p=0.117). However, significant differences in the use of individual terms were found. On average, experts used more frequently the term *dry* to describe the astringency of the wine samples (p=0.017) than trained assessors, while the opposite was found for the astringency sub-qualities *fine emery* (p<0.0001), *suede* (p<0.0001) and *velvety* (p=0.033) (Fig. 6.1).

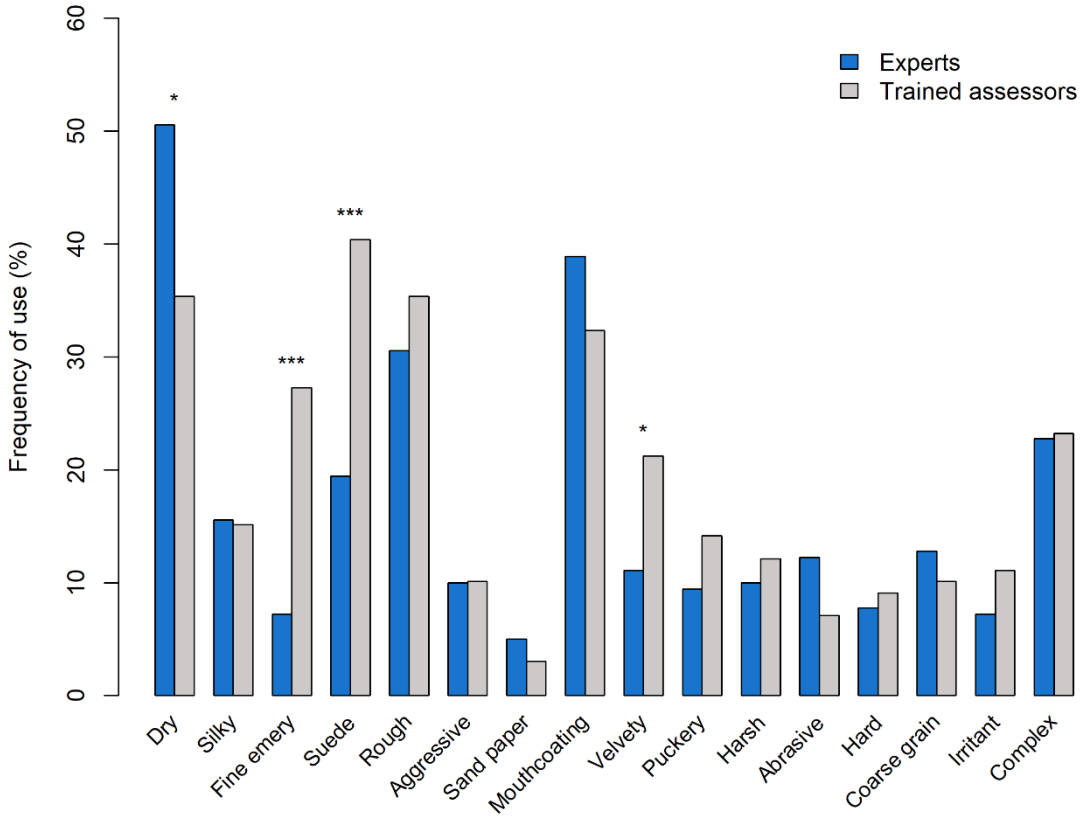

Fig. 6.1. Average frequency of use (%) of the sixteen astringency sub-qualities included in the check-all-that-apply question for trained assessors and experts. The frequency of use of sub-qualities marked with asterisks were significantly different for both panels according to Fisher's exact test (*, p<0.05; ***, p<0.001).

Table 6.3. Frequency of use (expressed in percentage) of each term included in the check-all-that-apply question to describe astringency sub-qualities of the six Tannat wine samples for trained assessors and experts. The p-values correspond to the sample effect of the Analysis of deviance.

Term	Sample						p-value*
	1	2	3	4	5	6	
Trained assessors							
Dry	38.9	37.5	18.8	35.3	38.9	42.9	0.746
Silky	$27.8^{a,b}$	43.8^{a}	$6.3^{b,c}$	0^{c}	$11.1^{b,c}$	0^{c}	**0.001**
Fine emery	27.8	12.5	37.5	41.2	27.8	14.3	0.333
Suede	$44.4^{a,b}$	62.5^{a}	$56.3^{a,b}$	$23.5^{b,c}$	$44.4^{a,b}$	7.1^{c}	**0.009**
Rough	22.2	25	37.5	58.8	27.8	42.9	0.224
Aggressive	$11.1^{a,b}$	$12.5^{a,b}$	0^{b}	5.9^{b}	0^{b}	35.7^{a}	**0.014**
Sand paper	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	21.4^{a}	**0.030**
Mouthcoating	22.2	31.3	31.3	23.5	44.4	42.9	0.642
Velvety	16.7	43.8	25	5.9	27.8	7.1	0.073
Puckery	$16.7^{a,b}$	$12.5^{a,b}$	0^{b}	5.9^{b}	11.1^{b}	42.9^{a}	**0.022**
Harsh	5.6^{b}	$6.3^{a,b}$	0^{b}	$17.6^{a,b}$	$11.1^{a,b}$	35.7^{a}	**0.046**
Abrasive	$5.6^{a,b}$	0^{b}	0^{b}	$11.8^{a,b}$	0^{b}	28.6^{a}	**0.017**
Hard	$5.6^{a,b}$	0^{b}	$6.3^{a,b}$	$17.6^{a,b}$	0^{b}	28.6^{a}	**0.028**
Coarse grain	0	6.3	6.3	11.8	16.7	21.4	0.239
Irritant	22.2	0	12.5	11.8	5.6	14.3	0.268
Complex	0^{c}	$12.5^{b,c}$	$18.8^{b,c}$	$17.6^{b,c}$	61.1^{a}	$28.6^{a,b}$	**<0.001**
Experts							
Dry	43.3	60.0	53.3	50.0	53.3	43.3	0.773
Silky	23.3^{a}	13.3^{a}	26.7^{a}	16.7^{a}	13.3^{a}	0^{b}	**0.015**
Fine emery	3.3	13.3	6.7	3.3	3.3	13.3	0.387
Suede	30.0	16.7	20.0	13.3	23.3	13.3	0.562
Rough	16.7	23.3	33.3	36.7	30.0	43.3	0.250
Aggressive	6.7	13.3	6.7	6.7	13.3	13.3	0.812
Sand paper	3.3	6.7	3.3	3.3	3.3	10.0	0.822
Mouthcoating	26.7	23.3	50.0	46.7	43.3	43.3	0.155
Velvety	13.3	6.7	16.7	16.7	13.3	0.0	0.084
Puckery	10.0	6.7	6.7	6.7	6.7	20.0	0.513
Harsh	3.3	10.0	13.3	6.7	13.3	13.3	0.642
Abrasive	3.3	10.0	13.3	16.7	13.3	16.7	0.519
Hard	3.3	3.3	6.7	10.0	10.0	13.3	0.610
Coarse grain	3.3^{b}	3.3^{b}	$13.3^{a,b}$	10.0^{b}	$13.3^{a,b}$	33.3^{a}	**<0.001**
Irritant	10.0	10.0	6.7	6.7	3.3	6.7	0.913
Complex	$16.7^{b,c}$	6.7^{c}	10.0^{c}	$20.0^{b,c}$	46.7^{a}	$36.7^{a,b}$	**<0.001**

* Values in bold are significant at a significance level of 5%.
For attributes that significantly differed among samples, frequencies of use (%) with different superscripts are significantly different according to the sign test at a significance level of 5%.

Table 6.3 shows the frequency of use of the different terms to describe the astringency of Tannat wine samples for both panels, as well as results from the analysis of deviance for the sample effect. Trained assessors showed higher discriminative ability than experts when describing the astringency of Tannat wine. Significant differences among samples were found for 9 out of 16 terms, while according to the experts' data samples differed significantly only in 3 terms (coarse grain, silky and complex). Astringency sub-qualities dry, rough and mouthcoating were relevant to describe most samples, and no significant differences among samples were established neither for experts nor for trained assessors.

As shown in Table 6.3, astringency description using CATA questions was different for experts and trained assessors. For trained assessors, the astringency of samples 1 and 2 was mainly described with sub-qualities related to soft textures, such as *silky* (28% and 44%) and *suede* (44% and 63%), with *velvety* being also very frequently used to describe sample 2 (44%). For experts, the astringency of sample 1 was also related to such soft textures (*silky* 23% and *suede* 30%), but for sample 2 only *dry* (60%), *rough* (23%) and *mouthcoating* (23%) were used by more than 20% of experts. Sample 3 showed a more complex profile when assessed by trained assessors as its astringency was described not only by soft texture attributes (*suede* 56% and *velvety* 25%) but also by *fine emery* (38%) and rough (38%). Although there were no significant differences among samples in the attribute *dry*, the frequency of use of that sub-quality to describe the astringency of sample 3 was less than 20%. On the contrary, when experts evaluated sample 3, they used the attribute *dry* as frequently as for other samples. Other relevant sub-qualities to describe its astringency were *mouthcoating* (50%), *rough* (33%), *silky* (27%) and *suede* (20%). The astringency of sample 4 was described mainly as *rough* (59%) and *fine emery* (41%) by trained assessors, while for experts *mouthcoating* (47%) and *rough* (37%) were the most frequently used attributes. For both panels, sample 5 showed the highest frequency of use of *complex* (61% for trained assessors and 47% for experts), although it did not significantly differ from sample 6. *Rough* and *mouthcoating* were also frequently used by both panels to describe sample 5's astringency, but for trained assessors a larger number of descriptors were relevant: *suede* (44%), *fine emery* (28%) and *velvety* (28%). Finally, both panels agreed that sample 6 had a clearly different astringency, although there were some differences in the terms used to describe it. For both panels the astringency of sample 6 could be described as *complex* (trained assessors 29% and experts 37%), *rough* (43% for both) and *mouthcoating* (43% for both). Also, neither for trained assessors nor for experts were attributes related to soft textures (*silky, suede, velvety*) relevant to describe the

astringency of sample 6 (frequencies equal or lower to 13%). For trained assessors, *puckery* (43%) and terms related to rough textures and excessive astringency, *aggressive* (36%), *harsh* (36%), *abrasive* (29%), *hard* (29%) and *sand paper* (21%), were related to sample 6. On the other hand, for experts the astringency sub-quality *coarse grain* (33%) was relevant for sample 6.

6.3.3. Astringency quality perception

As shown in Table 6.2, astringency quality assessments by experts significantly differed among samples (p=0.025). Astringency quality was significantly correlated to astringency intensity assessed by experts (r=0.86, p=0.027), but not to any time-intensity parameter obtained in the trained assessors' evaluation of astringency (r≤0.69, p≥0.129).

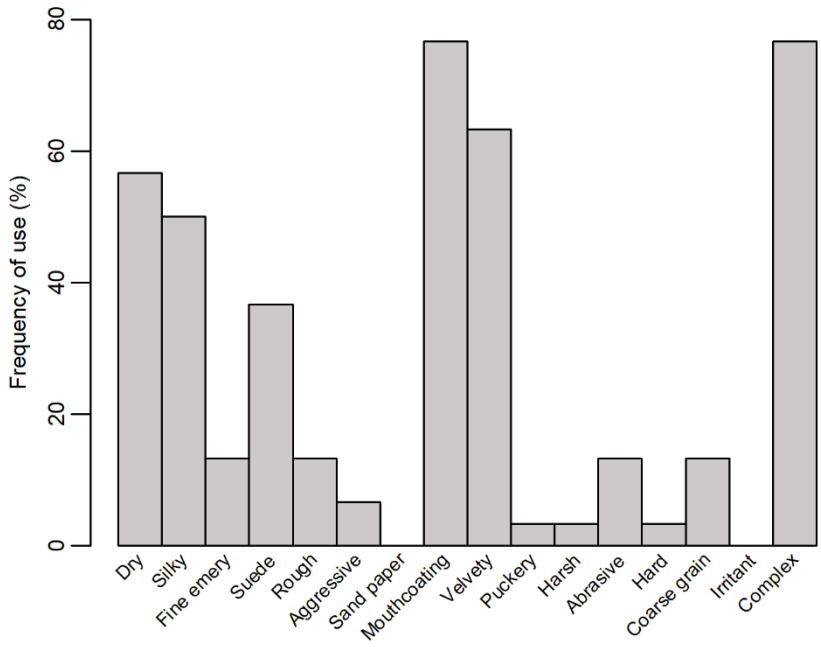

Fig. 6.2. Frequency of use of sixteen astringency sub-qualities to describe the astringency of an ideal high quality Tannat wine by wine experts.

The frequency of use of the 16 astringency sub-qualities included in the CATA question to describe the astringency of an ideal high quality Tannat wine is shown in Fig. 6.2. The astringency of the ideal Tannat wine was mainly described as *mouthcoating*, *complex*, *velvety*, *dry* and *silky*. The experts also indicated that the ideal Tannat wine should have a moderately high intensity (5.5 on a 9 point scale). It is interesting to note that sample 5 had the highest astringency quality score and an average astringency intensity of 5.5 according to experts' assessments. Also, *complex* and *mouthcoating* were the most frequently used astringency sub-qualities to describe sample 5 for both panels, while soft textures such as *velvety* and *suede* were frequently mentioned by

trained assessors. As shown in Table 6.1, sample 5 was the most expensive Tannat wine considered in the study, and also the only one that had been aged in oak barrels.

The penalty-lift analysis performed on astringency quality scores and astringency description using CATA questions revealed different insights. The sub-qualities *complex* and *suede* were the significant positive drivers of astringency quality, whereas the sub-qualities *puckery*, *sand paper* and *irritant* were significant negative drivers, leading to lower quality scores (Fig. 6.3).

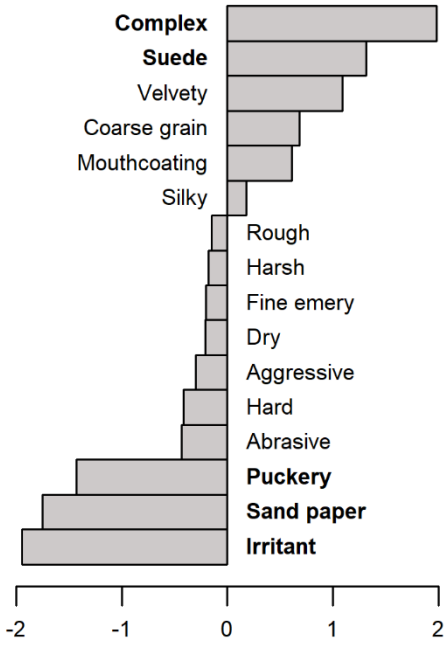

Fig. 6.3. Penalty-lift analysis based on average astringency quality scores and CATA data from experts. Astringency sub-qualities in bold were significant in the penalty-lift analysis.

6.3.3. Correlation between astringency and physicochemical parameters

Both total polyphenol index and tannin concentration were significantly correlated to expert's astringency intensity scores ($r \geq 0.89$, $p \leq 0.020$) and maximum astringency intensity from trained assessors ($r \geq 0.94$, $p \leq 0.005$), but only tannin concentration was significantly correlated to astringency quality scores ($r = 0.85$, $p = 0.032$).

6.4. DISCUSSION

Wine experts assessments are still an important source of information for both the winemaking industry and wine consumers. Wine experts tend to rely on both objective and subjective considerations, as well as on their tasting experience to provide a holistic judgment of the sensory characteristics and quality of wine (Leeschaeve & Noble, 2010). In the present research, experts' assessments of Tannat wine astringency were explored, and compared to those of a trained assessors' panel. Both panels

evaluated total astringency intensity of six commercial samples, though using different methodologies, and described astringency sub-qualities using check-all-that-apply questions.

Astringency intensity evaluations of trained assessors and experts were partly related, suggesting some agreement between panels when assessing total astringency intensity. Both panels agreed in which samples exhibited the lowest and highest astringency intensities. However, sample discrimination was greater for experts than for trained assessors. This may be related to the difference in the methodologies and the criteria used by both panels to assess astringency intensity. Trained assessors had been extensively trained to evaluate astringency intensity for more than a year, and used time-intensity, to account for the well-known temporal evolution of the attribute. On the contrary, experts performed a static evaluation of astringency and were not trained nor given any reference for scaling. Instead, they performed the evaluation using their own mental representations of astringency intensity, based on their previous tasting experiences. Thus, it is likely that their total astringency intensity assessments were not based only on astringency intensity, but also on qualitative aspects of astringency. Samples with lowest astringency intensity scores from experts (samples 1 and 2) were those described by the trained panel as eliciting astringency sub-qualities related to softer textures (*silky*, *suede* and *velvety*). Although previous studies on astringency sensory characterization of Tannat wines showed a certain relationship of astringency sub-qualities with total astringency intensity, total intensity and the frequency of use of astringency sub-qualities provided different information about red wine astringency (Vidal et al., 2017).

Wine astringency has been traditionally described by wine tasters and researchers using a wide range of subtle sensations, but the ability of wine experts to consistently use such terms is a relevant subject of study. In the present research trained assessors showed higher ability to discriminate samples according to astringency sub-qualities than experts (Table 6.3). This result may be linked to the panels' training level, and to the fact that the list of attributes used in the study was developed by consensus with the trained panelists (Vidal et al., 2017). Another possible explanation is that experts might be more familiar with less specific terms than those used by the trained panel. Zamora and Guirao (2004) conducted a study to compare wine assessments by a trained panel and by experts, and reported that experts tended to use rather abstract terms, which were not the same for each expert. Although those researchers considered only aroma and taste attributes, this tendency is likely to be true for mouthfeel attributes also.

Regardless of the differences found between astringency descriptions by trained assessors and experts, some similarities in how they perceived the differences among samples can be inferred. For both panels, the astringency of sample 1 was associated to soft texture sub-qualities, sample 5 was distinguished by its *complex* astringency, while the astringency of sample 6 was described using attributes related to rougher textures. This suggest that roughly both panels perceived the differences among samples but used different astringency sub-qualities to describe such differences. Similar results were found in the study conducted by Zamora and Guirao (2004). This result may be linked to different interpretations of the terms included in the CATA question. The lack of consensus among wine experts in the vocabulary used to communicate mouthfeel attributes is widely acknowledged, and was the main motivation for the development of the Mouth-feel wheel (Gawel et al., 2000). However, as stressed by Sáenz-Navajas et al. (2017), the lack of reference materials that illustrate specific mouthfeel properties makes it difficult for trained panels and experts to have a unified interpretation of the lexicon. Gawel et al. (2001) also reported discrepancies in the interpretation of astringency sub-qualities descriptors between trained wine experts and experienced wine-makers, especially when abstract and synthetic terms were considered. In addition, differences between panels might be related to their use of different frames of reference. Trained panelists refer to the standards provided during the training period, while experts probably rely on attributes stored in their long-term memory as a consequence of their exposure and experience in wine tasting (Zamora & Guirao, 2004).

Astringency quality scores given by wine experts were significantly correlated to their astringency intensity scores. This suggests that according to experts, astringency is a desired sensory characteristic for Tannat wine. In fact, when asked about the intensity of astringency a high quality Tannat wine should have, the average score provided by experts was 5.5 on a 9 point scale, which corresponds to a moderately high astringency intensity. Varela and Gámbaro (2006) also reported an association between astringency intensity of Tannat wines (assessed by a trained panel) and overall quality scores provided by highly-involved Uruguayan consumers. A positive relationship between astringency intensity and overall wine quality has also been reported for Spanish and Australian experts (Lattey et al., 2010; Sáenz-Navajas et al., 2016).

In addition to astringency intensity, the qualitative aspects are also relevant for wine quality. In the present research, *complex* and *suede* were identified in the penalty-lift analysis as the astringency sub-qualities that significantly increased quality scores. *Complex* and *suede* were also frequently used by experts to describe the astringency of

a high quality Tannat wine, together with other sub-qualities related to soft textures (*velvety* and *silky*) and *mouthcoating* (Fig. 6.2). In the study conducted by Sáenz-Navajas et al. (2013), complexity assessed in-mouth was linked to quality judgements of French and Spanish wine experts, as well as French consumers. Although the mentioned study was not focused solely on astringency as the present research, it seems that *complex*, which is a rather abstract and holistic term, is positively linked to quality assessments. *Velvety* is also regarded as a positive astringency sub-quality (Ferrer-Gallego et al., 2014), while according to Spanish experts' declarations, *round/smooth* and *soft* tannins are expected to be found in high quality wines (Sáenz-Navajas et al., 2016). Thus, it appears that wine experts have a relatively homogeneous conceptualization of the astringency sub-qualities that positively contribute to red wine quality, as previously stressed by Sáenz-Navajas et al. (2016). Penalty analysis also showed that *irritant*, *sand paper* and *puckery* significantly lowered experts' astringency quality scores.

Results from the penalty analysis are relevant for a first exploration of the relationship between astringency quality perception and specific astringency sub-qualities, but it should be noticed that it is a univariate analysis that considers each attribute individually. A closer investigation of astringency description of individual samples may provide further information on the role of astringency characteristics on quality perception. In this sense, it is interesting to note that, although sub-qualities related to soft textures appear to be important for astringency quality perception, samples 1 and 2, which were characterized by those sub-qualities received the lowest quality scores (Table 2). Both samples corresponded to relatively young wines (vintages 2013 and 2014) and were amongst the cheapest samples considered in this study. They also presented a lower polyphenol and tannin content, compared to the rest of the samples (Table 1). On the other hand, the samples with higher quality scores (5 and 6) were those exhibiting the highest polyphenol and tannin content, and thus, presumably, were those with higher potential to be high quality wines. They were also the most expensive wines considered in this research (Table 1). In particular sample 5 was a 2006 vintage, which had been aged in oak barrels. During wine ageing, a softening of astringency is observed, which has been attributed to reactions between phenolic compounds (Cheynier & Sarni-Machado, 2010; Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006). However, in the present study, sample 5 was not only characterized by soft textures such as suede and velvety, but also by rough and mouthcoating, among other sub-qualities. Thus, it can be hypothesized that the changes in the phenolic profile which occur during wine ageing, and that enhances the quality of a red wine, do not just "softens" the astringency, but confers the wine a wider range of sub-qualities which adds

to the complexity of its astringency. All above considered, it seems that softer textures per se might not be drivers of astringency quality, unless they are perceived together with other subtle sensations that add to the complexity of astringency. Further research is necessary to confirm such hypothesis, considering a larger sample set with different quality levels. Also, studies on the evolution of astringency characteristics and astringency quality during wine ageing, both in oak barrels and in bottles could contribute to a better understanding of the subject.

In this research, astringency quality of the six samples was assessed as difference with the astringency of a high quality Tannat wine, to prevent experts from assessing overall quality instead of focusing specifically in astringency sensations. The maximum average quality score given by experts was obtained for sample 5. Although this sample had an average astringency intensity equal to the expected astringency intensity of high quality Tannat wine, and was similarly described, its average quality score was 5.6 on a 9 point scale. Thus, there seems to be a misalignment between experts' expectations of the astringency sub-qualities of a high quality Tannat wine, and their ability to perceive and recognize those attributes when tasting a wine sample. This result can be related to the fact that experts are not used to assess solely wine astringency, especially when assessing wine quality. In line with this, Sáenz-Navajas et al. (2016) reported that although there was consensus among experts in the conceptualization in-mouth quality (related only to taste and trigeminal sensations), experts showed a generalized disagreement when rating the in-mouth quality of sixteen Spanish red wines. The authors attributed such disagreement to the absence of mental prototypes of quality based exclusively on taste and mouthfeel sensations, as experts usually evaluate them while simultaneously perceiving olfactory and/or visual cues. This also represents a potential limitation of the present study. As experts were asked to focus solely on astringency perception, there is some risk of a dumping bias effect. However, the fact that no significant differences were found in the total average use of the terms from the CATA question between experts and trained panelist suggest that this effect was not important in this study.

Another interesting avenue for further research would be to study the relationship between red wine astringency characteristics and astringency quality as perceived by consumers, considering that the quality concepts expressed by wine experts are not necessarily aligned with those from consumers (Lattey et al., 2010). However, research on how many astringency characteristics can be evaluated by consumers and on consumer understanding of astringency sub-qualities needs to be conducted first. In this sense, qualitative studies with Uruguayan consumers have shown that although they

understand the meaning of wine astringency and are able to accurately define the sensation, the vocabulary they use to describe it is limited (Vidal, Giménez, Medina, Boido, & Ares, 2015).

6.5. CONCLUSIONS

Astringency characterization of Tannat wines was performed by trained assessors and experts. Although a certain agreement between panels was observed for astringency intensity evaluations, their description of astringency sub-qualities differed. Astringency quality as perceived by experts was not related only to astringency intensity; astringency sub-qualities complex and velvety were the main drivers of astringency quality, while puckery, sand paper and irritant significantly lowered quality scores. However, the balance of the different astringency sub-qualities elicited in red wines, together with astringency intensity, seem to play a major role in quality perception. Soft textures per se do not seem to be drivers of quality, as wines that only elicited this type of astringency received the lowest quality scores. This research was a first step to understanding the relationship between astringency characteristics and quality perception, but further research is necessary to confirm and extend the knowledge on this matter.

CONCLUSIONS

The general aim of this thesis was to characterize the astringency of commercial Uruguayan Tannat wines. Given that several authors have suggested that different sub-qualities should be assessed to fully characterize red wine astringency (Gawel, Illand, & Francis, 2000; Gawel, Oberholster, & Francis, 2001), the first specific objective was to determine an appropriate vocabulary of astringency sub-qualities, which should be relevant to Uruguayan fine wine consumers. Results presented in **CHAPTER 1** showed that Uruguayan consumers understand the meaning of wine astringency and are able to accurately define the sensation, although the vocabulary they use is limited. Most of the terms included in the Mouth-feel wheel (Gawel et al., 2000) do not seem adequate to communicate red wine astringency characteristics to Uruguayan consumers, who mainly selected terms related to *dryness*, rough textures (*rough*, *harsh*, *sand paper*) and aggressiveness (*aggressive*, *abrasive*, *irritant*) as appropriate to describe astringency. The astringency terms identified as relevant for Uruguayan consumers served as input to define the vocabulary used by a panel of trained assessors to describe the astringency of commercial Tannat wines. Additional terms, including those related to softer textures (*silky*, *velvety* and *suede*), were also included for subsequent studies, to enable the trained assessors to describe a broader range of astringency-related sensations.

The complex nature of astringency perception, and the challenges that are faced to fully characterize red wine astringency, were reflected in this thesis, including the several considerations that are necessary during experimental design of sensory evaluation of astringency (**CHAPTER 2**), and the relevance of considering the temporal dependency of astringency, and going beyond total intensity (**CHAPTERS 3 and 5**).

Keeping all this in mind, the sensory astringency of commercial Uruguayan Tannat wines was characterized by a trained panel, considering both the development of total astringency intensity over the evaluation period, and the description of astringency sub-qualities (**CHAPTER 3**). In terms of global astringency, samples mainly differed in parameters related to the intensity of the sensation rather than its development over time. Although the variability in astringency intensity was moderate in general, a small group of samples was characterized by its low astringency intensity, while another small group presented higher astringency intensity than the rest. Samples also differed in the terms used to describe their astringency, with sub-qualities ranging from soft textures (*silky*, *velvety* and *suede*) to those related to excessive astringency (*harsh*, *hard* and *aggressive*), suggesting that different styles of Tannat wine exist in the Uruguayan marketplace.

The relationship between sensory astringency and the composition of the commercial Tannat wines, with special emphasis on the phenolic profile, was assessed using a novel predictive method, boosted regression trees (**CHAPTER 4**). Although the predictive accuracy was much higher for astringency intensity than for the frequency of mention of astringency sub-qualities, the statistical strategy applied allowed to point out to some compositional variables most likely involved in wine astringency perception. Among the basic compositional parameters, only condensed tannin concentration, determined by a relatively simple assay, was a relevant predictor of sensory astringency. Regarding the phenolic compounds determined by HPLC-MS, flavan-3-ols presented the highest contribution to astringency, particularly some dimers, trimers and the sum of non-galloylated tetramers, and their effect was different depending on the astringency sub-quality considered.

The sensory quality of wine is determined by wine composition (Peynaud, 1983), which is affected by several factors, including the grape variety, the region where the grapes are grown, the vintage, as well as viticulture practices and winemaking techniques, such as maceration and ageing in oak barrels (Garrido & Borges, 2013; Kennedy, Saucier, & Glories, 2006; Smith, McRae, & Bindon, 2015). All these factors lead to different categories of wines in terms of quality, which is supposed to be reflected in the price of the final product (Cáceres-Mella et al., 2012). However, in the present thesis, no relationship between the samples' astringency characteristics and their vintage, price range and ageing in oak barrels could be established. This could be a limitation of the specific sample set of Tannat wines, as the different vintages, price ranges and the characteristic of being aged in oak barrel were not balanced in the sample set. Besides, a wide range of vineyards and winemaking techniques were represented in the sample set, as samples corresponded to different brands of Tannat fine wine from 15 different wineries. Furthermore, the Uruguayan legislation allows commercial monovarietal wines to present up to 15% of other varieties (González-Neves, Gómez-Cordovés, & Barreiro, 2001), which adds variability to the sample set. Further research is necessary to better understand how astringency characteristics are influenced by production variables. In this sense, although studies involving commercial samples have been encouraged (Scollary, Pásti, Kállay, Blackman, & Clark, 2012), the use of experimental wines with controlled production variables seems to be a good alternative to achieve such goal.

Results presented in **CHAPTER 5** highlight the relevance of considering the dynamic aspects of astringency perception. The Temporal Dominance of Sensations of astringency sub-qualities was determined for a subset of Tannat wines. Samples showed

temporal profiles that differed in the dominance of the terms and the time elapsed until they became dominant, in line with results from Lee and Lawless (1991), who showed that different astringency sub-qualities are perceived in different moments and with different intensity. Moreover, wines which did not significantly differ in their (static) average astringency intensity showed different dynamic astringency profiles. Taking into account that the sensory temporal profile has a large impact on consumer liking (Lawless & Heymann, 2010) and on the subjective quality perception of wine (Lesschaeve & Noble, 2010), the dynamic profile of Tannat wine astringency could be a better predictor of consumer's liking and quality perception than static measures of astringency and astringency sub-qualities.

Astringency characterization of Tannat wine performed by the trained panel was compared to astringency perception of wine experts (**CHAPTER 6**). Most wineries rely on the judgment of wine experts for decision making, and experts' opinions of the sensory characteristics and quality of wine are also a valued source of information for consumers'. Results showed certain agreement between trained assessors and experts in astringency intensity evaluations, but their description of astringency sub-qualities differed. In rough terms both panels perceived the differences among samples, but used different astringency sub-qualities to describe those differences. Besides, trained assessors showed higher sample discrimination in terms of astringency description. The study also provided insights on the astringency sub-qualities linked to expert's perception of astringency quality. C*omplex* and *velvety* were the positive drivers of Tannat wine astringency quality, while *puckery*, *sand paper* and *irritant* significantly lowered quality scores.

Experts are known to have better abilities to assess and describe wine's sensory characteristics (Gawel, 1997) and to act more analytically than consumers when assessing wine quality (Hopfer & Heymann, 2014). However, the quality concepts expressed by wine experts are not necessarily aligned with those from consumers (Lattey, Bramley, & Francis, 2010). Thus, an interesting approach for future research would be to assess the astringency characteristics of Tannat wine working directly with consumers. As suggested in **CHAPTER 1**, it would be interesting to explore how consumers understand the different sub-qualities of astringency and what sensations they expect from wines described with those characteristics. Research comparing consumers' and trained assessors' descriptions of wine astringency seems necessary. However, in order to accomplish this, careful methodological choices ought to be selected, and studies to determine which and how many astringency characteristics can be evaluated by consumers seem mandatory.

Overall, results from the present thesis provided on one side methodological insights about astringency evaluation of red wine, including the identification of vocabulary to describe astringency sub-qualities that is relevant for Uruguayan consumers; the definition of an adequate palate cleanser; and the applicability of sensory methods to assess the static and the dynamic profile of red wine astringency. On the other side, applied information about the astringency of Uruguayan commercial Tannat wines was obtained, which could be highly valuable for the Uruguayan wine industry.

REFERENCES

Adams, J., Williams, A., Lancaster, B., & Foley, M. (2007). Advantages and uses of check-all-that-apply response compared to traditional scaling of attributes for salty snacks. In *7th Pangborn sensory science symposium*, *August 12–16. Minneapolis, USA.*

Alcalde-Eon, C., Boido, E., Carrau, F., Dellacassa, E., & Rivas-Gonzalo, J.C. (2006). Pigment profiles in monovarietal wines produced in Uruguay. *American Journal of Enology and Viticulture, 57*, 449–459.

Ares, G., Barreiro, C., Deliza, R., & Gámbaro, A. (2009). Alternatives to reduce the bitterness, astringency and characteristic flavour of antioxidant extracts. *Food Research International*, 42*,* 871-878.

Ares, G., Bruzzone, F., Vidal, L., Cadena, R. S., Giménez, A., Pineau, B., ... Jaeger, S. R. (2014). Evaluation of a rating-based variant of check-all-that-apply questions: Rate-all-that-apply (RATA). *Food Quality and Preference*, 36, 87–95.

Ares, G., & Jaeger, S.R. (2015). Check-all-that-apply (CATA) questions with consumers in practice. Experimental considerations and impact on outcome. In J. Delarue, J. B. Lawlor, & M. Rogeaux (Eds.), *Rapid sensory profiling techniques and related methods* (pp. 227–245). Sawston, Cambridge: Woodhead Publishing.

Ares, G., Jaeger, S.R., Antúnez, L., Vidal, L., Giménez, A., Coste, B., … Castura, J.C. (2015). Comparison of TCATA and TDS for dynamic sensory characterization of food products. *Food Research International, 78*, 148-158.

Arnold, R.A., Noble, A.C., & Singleton, V.L. (1980). Bitterness and astringency of phenolic fractions in wine. *Agricultural and Food Chemistry*, 28, 675-678.

ASTM (2004). *Standard definitions of terms relating to sensory evaluation of materials and products. Annual book of ASTM standards.* Philadelphia: American Society for Testing and Materials.

Bajec, M.R., & Pickering, G.J. (2008). Astringency: mechanisms and perception. *Critical Reviews in Food Science & Nutrition*, 48, 858-875.

Ballester, J., Patris, B., Symoneaux, R., & Valentin, D. (2008). Conceptual vs. perceptual wine spaces : Does expertise matter? *Food Quality and Preference,* 19, 267-276.

Bartlett, M.S. (1950). Tests of significance in factor analysis. *British Journal of Psychology*, 3, 77-85.

Bate-Smith, E.C. (1954). Astringency in foods. *Food*, 23, 124–127.

Beecher, J.W., Drake, M.A., Luck, P.J., & Foegeding, E.A. (2006). Factors regulating astringency of whey protein beverages. *Journal of Dairy Science*, 91, 2553–2560.

Bell, R., & Marshall, D.W. (2003). The construct of food involvement in behavioral research: scale development and validation. *Appetite*, 40, 235–244.

Bindon, K., Holt, H., Williamson, P.O., Varela, C., Herderich, M., & Francis, I.L. (2014). Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet Sauvignon 2. Wine sensory properties and consumer preference. *Food Chemistry*, 154, 90-101.

Blanchard, P. (1999). *Tannat en Pyreneos Atlanticos.* An 94-99. Cámara de Agricultura de Pirineos Atlánticos.

Boido, E., Alcalde-Eon, C., Carrau, F., Dellacassa, E., & Rivas-Gonzalo, J.C. (2006). Aging effect on the pigment composition and color of Vitis vinifera L. Cv. Tannat wines. Contribution of the main pigment families to wine color. *Journal of Agricultural and Food Chemistry*, 54, 6692-6704.

Boido, E., Fariña, L., Carrau, F., Dellacassa, E., & Cozzolino, D. (2013). Characterization of Glycosylated Aroma Compounds in Tannat Grapes and Feasibility of the Near Infrared Spectroscopy Application for Their Prediction. *Food Analytical Methods*, 6, 100-111.

Boido, E., García-Marino, M., Dellacassa, E., Carrau, F., Rivas-Gonzalo, J., & Escribano-Bailón, M.T. (2011). Characterization and evolution of grape polyphenol profiles of Vitis vinifera L. cv. Tannat during ripening and vinification. *Australian Journal of Grape and Wine Research, 17*, 383-393.

Boido, E., Lloret, A., Medina, K., Farñia, L., Carrau, F., Versini, G., & Dellacassa, E. (2003). Aroma composition of Vitis vinifera cv. Tannat: The typical red wine from Uruguay. *Journal of Agricultural and Food Chemistry*, 51, 5408-5413.

Boidron, R., Boursiquot, J.-M., Doazan, J.-P., Leclair, P., Leguay, M., & Walter, B. (1995). *Catalogue des variétés et clones de vigne cultivés en France, 1a ed.* Le Grau du Roi: ENTAV.

Boulet, J.-C., Trarieux, C., Souquet, J.-M., Ducasse, M.-A., Caillé, S., Samson, A., … Cheynier, V. (2016). Models based on ultraviolet spectroscopy, polyphenols, oligosaccharides and polysaccharides for prediction of wine astringency. *Food Chemistry*, 190, 357-363.

Brannan, G.D., Setser, C.S., & Kemp, K.E. (2001a). Effectiveness of rinses in alleviating bitterness and astringency residuals in model solutions. *Journal of Sensory Studies*, 16*,* 261–275.

Brannan, G.D., Setser, C.S., & Kemp, K.E. (2001b). Interaction of astringency and taste characteristics. *Journal of Sensory Studies*, 16, 179-197.

Breiman, L. (2001) Statistical modeling: the two cultures. *Statistical Science*, 16, 199–215.

Breslin, P.A.S., Gilmore, M.M., Beauchamp, G.K., & Green, B.G. (1993). Psychophysical evidence that oral astringency is a tactile sensation. *Chemical Senses*, 18, 405–417.

Brock, A., & Hofmann, T. (2008). Identification of the key astringent compounds in spinach (*Spinacia oleracea*) by means of the taste dilution analysis. *Chemosensory Perception*, 1, 268–281.

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*, 47, 392-402.

Brossaud, F., Cheynier, V., & Noble, A. C. (2001). Bitterness and astringency of grape and wine polyphenols. *Australian Journal of Grape and Wine Research*, 7, 33–39.

Bruzzone, F., Ares, G., & Giménez, A. (2013). Temporal aspects of yoghurt texture perception. *International Dairy Journal,* 29, 124-134.

Cáceres-Mella, A., Peña-Neira, A., Avilés-Gálvez, P., Medel-Marabolí, M., del Barrio-Galán, R., López-Solís, R., & Canals, J.M. (2014). Phenolic composition and mouthfeel characteristics resulting from blending Chilean red wines. *Journal of the Science of Food and Agriculture*, 94, 666–676.

Cáceres-Mella, A., Peña-Neira, A., Galvez, A., Obreque-Slier, E., López-Solís, R., & Canals, J.M. (2012). Phenolic Compositions of Grapes and Wines from Cultivar Cabernet Sauvignon Produced in Chile and Their Relationship to Commercial Value. *Journal of Agricultural and Food Chemistry*, 60, 8694-8702.

Cáceres-Mella, A., Peña-Neira, A., Narváez-Bastias, J., Jara-Campos, C., López-Solís, R., & Canals, J.M. (2013). Comparison of analytical methods for measuring proanthocyanidins in wines and their relationship with perceived astringency. *International Journal of Food Science and Technology*, 48, 2588–2594.

Cadena, R.S., Vidal, L., Ares, G., & Varela, P. (2014). Dynamic sensory descriptive methodologies. Time-intensity and Temporal dominance of sensations. In P. Varela, & G. Ares (Eds.), *Novel Techniques for sensory characterization and consumer profiling* (pp. 333-364). Boca Raton: CRC Press.

Cala, O., Dufourc, E. J., Fouquet, E., Manigand, C., Laguerre, M., & Pianet, I. (2012). The colloidal state of tannins impacts the nature of their interaction with proteins: The case of salivary proline-rich protein/procyanidins binding. *Langmuir*, 28(50), 17410–17418.

Campo, E., Ballester, J., Langlois, J., Dacremont, C., & Valentin, D. (2010). Comparison of conventional descriptive analysis and a citation frequency-based descriptive method for odor profiling: An application to Burgundy Pinot noir wines. *Food Quality and Preference*, 21, 44-55.

Carr, B.T., Craig-Petsinger, D., & Hadlich, S. (2001). A case study in relating sensory descriptive data to product concept fit and consumer vocabulary. *Food Quality and Preference*, 12, 407–412.

Carrau, F. (1997). The emergence of a new Uruguayan wine industry. *Journal of Wine Research, 8*, 179–185.

Castura, J.C., Antúnez, L., Giménez, A., & Ares, G. (2016). Temporal Check-All-That-Apply (TCATA): A novel dynamic method for characterizing products. *Food Quality and Preference, 47*, 79-90.

Charlton, A.J., Baxter, N.J., Khan, M.L., Moir, A.J., Haslam, E., Davies, A.P., & Williamson, M.P. (2002). Polyphenol/peptide binding and precipitation. *Journal of Agricultural and Food Chemistry*, 50, 1593–1601.

Charters, S., & Pettigrew, S. (2007). The dimensions of wine quality. *Food Quality and Preference*, 18, 997–1007.

Cheynier, V., & Sarni-Manchado, P. (2010). Wine taste and mouthfeel. In: A. Reynolds (Ed). Managing Wine Quality (pp.29-72). Cambridge: Woodhead Publishing Limited.

Chira, K., Jourdes, M., & Teissedre, P.-L. (2012). Cabernet sauvignon red wine astringency quality control by tannin characterization and polymerization during storage. European Food Research and Technology, 234, 253-261.

Chira, K., Pacella, N., Jourdes, M., & Teissedre, P.-L. (2011). Chemical and sensory evaluation of Bordeaux wines (Cabernet-Sauvignon and Merlot) and correlation with wine age. *Food Chemistry*, 126, 1971-1977.

Cliff, M.A., King, M.C., & Schlosser, J. (2007). Anthocyanin, phenolic composition, colour measurement and sensory analysis of BC commercial red wines. *Food Research International*, 40, 92-100.

Colonna, A.E., Adams, D.O., & Noble, A. (2004). Comparison of procedures for reducing astringency carry-over effects in evaluation of red wines. *Australian Journal of Grape and Wine Research*, 10, 26–31.

Condelli, N., Dinnella, C., Cerone, A., Monteleone, E., & Bertuccioli, M. (2006). Prediction of perceived astringency induced by a phenolic compounds II: criteria for panel selection and preliminary application on wine samples. *Food Quality and Preference*, 17, 96–107.

Coniberti, A., Ferrari, V., Fariña, L., Carrau, F., Dellacassa, E., Boido, E., & Disegna, E. (2012). Role of canopy management in controlling high pH in Tannat grapes and wines. *American Journal of Enology and Viticulture*, 63, 554-558.

Courregelongue, S., Schlich, P., & Noble, A.C. (1999). Using repeated ingestion to determine the effect of sweetness, viscosity and oiliness on temporal perception of soymilk astringency. *Food Quality and Preference*, 10, 273–279.

Critchley, H.D., & Rolls, E.T. (1996). Responses of Primate Taste Cortex Neurons to the Astringent Tastant Tannic Acid. *Chemical Senses*, 21, 135-145.

Cronbach, L.J. (1951). Coefficient alpha and the internal strucuture of tests. *Psychometrika*, 16, 297-334.

Crozier, A., Jaganath, I.B., & Clifford, M.N. (2006). Phenols, Polyphenols and Tannins: An Overview. In: A. Cozier, M. N. Clifford, & H. Ashihara (Eds.), *Plant Secondary Metabolites. Occurrence, Structure and Role in the Human Diet* (pp. 1-24). Singapore: Blackwell Publishing.

D'Alessandro, S., & Pecotich, A. (2013). Evaluation of wine by expert and novice consumers in the presence of variations in quality, brand and country of origin cues. *Food Quality and Preference*, 28, 287–303.

de Freitas, V., & Mateus, N. (2001). Nephelometric study of salivary protein-tannin aggregates. *Journal of the Science of Food and Agriculture*, 82, 113–199.

del Barrio-Galán, R., Pérez-Magariño, S., & Ortega-Heras, M. (2011). Techniques for improving or replacing ageing on lees of oak aged red wines: The effects on polysaccharides and the phenolic composition. *Food Chemistry*, 127, 528-540.

Demiglio, P., & Pickering, G.J. (2008).The influence of ethanol and pH on the taste and mouthfeel sensations elicited by red wine. *Journal of Food Agriculture and Environment*, 6, 143-150.

Denzin, N.K. (1978). *The research act: A theoretical introduction to sociological methods*. New York: McGraw-Hill Inc.

Dinnella, C., Recchia, A., Fia, G., Bertuccioli, M., & Monteleone, E. (2009). Saliva characteristics and individual sensitivity to phenolic astringent stimuli. *Chemical Senses*, 34, 295-304.

Dodd, T.H., Pinkelton, B.E., & Gustafson, A.W. (1996). External information sources of product enthusiasts. *Psychology and Marketing*, 13, 291-305.

Elith, J., Leathwick, J.R., & Hastie, T. (2008). A working guide to boosted regression trees. *Journal of Animal Ecology*, 77, 802–813.

Fariña, L., Carrau, F., Boido, E., Disegna, E., & Dellacassa, E. (2010). Carotenoid profile evolution in Vitis vinifera cv. Tannat grapes during ripening. *American Journal of Enology and Viticulture*, 61, 451-456.

Fariña, L., Villar, V., Ares, G., Carrau, F., Dellacassa, E., & Boido, E. (2015). Volatile composition and aroma profile of Uruguayan Tannat wines. *Food Research International*, 69, 244-255.

Favre, G., Peña-Neira, A., Baldi, C., Hernández, N., Traverso, S., Gil, G., & González-Neves, G. (2014). Low molecular-weight phenols in Tannat wines made by alternative winemaking procedures. *Food Chemistry*, 158, 504-512.

Feinerer, I., Hornik, K., & Meyer, D. (2008). Text Mining Infrastructure in R. *Journal of Statistical Software*, 25, 1-54.

Ferrer-Gallego, R., Brás, N.F., García-Estévez, I., Mateus, N., Rivas-Gonzalo, J.C., de Freitas, V., & Escribano-Bailón, M.T. (2016). Effect of flavonols on wine astringency and their interaction with human saliva. *Food Chemistry*, 209, 358-364.

Ferrer-Gallego, R., Gonçalves, R., Rivas-Gonzalo, J. C., Escribano-Bailon, M. T., & de Freitas, V. (2012). Interaction of phenolic compounds with bovine serum albumin (BSA) and alpha-amylase and their relationship to astringency perception. *Food Chemistry*, 135, 651–658.

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.

Fischer, U., Boulton, R.B., & Noble, A.C. (1994). Physiological factors contributing to the variability of sensory assessments: relationship between salivary flow rate and temporal perception of gustatory stimuli. *Food Quality and Preference*, 5, 55–64.

Fisher, R.A. (1954). *Statistical methods for research workers*. Edinburgh: Oliver and Boyd.

Fleming, E.E., Ziegler, G.R., & Hayes, J.E. (2015). Check-all-that-apply (CATA), sorting, and polarized sensory positioning (PSP) with astringent stimuli. *Food Quality and Preference*, 45, 41–49.

Fontoin, H., Saucier, C., Teissedre, P.-L., & Glories, Y. (2008). Effect of pH, ethanol and acidity on astringency and bitterness of grape seed tannin oligomers in model wine solution. *Food Quality and Preference*, 19, 286-291.

Francis, I.L., Gawel, R., Iland, P.G., Vidal, S., Cheynier, V., Guyot, S., … Waters, E.J. (2002). Characterising mouth-feel properties of red wines. *Australian and New Zealand Wine Industry Journal,* 17, 18–25.

Froehlich, D.A., Pangborn, R.M., & Whitaker, J.R. (1987). The effect of oral stimulation on human parotid salivary flow rate and alpha-amylase secretion. *Physiology & Behavior*, 41, 209-217.

Furlan, A.L., Jobin, M.-L., Pianet, I., Dufourc, E.J., & Géan, J. (2014). Flavanol-lipid interaction: a novel molecular perspective in the description of wine Astringency & Bitterness and antioxidant action. *Tetrahedron*, 71, 3143-3147.

Gámbaro, A., Boido, E., Zlotejablko, A., Medina, K., Lloret, A., Dellacassa, E., & Carrau, F. (2001). Effect of malolactic fermentation on the aroma properties of Tannat wine. *Australian Journal of Grape and Wine Research*, 7, 27-32.

Gambaro, A., Varela, P., Boido, E., Gimenez, A., Medina, K., & Carrau, F. (2003). Aroma characterization of commercial red wines of Uruguay. *Journal of Sensory Studies*, 18, 353-366.

Garrido, J., & Borges, F. (2013). Wine and grape polyphenols - A chemical perspective. *Food Research International*, 54, 1844–1858.

Gawel, R. (1997). The use of language by trained and untrained wine tasters. *Journal of Sensory Studies,* 12, 267-284.

Gawel, R. (1998). Red wine astringency: a review. *Australian Journal of Grape and Wine Research*, 4, 74–95.

Gawell, R., Francis, L., & Waters, E.J. (2007). Statistical correlations between the in-mouth textural characteristics and the chemical composition of Shiraz wines. *Journal of Agricultural and Food Chemistry,* 55, 2683-2687.

Gawel, R., & Godden, P.W. (2008). Evaluation of the consistency of wine quality assessments from expert wine tasters. *Australian Journal of Grape and Wine Research*, 14, 1-8.

Gawel, R., Iland, P.G., & Francis, I.L. (2001). Characterizing the astringency of red wine: a case study. *Food Quality and Preference,* 12, 83-94.

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, 203–207.

Gibbins, H.L., & Carpenter, G.H. (2013). Alternative mechanisms of astringency - What is the role of saliva? *Journal of Texture Studies*, 44, 364–375.

Gillespie, D.M. (2000). Sensory Profiles of Taste and Mouthfeel of Red Wines: Sourness, Bitterness and Astringency. MSc. thesis. Davis, California: University of California.

Goldner, M.C., & Zamora, M.C. (2010). Effect of polyphenol concentrations on astringency perception and its correlation with gelatin index of red wine. *Journal of Sensory Studies*, 25, 761–777.

Goldsmith, R.E., & d'Hauteville, F. (1998). Heavy wine consumption: Empirical and theoretical perspectives. *British Food Journal*, 100, 184-190.

González-Neves, G., Barreiro, L., Gil, G., Franco, J., Ferrer M., Moutounet, M., & Carbonneau, A. (2004). Anthocyanic composition of Tannat grapes from the south region of Uruguay. *Analytica Chimica Acta*, 513, 197–202.

González-Neves, G., Favre, G., & Gil, G. (2014). Effect of fining on the colour and pigment composition of young red wines. *Food Chemistry*, 157, 385–392.

González-Neves, G., Ferrer, M., & Gil, G. (2012). Differentiation of Tannat, Cabernet Sauvignon and Merlot grapes from Uruguay according to their general composition and polyphenolic potential. *Comunicata Scientiae*, 3(1), 41-49.

González-Neves, G., Gil, G., Barreiro, L., & Favre, G. (2010). Pigment profile of red wine cv. Tannat made with alternative winemaking techniques. *Journal of Food Composition and Analysis*, 23, 447–454.

González-Neves, G., Gil, G., Favre, G., & Ferrer, M. (2012). Influence of grape composition and winemaking on the anthocyanin composition of red wines of Tannat. *International Journal of Food Science and Technology*, 47, 900–909.

González-Neves, G., Gómez-Cordovés, C., & Barreiro, L. (2001). Anthocyanic composition of Tannat, Cabernet Sauvignon and Merlot young red wines from Uruguay. *Journal of Wine Research*, 12, 125-133.

Gonzalo-Diago, A., Dizy, M., & Fernández-Zurbano, P. (2014). Contribution of low molecular weight phenols to bitter taste and mouthfeel properties in red wines. *Food Chemistry*, 154, 187-198.

Green, B.G. (1993). Oral astringency: A tactile component of flavor. *Acta Psychologica,* 84, 119-125.

Guerrero, L., Claret, A., Verbeke, W., Enderli, G., Zakowska-Biemans, S., Vanhonacker, F., ... Hersleth, M. (2010). Perception of traditional food products in six European regions using free word association. *Food Quality and Preference*, 21, 225–233.

Guinard, J.-X., Pangborn, R.M., & Lewis, M.J. (1986). The time-course of astringency in wine upon repeated ingestion. *American Journal of Enology and Viticulture*, 37,184–189.

Guinard, J.-X., Zoumas-Morse, C., & Walchak, C. (1998). Relation between parotid saliva flow and composition and the perception of gustatory and trigeminal stimuli in foods. *Physiology & Behavior*, 63, 109–118.

Hastie, T., Tibshirani, R., & Friedman, J. (2009). *The elements of statistical learning. Data mining, inference and prediction. Second Edition*. New York: Springer.

Hijmans, R.J., Phillips, S., Leathwick, J., & Elith, J. Package 'dismo'. (2017). Available at: https://cran.r-project.org/web/packages/dismo/dismo.pdf. Accessed: (15.11.17).

Hodgson, R.T. (2008). An examination of judge reliability at a major U.S. wine competition. *Journal of Wine Economics*, 3, 105–113.

Hollebeek, L.D., Jaeger, S.R., Brodie, R.J., & Balemi, A. (2007). The influence of involvement on purchase intention for new world wine. *Food Quality and Preference,* 18, 1033-1049.

Holt, H.E., Francis, I.L., Field, J., Herderich, M.J., & Iland, P.G. (2008). Relationships between wine phenolic composition and wine sensory properties for Cabernet Sauvignon (*Vitis vinifera* L.). *Australian Journal of Grape and Wine Research*, 14, 162-176.

Hopfer, H., Heymann, H. (2014). Judging wine quality: Do we need experts, consumers or trained panelists? *Food Quality and Preference*, 32, 221-223.

Horn, J.L. (1965). A rationale and test for the number of factors in factor analysis. *Psychometrika*, 30, 179–185.

Hough, G., & Ferraris, D. (2010). Free listing: A method to gain initial insight of a food category. *Food Quality and Preference*, 21, 295–301.

Hufnagel, J.C., & Hofmann, T. (2008). Orosensory directed identification of astringent mouthfeel and bitter-tasting compounds in red wine. *Journal of Agricultural and Food Chemistry*, 56, 1376–1386.

Iland, P., Ewart, A., & Sitters, J. (1993). *Techniques for chemical analysis and stability tests of grape juice and wine*. Campbelltown: Patrick Iland Wine Promotions.

Instituto Nacional de Vitivinicultura (INAVI). Estadísticas de viñedos 2017. Datos Nacionales. (2017). Available at: http://www.inavi.com.uy/articulos/657840-38-estada-sticas-de-via-edos-datos-nacionales-2017.html. (Accessed: 07.12.17).

Ishikawa, T. & Noble, A.C. (1995). Temporal perception of astringency and sweetness in red wine. *Food Quality and Preference*, 6, 27–33.

ISO. (1977). Sensory analysis – apparatus – wine-tasting. *ISO Standard 3591:1977*. Geneva, Switzerland: International Organization for Standardization.

ISO. (2007). Sensory Analysis: General Guidance for the Design of Test Rooms, *ISO Standard 8589*. Geneva, Switzerland: International Organization for Standardization.

ISO. (2012). Sensory analysis—General guidance for the selection, training, and monitoring of selected assessors and expert sensory assessors. *ISO Standard 8586:2012*. Geneva, Switzerland: International Organization for Standardization.

Ivanova, V., Dörnyei, Á., Márk, L., Vojnoski, B., Stafilov, T., Stefova, M., & Kilár, F. (2011). Polyphenolic content of Vranec wines produced by different vinification conditions. *Food Chemistry*, 124, 316-325.

Jackson, R.S. (2014). *Wine Science. Principles and Applications*. 4th ed. San Diego, CA: 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, 923-950.

Joslyn, M.A., & Goldstein, J.L. (1964). Astringency of fruits and fruit products in relation to phenolic content. *Advances in Food Research*, 13, 179–217.

Kaiser, H.F. (1970). A second generation little jiffy. *Psychometrika*, 35, 401–415.

Kaiser, H.F., & Rice, J. (1974). Little jiffy, mark iv. *Educational and Psychological Measurement*, 34, 111–117.

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

Kallithraka, S., Bakker, J., & Clifford, M.N. (1997b). Evaluation of bitterness and astringency of (+)-catechin and (-)-epicatechin in red wine and in model solution. *Journal of Sensory Studies*, 12, 25-37.

Kallithraka, S., Bakker, J., & Clifford, M.N. (1997c). Red wine and model wine astringency as affected by malic and lactic acid. *Journal of Food Science*, 62, 416-420.

Kallithraka, S., Bakker, J., & Clifford, M.N. (1998). Evidence that salivary proteins are involved in astringency. *Journal of Sensory Studies*, 13, 29-43.

Kallithraka, S., Kim, D., Tsakiris, A., Paraskevopoulos, I., & Soleas, G. (2011). Sensory assessment and chemical measurement of astringency of Greek wines: Correlations with analytical polyphenolic composition. *Food Chemistry*, 126, 1953-1958.

Kawamura, Y., Funakoshi, M., Kasahara, Y., & Yamamoto, T. (1969). A neurophysiological study on astringent taste. *The Japanese Journal of Physiology*, 19, 851-865.

Kelebek, H. (2016). LC-DAD–ESI-MS/MS characterization of phenolic constituents in Turkish black tea: Effect of infusion time and temperature. *Food Chemistry*, 204, 227-238.

Kennedy, J.A., Saucier, C., & Glories, Y. (2006). Grape and Wine Phenolics: History and Perspective. *American Journal of Enology and Viticulture*, 57(3), 239-248.

Kielhorn, S. & Thorngate III, J.H. (1999). Oral sensations associated with the flavan-3-ols (+)catechin and (-)-epicatechin. *Food Quality and Preference,* 10,109–116.

Kishi, M., Sadachi, H., Nakamura, J., & Tonoike, M. (2017). Functional magnetic resonance imaging investigation of brain regionsassociated with astringency. *Neuroscience Research*, 122, 9–16.

Krippendorff, K. (2004). *Content analysis: An introduction to its methodology* (2nd Ed.).Thousand Oaks, CA: Sage Publications.

Kuznetsova, A., Brockhoff, P.B., & Christensen, R.H.B. Package 'lmerTest'. (2017). Available at: https://cran.r-project.org/web/packages/lmerTest/lmerTest.pdf. (Accessed: 11.12.17).

Labbe, A., Schlich, P., Pineau, N., Gilbert, F., & Martin, N. (2009). Temporal dominance of sensations and sensory profiling: a comparative study. *Food Quality and Preference,* 20, 216-221.

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., Sarkar, A., Bryant, M.G., Breadling, A.R., Bartolomé, B., & Moreno-Arribas, M.V. (2017). Exploring mouthfeel in model wines: Sensory-to-instrumental approaches. *Food Research International*, 102, 478-486.

Llaudy, M.C., Canals, R., Canals, J.-M., Rozés, N., Arola, L., & Zamora, F. (2004). New method for evaluating astringency in red wine. *Journal of Agricultural and Food Chemistry*, 52, 742-746.

Langstaff, S.A., Guinard, X.J., & Lewis, M.J. (1991). Sensory evaluation of the mouthfeel of beer. *ASBC Journal,* 49, 54-59.

Lattey, K.A., Bramley, B.R., & Francis, I.L. (2010). Consumer acceptability, sensory properties and expert quality judgements of Australian Cabernet Sauvignon and Shiraz wines. *Australian Journal of Grape and Wine Research*, 16, 189–202.

Lawless, H.T. (1984). Flavor description of white wine by "expert" and non expert wine consumers. *Journal of Food Science*, 49, 120-123.

Lawless, H.T., & Clark, C.C. (1992). Psychological biases in time-intensity scaling. *Food Technology*, 46, 81–90.

Lawless, H.T., Corrigan, C.J., & Lee, C.B. (1994). Interactions of astringent substances. *Chemical Senses,* 19, 141,154.

Lawless, L.J.R. & Civille, G.V. (2013). Developing Lexicons: A Review. *Journal of Sensory Studies,* 28, 270-281.

Lawless, H.T., & Heymann, H. (2010). *Sensory evaluation of food: Principles and practices.* 2nd Ed. New York: Springer.

Lezaeta, A., Bordeu, E., Næs, T., & Varela, P. (2017). Exploration of consumer perception of Sauvignon Blanc wines with enhanced aroma properties using two different descriptive methods. *Food Research International*, 99, 186-197.

Lê, S., Josse, J., & Husson, F. (2008). FactoMineR: An R package for multivariate analysis. *Journal of Statistical Software*, 25(1), 1–18.

Lea, A.G.H., & Arnold, G.M. (1978). The phenolics of ciders: bitterness and astringency. *Journal of the Science of Food and Agriculture*, 29, 478–483.

Lee, C.B. & Lawless, H.T. (1991). Time-course of astringent sensations. *Chemical Senses,* 16, 225–238.

Lee, C.A., & Vickers, Z.M. (2012). Astringency of foods may not be directly related to salivary lubricity. *Journal of Food Science*, 77, S302–S306.

Lee, C.A., & Vickers, Z.M. (2008). The astringency of whey protein beverages iscaused by their acidity. *International Dairy Journal,* 18, 1153–1156.

Lee, C.A., & Vickers, Z.M. (2010). Discrimination among astringent samples is affected by choice of palate cleanser. *Food Quality and Preference,* 21, 93-99.

Lehrer, A. 1975. Talking about wine. *Language,* 51, 901-923.

Lemieux, L., & Simard, R.E. (1994). Astringency, a textural defect in dairy products. *Lait*, 74(3), 217-240.

Lenfant, F., Loret, C., Pineau, N., Hartmann, C., & Martin, N. (2009). Perception of oral food breakdown. The concept of sensory trajectory. *Appetite,* 52, 659-667.

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, 330S-335S.

Lesschaeve, I., & Noble, A.C. (2010). Sensory analysis of wine. In A. Reynolds (Ed.). *Managing Wine Quality* (pp.189-217). Cambridge : Woodhead Publishing Limited.

Liang, N.-N., Zhu, B.-Q., Han, S., Wang, J.-H., Pan, Q.-H., Reeves, M.J., … He, F. (2014). Regional characteristics of anthocyanin and flavonol compounds from grapes of four Vitis vinifera varieties in five wine regions of China. *Food Research International*, 64, 264-274.

Lloret, A., Boido, E., Carrau, F., Disegna, E., Menendez, M., & Dellacassa, E. (2003). Evaluación del contenido y perfil de componentes antociánicos durante la maduración de uvas Tannat con respecto a otras variedades tintas. *Proccedings for the IX Congreso Latinoamericano de Viticultura y Enología* (pp. 64-65). (Santiago de Chile, Chile).

Lockshin, L. (1998). Involved or not involved. *Australian and New Zealand Wine Industry Journal,* 13(3), 302–303.

Lockshin, L., Quester, P., & Spawton, T. (2001). Segmentation by involvement or nationality for global retailing: A cross-national comparative study of wine shopping behaviours. *Journal of Wine Research*, 12(3), 223–236.

Lockshin, L.S., Spawton, A.L., & MacIntosh, G. (1997). Using product, brand and purchasing involvement for retail segmentation. *Journal of Retailing and Consumer Services*, 4(3), 171–183.

Lucak, C.L., & Delwiche, J.F. (2009). Efficacy of various palate cleansers with representative foods. *Chemosensory Perception*, 2, 32-39.

Luck, G., Liao, H., Murray, N., Grimmer, H., Warminski, E., Williamson, M., … Haslam, E. (1994). Polyphenols,astringency and proline-rich proteins. *Phytochemistry*, 37, 357–371.

Lyman, B.J. & Green, B.G. (1990). Oral astringency: Effects of repeated exposure and interactions with sweeteners. *Chemical Senses*, 15, 151-164.

Ma, W., Guo, A., Zhang, Y., Wang, H., Liu, Y., & Li., H. (2014). A review on astringency and bitterness perception of tannins in wine. *Trend in Food Science & Technology*, 40, 6-19.

Marshall, D., & Bell, R. (2004). Relating the food involvement scale to demographic variables, food choice and other constructs. *Food Quality and Preference*, 15, 871–879.

McRae, J.M., & Kennedy, J.A. (2011). Wine and Grape Tannin Interactions with Salivary Proteins and Their Impact on Astringency: A Review of Current Research. *Molecules*, 16, 2348-2364.

Meilgaard, M.C., & Muller, J.E. (1987). Progress in descriptive analysis of beer and brewing products. *Technical Quarterly of the Master Brewers Association of America*, 24, 79-85.

Meillon, S., Urbano, C., & Schlich, P. (2009). Contribution of the Temporal Dominance of Sensations (TDS) method to the sensory description of subtle differences in partially dealcoholized red wines. *Food Quality and Preference,* 20, 490-499.

Meyners, M., & Castura, J.C. (2016). Randomization of CATA attributes: Should attribute lists be allocated to assessors or to samples? *Food Quality and Preference*, 48, 210-215.

Meyners, M., Castura, J.C., & Carr, B.T. (2013). Existing and new approaches for the analysis of CATA data. *Food Quality and Preference*, 30, 309–319.

Misnawi, A., Jinap, S., Jamilah, B., & Nazamid, S. (2004). Sensory properties of cocoa liquor as affected by polyphenol concentration and duration of roasting. *Food Quality and Preference*, 15, 403–409.

Mittal, B., & Lee, M.-S. (1989). A causal model of consumer involvement. *Journal of Economic Psychology,* 10, 363-389.

Modell, S. (2005). Triangulation between case study and survey methods in management accounting research: An assessment of validity implications. *Management Accounting Research,* 16, 231–254.

Monteleone, E., Condelli, N., Dinnella, C., & Bertuccioli, M. (2004). Prediction of perceived astringency induced by phenolic compounds. *Food Quality and Preference*, 15, 761-769.

Mouret, M., Lo Monaco, G., Urdapilleta, I., & Parr, W.V. (2013). Social representations of wine and culture: A comparison between France and New Zealand. *Food Quality and Preference,* 30, 102–107.

Niimi, J., Liu, M., & Bastian, S.E.P. (2017). Flavour-tactile cross-modal sensory interactions: The case for astringency. *Food Quality and Preference*, 62, 106–110.

Noble, A. C. (1995). Application of time-intensity procedures for the evaluation of taste and mouthfeel. *American Journal of Enology and Viticulture,* 46,128–133.

Noble, A.C. (2002). Astringency and bitterness of flavonoid phenols. In P. Given, & D. Paredes (Eds.). *Chemistry of Taste. Mechanisms, Behaviors and Mimics* (pp. 192-199). Washington, D.C: American Chemical Society.

Oberholster, A., Elmendorf, B.L., Lerno, L.A., King, E.S., Heymann, H., Brenneman, C.E., & Boulton, R.B. (2015). Barrel maturation, oak alternatives and micro-oxygenation: Influence on red wine ageing and quality. *Food Chemistry*, 173, 1250-1258.

Obreque-Slier, E., López-Solís, R., Peña-Neira, Á., & Zamora-Marín, F. (2010). Tannin-protein interaction is more closely associated with astringency than tannin-protein precipitation: experience with two oenological tannins and a gelatin. *International Journal of Food Science & Technology*, 45, 2629-2636.

Obreque-Slier, E., Peña-Neira, A., & López-Solís, R. (2010a). Enhancement of both salivary protein-enological tannin interactions and astringency perception by ethanol. *Journal of Agricultural and Food Chemistry*, 58(6), 3729−3735.

Obreque-Slier, E., Peña-Neira, Á., & López-Solís, R. (2010b). Interactions of enological tannins with the protein fraction of saliva and astringency perception are affected by pH. *LWT - Food Science and Technology*, 45, 88-93.

Ortega-Heras, M., Pérez-Magariño, S., Cano-Mozo, E., & González-San José, M.L. (2010). Differences in the phenolic composition and sensory profile between red wines aged in oak barrels and wines aged with oak chips. *LWT - Food Science and Technology,* 43, 1533-1541.

Ospina, R., & Ferrari, S.L.P. (2010). Inflated beta distributions. *Statistical Papers*, 51, 111-126.

Ovejero-López, I., Bro, R., & Bredie, W.L.P. (2005). Univariate and multivariate modelling of flavour release in chewing gum using time-intensity: A comparison of data analytical methods. *Food Quality and Preference*, 16, 327–343.

Parr, W.V., Ballester, J., Peyron, D., Grose, C., & Valentin, D. (2015). Perceived minerality in Sauvignon wines: Influence of culture and perception mode. *Food Quality and Preference,* 41, 121–132.

Payne, C., Bowyer, P.K., Herderich, M., & Bastian, S.E.P. (2009). Interaction of astringent grape seed procyanidins with oral epithelial cells. *Food Chemistry*, 115, 551-557.

Peleg, H., Gacon, K., Schlich, P., & Noble, A. C. (1999). Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. *Journal of the Science of Food and Agriculture*, 79, 1123-1128.

Peynaud, E. (1987). *The taste of wine: the art and science of wine appreciation.* London: Macdonald Orbis.

Peyvieux, C., & Dijksterhuis, G. (2001). Training a sensory panel for TI: a case study. *Food Quality and Preference*, 12, 19-28.

Pickering, G.J., & Robert, G. (2006). Perception of mouthfeel sensations elicited by red wine is associated with sensitivity to 6-n-propylthiouracil (PROP). *Journal of Sensory Studies,* 21, 249–265.

Pickering, G.J., Simunkova, K., & DiBattista, D. (2004). Intensity of taste and astringency sensations elicited by red wines is associated with sensitivity to PROP (6-n-propylthiouracil). *Food Quality and Preference*, 15, 147-154.

Pineau, N., Schlich, P., Cordelle, S., Mathonnière, C., Issanchou, S., Imbert, A., … Köster, E. (2009). Temporal Dominance of Sensations: Construction of the TDS curves and comparison with time–intensity. *Food Quality and Preference*, 20, 450–455.

Podio, N.S., Baroni, M.V., & Wunderlin, D.A. (2017). Relation between polyphenol profile and antioxidant capacity of different Argentinean wheat varieties. A Boosted Regression Trees study. *Food Chemistry*, 232, 79-88.

Podio, N.S., López-Froilán, R., Ramirez-Moreno, E., Bertrand, L., Baroni, M.V., Pérez-Rodríguez, M.L., … Wunderlin, D.A. (2015). Matching in Vitro Bioaccessibility of Polyphenols and Antioxidant Capacity of Soluble Coffee by Boosted Regression Trees. *Journal of Agricultural and Food Chemistry*, 63, 9572−9582.

Preys, S., Mazerolles, G., Courcoux, P., Samson, A., Fischer, U., Hanafi, M., … Cheynier, V. (2006). Relationship between polyphenolic composition and some sensory properties in red wines using multiway analyses. *Analytica Chimica Acta*, 563, 126-136.

Quijada-Morín, N., Regueiro, J., Simal-Gándara, J., Tomás, E., Rivas-Gonzalo, J.C., & Escribano-Bailón, M.T. (2012). Relationship between the Sensory-Determined Astringency and the Flavanolic Composition of Red Wines.*Journal of Agricultural and Food Chemistry*, 60, 12355-12361.

Quijada-Morín, N., Williams, P., Rivas-Gonzalo, J.C., Doco, T., & Escribano-Bailón, M.T. (2014). Polyphenolic, polysaccharide and oligosaccharide composition of Tempranillo red wines and their relationship with the perceived astringency. *Food Chemistry*, 154, 44–51.

R Core Team (2017). *R: A language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing.

Reinbach, H.C., Giacalone, D., Ribeiro, L.M., Bredie, W.L.P., & Frøst, M.B. (2014). Comparison of three sensory profiling methods based on consumer perception: CATA, CATA with intensity and Napping®. *Food Quality and Preference*, 32, 160–166.

Rendall, R., Pereira, A.C., & Reis, M.S. (2017). Advanced predictive methods for wine age prediction: Part I – A comparison study of single-block regression approaches based on variable selection, penalized regression, latent variables and tree-based ensemble methods. *Talanta*, 171, 341-350.

Revelle, W. (2013) psych: Procedures for Personality and Psychological Research, Evanston, Illinois: Northwestern University. Available at: http://CRAN.R-project.org/package=psych Version = 1.4.1.

Ribéreau-Gayon, P., Glories, Y., Maujean, A. & Dubourdieu, D. (2006). *Handbook of Enology. Volume 2. The Chemistry of Wine. Stabilization and Treatments*. 2nd Edition. Chippenham, Wiltshire: John Wiley & Sons.

Ribéreau-Gayon, P., & Stonestreet, E. (1966). Le dosage des tanins du vin rouge et determination de leur structure. *Chemical Analysis*, 48, 188-192.

Ridgeway, G. Package 'gbm'. (2017). Available at: https://cran.r-project.org/web/packages/gbm/gbm.pdf. (Accessed: 15.11.17).

Robichaud, J.L. & Noble, A.C. (1990). Astringency and bitterness of selected phenolics in wine. *Journal of the Science of Food and Agriculture*, 53, 343–353.

Ross, C.F., Hinken, C., & Weller, K. (2007). Efficacy of palate cleansers for reduction of astringency carryover during repeated ingestions of red wine. *Journal of Sensory Studies*, 22, 293-312.

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, 1984-1992.

Rusell Bernard, H. (2005). Free listing. In H. Rusell Bernard (Ed.), *Research methods in anthropology: Qualitative and quantitative approaches* (pp. 301–311). 4th ed. Lanham, MD: AltaMiraPress.

Sáenz-Navajas, M.-P., Avizcuri, J.-M., Ballester, J., Fernández-Zurbano, P., Ferreira, V., Peyron, D., & Valentin, D. (2015). Sensory-active compounds influencing wine experts' and consumers' perception of red wine intrinsic quality. *LWT - Food Science and Technology*, 60, 400-411.

Sáenz-Navajas, M.P., Avizcuri, J.M., Echávarri, J.F., Ferreira, V., Fernández-Zurbano, P., & Valentin, D. (2016). Understanding quality judgements of red wines by experts: Effect of evaluation condition. *Food Quality and Preference*, 16, 216–227.

Sáenz-Navajas, M.-P., Avizcuri, J.-M., Ferreira, V., & Fernández-Zurbano, P. (2012). Insights on the chemical basis of the astringency of Spanish red wines. *Food Chemistry*, 134, 1484-1493.

Sáenz-Navajas, M.P., Avizcurri, J.M., Ferreria, V., & Fernández-Zurbano, P. (2014). Sensory changes during bottle storage of Spanish red wines under different initial oxygen doses. *Food Research International*, 66, 235-246.

Sáenz-Navajas, M.-P., Avizcuri, J.-M., Ferrero-del-Teso, S., Valentin, D., Ferreira, V., & Fernández-Zurbano, P. (2017). Chemo-sensory characterization of fractions driving different mouthfeel properties in red wines. *Food Research International*, 94, 54-64.

Sáenz-Navajas, M.P., Ballester, J., Pêcher, C., Peyron, D., & Valentin, D. (2013). Sensory drivers of intrinsic quality of red wines. Effect of culture and level of expertise. *Food Research International*, 54, 1506–1518.

Sáenz-Navajas, M.P., Campo, E., Fernández-Zurbano, P., Valentin, D., & Ferreira, V. (2010). An assessment of the effects of wine volatiles on the perception of taste and astringency in wine. *Food Chemistry*, 121, 1139–1149.

Sáenz-Navajas, M.P., Tao, Y.S., Dizy, M., Ferreira, V., & Fernández-Zurbano, P. (2010). Relationship between nonvolatile composition and sensory properties of Premium Spanish red wines and their correlation to quality perception. *Journal of Agricultural and Food Chemistry*, 58(23), 12407–12416.

Sarnoski, P.J., Johnson, J.V., Reed, K.A., Tanko, J.M., & O'Keefe, S.F. (2012). Separation and characterization of proanthocyanidins in Virginia type peanut skins by LC-MSn. *Food Chemistry*, 131, 927-939.

Scharbert, S., Holzmann, N., & Hofmann, T. (2004). Identification of astringent taste compounds in black tea infusions by combining instrumental analysis and human bioresponse. *Journal of Agricultural and Food Chemistry*, 52, 2498–2508.

Schiffman, S.S., Suggs, M.S., Sostman, A.L., Simon, S.A. (1992). Chorda tympani and lingual nerve responses to astringent compounds in rodents. *Physiology & Behavior*, 51, 55–63.

Schöbel, N., Radtke, D., Kyereme, J., Wollmann, N., Cichy, A., Obst, K., … Hatt, H. (2014). Astringency is a trigeminal sensation that involves the activation of G protein-coupled signaling by phenolic compounds. *Chemical Senses*, 39, 471-487.

Scollary, G.R., Pásti, G., Kállay, M., Blackman, J., & Clark, A.C. (2012). Astringency response of red wines: Potential role of molecular assembly. *Trends in Food Science & Technology*, 27, 25-36.

Smith, A.K., June, H., & Noble, A.C. (1996). Effects of viscosity on the bitterness and astringency of grape seed tannin. *Food Quality and Preference*, 7, 161-166.

Smith, P.A., McRae, J.M., & Bindon, K.A. (2015). Impact of winemaking practices on the concentration and composition of tannins in red wine. *Australian Journal of Grape and Wine Research*, 21, 601–614.

Smith, V., Møgelvang-Hansen, P., & Hyldigc, G. (2010). Spin versus fair speak in food labelling: A matter of taste? *Food Quality and Preference,* 21, 1016-1025.

Sokolowsky, M., & Fischer, U. (2012). Evaluation of bitterness in white wine applying descriptive analysis, time-intensity analysis, and temporal dominance of sensations analysis. *Analytica Chimica Acta*, 732, 46–52.

Solomon, G.E.A. (1990). Psychology of novice and expert wine talk*. American Journal of Psychology,* 103, 495-517.

Sowalsky, R.A., & Noble, A.C. (1998). Comparison of the effects of concentration, pH and anion species on astringency and sourness of organic acids. *Chemical Senses,* 23, 343–349.

Stone, H., & Sidel, J. L. (2004). *Sensory evaluation practices* (3rd ed.). San Diego: Academic Press.

Swahn, J., Öström, Å., Larsson, U., & Gustafsson, I.-B. (2010). Sensory and semantic language model for red apples. *Journal of Sensory Studies*, 25, 591-615.

Symoneaux, R., & Galmarini, M.V. (2014). Open-ended questions. In P. Varela & G. Ares (Eds.), *Novel techniques in sensory characterization and consumer profiling*. Boca Ratón: CRC Press.

ten Kleij, F., & Musters, P.A.D. (2003). Text analysis of open ended survey responses: A complementary method to preference mapping. *Food Quality and Preference,* 14, 43–52.

Thorngate, J.H., & Noble, A.C. (1995). Sensory evaluation of bitterness and astringency of 3R(-)-epicatechin and 3S(+)-catequin. *Journal of the Science of Food and Agriculture*, 67, 531-535.

Troszyńska, A., Amarowicz, R., Lamparski, G., Wołejszo, A., & Baryłko-Pikielna, N. (2006). Investigation of astringency of extracts obtained from selected tannins-rich legume seeds. *Food Quality and Preference*, 17, 31-35.

Valentin, D., Chollet, S., Lelievre, M., & Abdi, H. (2012). Quick and dirty but still pretty good: A review of new descriptive methods in food science. *International Journal of Food Science & Technology*, 47, 1563–1578.

Valentová, H., Skrovánková, S., Panovská, Z., & Pokorný, J. (2001). Determination of astringent taste in model solutions and in beverages. *Czech Journal of Food Science*, 19, 196–200.

Varela, P., & Ares, G. (2012). Sensory profiling, the blurred line between sensory and consumer science. A review of novel methods for product characterization. *Food Research International*, 48, 893–908.

Varela, P., & Gámbaro, A. (2006). Sensory descriptive analysis of Uruguayan Tannat wine: correlation to quality assessment. *Journal of Sensory Studies*, 21, 203-217.

Viala, P., & Vernorell, V. (1903). *Traité général de viticulture. Tomo IV - Ampèlographie*, 1a ed. París: Masson et cie.

Vidal, L., Antúnez, L., Giménez, A., & Ares, G. (2016). Evaluation of palate cleansers for astringency evaluation of red wines. *Journal of Sensory Studies*, 31, 93-100.

Vidal, L., Antúnez, L., Giménez, A., Medina, K., Boido, E., Ares, G. (2017). Sensory characterization of the astringency of commercial Uruguayan Tannat wines. *Food Research International*, 102, 425-434.

Vidal, S., Courcoux, P., Francis, L., Kwiatkowski, M., Gawel, R., Williams, P., … Cheynier, V. (2004). Use of an experimental design approach for evaluation of key wine components on mouth-feel perception. *Food Quality and Preference*, 15, 209-217.

Vidal, S., Francis, L., Guyot, S., Marnet, N., Kwiatkowski, M., Gawel, R., ... Waters, E. J. (2003). The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. *Journal of the Science of Food and Agriculture*, 83, 564–573.

Vidal, S., Francis, L., Noble, A., Kwiatkowski, M., Cheynier, V, & Waters, E. (2004). Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. *Analytica Chimica Acta*, 513, 57–65.

Vidal, S., Francis, L., Williams, P., Kwiatkowski, M., Gawel, R., Cheynier, V, & Waters, E. (2004). The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium. *Food Chemistry*, 85, 519-525.

Vidal, L., Giménez, A., Medina, K., Boido, E., & Ares, G. (2015). How do consumers describe wine astringency? *Food Research International*, 78, 321-326.

Wansink, B. & Painter, J. (2001). Descriptive menu labels effect on sales. *Cornell Hotel And Restaurant Administration Quarterly*, 42, 68–72.

Zamora, M.C., & Guirao, M. (2004). Performance comparison between trained assessors and wine experts using specific sensory attributes. *Journal of Sensory Studies*, 19, 530-545.

APPENDIX

Table A1. List of compounds (tentatively) identified and cuantified by HPLC-MS in the 40 samples of Tannat wine.

Compound	RT (min)	MS (m/z)	Fragments MS^2 (m/z)	Identification
Flavan-3-ols				
Prodelphinidin dimer	3.9	$[M+H]^+$ 595	-	Tentative
Procyanidin trimer	3.9	$[M-H]^-$ 865	695/713/739/572/451/407/289	MS^2
Prodelphinidin dimer (2)	4.5	$[M+H]^+$ 595	-	Tentative
(+)-gallocatechin	5.5	$[M-H]^-$ 305	-	Tentative
Prodelphinidin dimer (3)	5.9	$[M+H]^+$ 595	-	Tentative
Prodelphinidin trimer	8.7	$[M-H]^-$ 881	-	Tentative
Prodelphinidin dimer (4)	9	$[M+H]^+$ 595	-	Tentative
Prodelphinidin trimer (2)	9.3	$[M-H]^-$ 881	-	Tentative
Prodelphinidin trimer (3)	10.8	$[M-H]^-$ 881	-	Tentative
Prodelphinidin trimer (4)	11.5	$[M-H]^-$ 881	-	Tentative
Procyanidin tetramer	11.9	$[M+H]^+$ 1155	-	Tentative
Procyanidin dimer B-type	12.5	$[M-H]^-$ 577	425/407/289/287	MS^2
Procyanidin dimer B-type (2)	12.7	$[M-H]^-$ 577	425/407/289/287	MS^2
Prodelphinidin dimer (5)	13.9	$[M+H]^+$ 595	-	Tentative
Prodelphinidin trimer (5)	14.4	$[M-H]^-$ 881	-	Tentative
(-)-epigallocatechin	14.5	$[M-H]^-$ 305	-	Tentative
Prodelphinidin trimer (6)	15.7	$[M-H]^-$ 881	-	Tentative
(+)-catechin	15.9	$[M+H]^+$ 291	-	Standard
Procyanidin tetramer (2)	16.2	$[M+H]^+$ 1155	-	Tentative
Prodelphinidin trimer (7)	16.5	$[M-H]^-$ 881	-	Tentative
Procyanidin dimer B-type (3)	17.8	$[M-H]^-$ 577	425/407/289/287	MS^2
Procyanidin tetramer (3)	17.8	$[M+H]^+$ 1155	-	Tentative
Procyanidin trimer (2)	17.9	$[M-H]^-$ 865	695/713/739/572/451/407/289	MS^2
Prodelphinidin trimer (8)	18.3	$[M-H]^-$ 881	-	Tentative
Procyanidin tetramer (4)	18.6	$[M+H]^+$ 1155	-	Tentative
Procyanidin dimer B-type (4)	19	$[M-H]^-$ 577	425/407/289/287	MS^2
Procyanidin trimer (3)	19	$[M-H]^-$ 865	695/713/739/572/451/407/289	MS^2
Prodelphinidin trimer (9)	19.1	$[M-H]^-$ 881	-	Tentative
Procyanidin trimer (4)	19.1	$[M-H]^-$ 865	695/713/739/572/451/407/289	MS^2
Prodelphinidin trimer (10)	19.5	$[M-H]^-$ 881	-	Tentative
Procyanidin tetramer (5)	19.9	$[M+H]^+$ 1155	-	Tentative
(-)-epicatechin	20.4	$[M+H]^+$ 291	-	Tentative
Prodelphinidin trimer (11)	20.5	$[M-H]^-$ 881	-	Tentative
Procyanidin tetramer (6)	21.1	$[M+H]^+$ 1155	-	Tentative
Procyanidin trimer (5)	21.3	$[M-H]^-$ 865	695/713/739/572/451/407/289	MS^2
Procyanidin dimer B-type (5)	21.7	$[M-H]^-$ 577	425/407/289/287	MS^2
Procyanidin tetramer (7)	21.9	$[M+H]^+$ 1155	-	Tentative
Procyanidin trimer (6)	22.1	$[M-H]^-$ 865	695/713/739/572/451/407/289	MS^2
Procyanidin tetramer (8)	22.6	$[M+H]^+$ 1155	-	Tentative
Procyanidin trimer (7)	23.7	$[M-H]^-$ 865	695/713/739/572/451/407/289	MS^2
Procyanidin dimer B-type (6)	23.8	$[M-H]^-$ 577	425/407/289/287	MS^2
(-)-epicatechin gallate	23.9	$[M+H]^+$ 443	-	Tentative
Procyanidin dimer B2-3'-O-gallate	25	$[M+H]^+$ 731	-	Tentative
Procyanidin dimer B-type (7)	27.7	$[M-H]^-$ 577	425/407/289/287	MS^2

Table A1. *(Continued)* List of compounds (tentatively) identified and cuantified by HPLC-MS in the 40 samples of Tannat wine.

Compound	RT (min)	MS (m/z)	Fragments MS^2 (m/z)	Identification
Phenolic acids				
Gallic acid	3.2	$[M-H]^-$ 169	-	Standard
Protocatechuic acid	6.2	$[M-H]^-$ 153	-	Tentative
Methyl gallate	8.5	$[M-H]^-$ 183	-	Tentative
cis-caftaric acid	10.4	$[M-H]^-$ 311	-	Tentative
trans-fertaric acid	15.8	$[M-H]^-$ 325	-	Tentative
trans-caftaric acid	15.9	$[M-H]^-$ 311	-	Tentative
p-coumaric acid	17.3	$[M-H]^-$ 163	-	Tentative
p-coumaroyl hexose	17.7	$[M-H]^-$ 325	-	Tentative
trans-caffeic acid	17.7	$[M-H]^-$ 179	-	Tentative
p-coumaroyl hexose (2)	20.4	$[M-H]^-$ 325	-	Tentative
p-coumaroyl hexose (3)	20.9	$[M-H]^-$ 325	-	Tentative
Anthocyanins				
Delphinidin-3-O-glucoside	19.2	$[M]^+$ 465	-	Tentative
Cyanidin-3- O-glucoside	20.2	$[M]^+$ 449	-	Tentative
Petunidin-3- O-glucoside	20.7	$[M]^+$ 479	317	MS^2
Peonidin-3- O-glucoside	21.6	$[M]^+$ 463	-	Standard
Malvidin-3- O-glucoside	21.9	$[M]^+$ 493	-	Standard
Delphinidin-3- O-acetylglucoside	22.6	$[M]^+$ 507	303	MS^2
Cyanidin-3- O-acetylglucoside	23.6	$[M]^+$ 491	-	Tentative
Petunidin-3- O-acetylglucoside	23.8	$[M]^+$ 521	-	Tentative
Peonidin-3- O-acetylglucoside	24.7	$[M]^+$ 505	301	MS^2
Malvidin-3- O-acetylglucoside	24.8	$[M]^+$ 353	-	Tentative
Delphinidin-3- O-(p-coumaroyl)- glucoside	25.6	$[M]^+$ 611	-	Tentative
Petunidin-3- O-(p-coumaroyl)- glucoside	26	$[M]^+$ 625	317	MS^2
Cyanidin-3- O-(p-coumaroyl)- glucoside	26.7	$[M]^+$ 595	-	Tentative
Peonidin-3- O-(p-coumaroyl)- glucoside	26.9	$[M]^+$ 609	301	MS^2
Malvidin-3- O-(p-coumaroyl)- glucoside	26.9	$[M]^+$ 639	-	Tentative
Flavonols				
Quercetin-3-O-glucuronide	20.6	$[M-H]^-$ 477	301	MS^2
Myricetin-3-O- glucoside	22.6	$[M+H]^+$ 481	-	Tentative
Quercetin-3-O- glucoside	23.8	$[M+H]^+$ 465	-	Tentative
Isorhamnetin-3-O- glucoside	24.4	$[M-H]^-$ 477	315	MS^2
Laricetrin-3-O- glucoside	24.4	$[M+H]^+$ 495	-	Tentative
Siringetin-3-O- glucoside	25.8	$[M-H]^-$ 507	-	Tentative
Myricetin aglycone	26.7	$[M-H]^-$ 317	-	Tentative
Quercetin aglycone	29.7	$[M-H]^-$ 301	-	Standard
Laricetrin aglycone	29.9	$[M-H]^-$ 331	-	Tentative

Table A2. List of variables that were considered as predictors in the Boosted Regression Trees models. p-Values for the sample effect of the Analysis of Variance performed for each variable is shown.

	Predictor variable	p value
	Oenological parameters	
1	Ethanol	<0.0001
2	Total acidity	<0.0001
3	Volatile acidity	<0.0001
4	pH	<0.0001
5	Tannin concentration	<0.0001
	Flavan-3-ols (mg/L)	
6	(-)-epigallocatechin	<0.0001
7	(+)-catechin	<0.0001
8	(-)-epicatechin	<0.0001
9	Procyanidin dimer B-type	0.0356
10	Procyanidin dimer B-type (2)	<0.0001
11	Procyanidin dimer B-type (3)	0.0014
12	Procyanidin dimer B-type (4)	<0.0001
13	Procyanidin dimer B-type (6)	<0.0001
14	Prodelphinidin dimer	<0.0001
15	Prodelphinidin dimer (2)	<0.0001
16	Prodelphinidin dimer (3)	<0.0001
17	Prodelphinidin dimer (4)	<0.0001
18	Prodelphinidin dimer (5)	<0.0001
19	Procyanidin trimer	<0.0001
20	Procyanidin trimer (2)	<0.0001
21	Procyanidin trimer (3)	<0.0001
22	Procyanidin trimer (5)	<0.0001
23	Procyanidin trimer (6)	<0.0001
24	Procyanidin trimer (7)	0.0060
25	Prodelphinidin trimer	0.0001
26	Prodelphinidin trimer (3)	<0.0001
27	Prodelphinidin trimer (4)	<0.0001
28	Prodelphinidin trimer (5)	<0.0001
29	Prodelphinidin trimer (7)	<0.0001
30	Prodelphinidin trimer (8)	<0.0001
31	Prodelphinidin trimer (9)	<0.0001
32	Prodelphinidin trimer (10)	<0.0001
33	Prodelphinidin trimer (11)	<0.0001
34	Procyanidin tetramer	<0.0001
35	Procyanidin tetramer (3)	0.0011
36	Procyanidin tetramer (4)	0.0002
37	Procyanidin tetramer (5)	0.0013
38	ΣTrimer non-galloylated	<0.0001
39	ΣTetramer non-galloylated	<0.0001
40	Σnon-galloylated	<0.0001
41	∑ procyanidin	0.0332
42	∑ prodelphinidin	<0.0001
43	∑ monomer	<0.0001
44	∑ oligomer	<0.0001
45	∑ flavanol	<0.0001

Table A2. *(Continued)* List of variables that were considered as predictors in the Boosted Regression Trees models. p-Values for the sample effect of the Analysis of Variance performed for each variable is shown.

	Predictor variable	p value
	Phenolic acids (mg/L)	
46	Gallic acid	<0.0001
47	*cis*-caftaric acid	<0.0001
48	trans-fertaric acid	0.0001
49	trans-caftaric acid	<0.0001
50	p-coumaroyl hexose	<0.0001
51	trans-caffeic acid	<0.0001
52	p-coumaroyl hexose (3)	<0.0001
53	∑ hydroxybenzoic acid	<0.0001
54	∑ hydroxycinnamic acid	<0.0001
55	∑ phenolic acid	0.0003
	Anthocyanins (mg/L)	
56	Delphinidin-3-O-glucoside	<0.0001
57	Cyanidin-3-O-glucoside	<0.0001
58	Petunidin-3-O-glucoside	<0.0001
59	Peonidin-3-O-glucoside	<0.0001
60	Malvidin-3-O-glucoside	<0.0001
61	Delphinidin-3-O-acetylglucoside	<0.0001
62	Cyanidin-3-O-acetylglucoside	<0.0001
63	Petunidin-3-O-acetylglucoside	<0.0001
64	Peonidin-3-O-acetylglucoside	<0.0001
65	Malvidin-3-O-acetylglucoside	<0.0001
66	Delphinidin-3-O-(p-cumaroyl)glucoside	<0.0001
67	Petunidin-3-O-(p-cumaroyl)glucoside	0.0003
68	Cyanidin-3-O-(p-cumaroyl)glucoside	0.0039
69	Peonidin-3-O-(p-cumaroyl)glucoside	0.0057
70	Malvidin-3-O-(p-cumaroyl)glucoside	<0.0001
71	∑ glucoside	<0.0001
72	∑ acetylglucoside	<0.0001
73	∑ coumaroylglucoside	<0.0001
74	∑ anthocyanin	<0.0001
	Flavonols (mg/L)	
75	Quercetin-3-O-glucuronide	<0.0001
76	Myricetin-3-O- glucoside	<0.0001
77	Quercetin-3-O- glucoside	<0.0001
78	Isorhamnetin-3-O-glucoside	<0.0001
79	Laricetrin-3-O-glucoside	<0.0001
80	Siringetin-3-O- glucoside	<0.0001
81	Myricetin aglycone	0.0294
82	Quercetin aglycone	<0.0001
83	Laricetrin aglycone	<0.0001
84	∑ flavonol	<0.0001

Table A3. Median and range (between brackets) of the predictor's relative importance (%) of the 50 Boosted Regression Trees models that were built for each response variable: astringency maximum intensity (Imax) determined by time-intensity method and percentage of use of each term included in the check-all-that-apply question to describe astringency sub-qualities of Tannat wine samples. Only the 34 predictors that were amongst the five predictors with highest median relative importance for at least one of the responses are shown.

Variable	Imax	Dry	Silky	Fine emery	Suede	Rough	Aggressive	Sand paper	Mouthcoating	Velvety	Puckery	Harsh	Abrasive	Hard	Coarse grain	Irritant	Complex
Oenological parameters																	
Ethanol	0 (0-3)	3 (0-13)	0 (0-4)	0 (0-1)	0 (0-1)	0 (0-15)	0	0 (0-1)	1 (0-8)	0 (0-1)	0 (0-17)	0 (0-1)	0 (0-8)	0 (0-2)	0 (0-5)	3 (0-15)	**7 (0-40)**
Total acidity	0 (0-2)	0 (0-1)	0 (0-6)	0 (0-16)	0 (0-1)	0 (0-2)	0	2 (0-26)	0 (0-2)	**6 (0-35)**	0 (0-4)	0 (0-5)	**24 (6-52)**	1 (0-25)	0 (0-6)	0 (0-2)	0 (0-7)
Volatile acidity	2 (0-25)	**4 (0-40)**	0 (0-11)	0 (0-9)	0 (0-8)	0 (0-7)	0 (0-8)	0 (0-10)	**10 (0-55)**	0 (0-3)	0	0 (0-10)	1 (0-47)	1 (0-46)	1 (0-9)	0 (0-5)	0 (0-3)
pH	0 (0-5)	0 (0-1)	0 (0-9)	1 (0-14)	0 (0-8)	0 (0-14)	3 (0-12)	0	0 (0-2)	0 (0-4)	1 (0-16)	1 (0-13)	**9 (0-38)**	0 (0-1)	1 (0-15)	**3 (0-22)**	0 (0-1)
Tannin concentration	**17 (4-37)**	1 (0-8)	**35 (11-74)**	**10 (0-41)**	**22 (3-66)**	**13 (1-48)**	**31 (4-57)**	**6 (0-40)**	**6 (0-23)**	2 (0-23)	**5 (0-30)**	1 (0-6)	**8 (0-32)**	1 (0-10)	**13 (3-61)**	0 (0-6)	0 (0-1)
Flavan-3-ols (mg/L)																	
(-)-epicatechin	0 (0-1)	0	1 (0-24)	0 (0-1)	0 (0-15)	0 (0-2)	0 (0-2)	0 (0-2)	2 (0-33)	1 (0-34)	0	0 (0-5)	0	2 (0-33)	3 (0-18)	0	**6 (0-29)**
(-)-epigallocatechin	0 (0-1)	0 (0-5)	0 (0-2)	0	1 (0-9)	0 (0-3)	0 (0-3)	0 (0-7)	0 (0-2)	0 (0-2)	**5 (0-25)**	0 (0-2)	0	0 (0-1)	0 (0-2)	2 (0-13)	0 (0-1)
Procyanidin dimer B-type (2)	0 (0-2)	**18 (1-61)**	0 (0-4)	0 (0-5)	**3 (0-17)**	0 (0-4)	0 (0-1)	0 (0-10)	**3 (1-51)**	0 (0-10)	0	**26 (5-56)**	0 (0-7)	0 (0-2)	0 (0-16)	0 (0-7)	0 (0-5)
Procyanidin dimer B-type (3)	0 (0-4)	0 (0-7)	0 (0-40)	1 (0-50)	1 (0-8)	2 (0-74)	0 (0-4)	0 (0-2)	0	0 (0-5)	0 (0-18)	0 (0-1)	0 (0-2)	**6 (0-26)**	0 (0-3)	0 (0-1)	1 (0-32)
Prodelphinidin dimer (4)	0 (0-2)	0 (0-2)	0 (0-8)	**4 (0-30)**	0 (0-1)	0 (0-1)	0 (0-3)	0 (0-6)	1 (0-10)	0 (0-1)	0	0 (0-4)	0 (0-2)	0 (0-1)	0 (0-4)	**9 (0-32)**	0 (0-1)
Procyanidin trimer (3)	0 (0-1)	0 (0-5)	0 (0-1)	0 (0-4)	0	0 (0-1)	1 (0-8)	2 (0-19)	3 (0-31)	0 (0-1)	0 (0-14)	0 (0-10)	0 (0-6)	0 (0-1)	1 (0-22)	**18 (2-70)**	0 (0-7)
Procyanidin trimer (7)	0 (0-1)	1 (0-23)	**2 (0-23)**	1 (0-13)	0 (0-9)	0 (0-4)	0 (0-4)	**2 (0-21)**	0	**54 (0-79)**	0 (0-2)	1 (0-16)	4 (0-18)	0 (0-4)	**8 (0-42)**	0	1 (0-9)
Prodelphinidin trimer (7)	**18 (0-39)**	0 (0-7)	**2 (0-15)**	0 (0-4)	0 (0-2)	0 (0-1)	0 (0-1)	1 (0-10)	0 (0-2)	0 (0-5)	2 (0-15)	0 (0-4)	0	0 (0-3)	1 (0-8)	**5 (0-33)**	0 (0-8)
Prodelphinidin trimer (8)	0 (0-4)	0 (0-10)	0 (0-4)	**12 (0-47)**	0 (0-1)	0 (0-23)	0	0 (0-1)	2 (0-14)	**4 (0-26)**	0 (0-5)	0 (0-1)	3 (0-15)	0 (0-13)	1 (0-19)	0 (0-10)	0 (0-1)
Prodelphinidin trimer (9)	**12 (0-36)**	1 (0-17)	0 (0-4)	0 (0-1)	0 (0-11)	0 (0-12)	0 (0-2)	0 (0-16)	0 (0-4)	0 (0-7)	**4 (0-22)**	0 (0-1)	0 (0-9)	2 (0-20)	0	0 (0-1)	0 (0-2)
Prodelphinidin trimer (10)	2 (0-17)	2 (0-36)	1 (0-15)	0 (0-9)	0 (0-14)	**6 (0-23)**	0 (0-2)	0 (0-1)	0 (0-4)	0 (0-7)	0 (0-11)	0 (0-1)	0 (0-2)	0 (0-4)	0 (0-2)	1 (0-11)	0 (0-1)
Procyanidin tetramer (3)	2 (0-10)	0 (0-3)	0 (0-4)	0 (0-1)	0 (0-3)	0	0 (0-9)	**14 (1-58)**	1 (0-10)	**3 (0-55)**	0 (0-7)	1 (0-6)	1 (0-24)	2 (0-23)	**6 (0-28)**	0 (0-2)	0
Procyanidin tetramer (4)	0 (0-2)	0 (0-5)	0	0 (0-28)	0 (0-2)	0 (0-11)	0 (0-2)	0 (0-3)	0 (0-1)	0 (0-4)	0 (0-9)	0 (0-2)	0 (0-2)	0 (0-3)	**4 (0-23)**	2 (0-19)	0 (0-2)
ΣTetramer non-galloylated	3 (0-17)	0	0 (0-7)	1 (0-17)	0 (0-4)	0 (0-2)	**4 (0-22)**	0 (0-8)	0 (0-2)	**2 (0-26)**	1 (0-20)	**17 (0-43)**	1 (0-14)	**6 (0-28)**	3 (0-22)	0 (0-1)	0 (0-2)
Σmonomer	0 (0-3)	0 (0-1)	0 (0-3)	0	1 (0-20)	0 (0-1)	0 (0-6)	0 (0-22)	0 (0-5)	0 (0-1)	0 (0-3)	0	0 (0-1)	**5 (0-34)**	0 (0-1)	0	**9 (0-34)**
Phenolic acids (mg/L)																	
Gallic acid	2 (0-14)	1 (0-6)	0 (0-1)	0 (0-6)	3 (0-14)	2 (0-14)	**8 (0-36)**	2 (0-48)	0 (0-11)	0 (0-1)	0 (0-17)	0 (0-1)	0	0 (0-1)	1 (0-15)	0 (0-1)	2 (0-26)
cis-caftaric acid	0 (0-1)	1 (0-6)	0 (0-5)	1 (0-13)	0 (0-3)	0 (0-3)	0 (0-2)	0 (0-1)	0 (0-2)	0 (0-3)	0 (0-1)	1 (0-25)	0 (0-5)	1 (0-14)	0 (0-2)	0 (0-1)	**6 (0-20)**
trans-caffeic acid	0 (0-1)	0 (0-1)	0 (0-3)	0 (0-5)	**4 (0-22)**	1 (0-14)	0 (0-2)	0 (0-1)	0 (0-8)	0 (0-6)	0 (0-10)	**3 (0-13)**	0 (0-6)	**6 (0-29)**	0 (0-14)	0 (0-5)	2 (0-20)
Σhydroxybenzoic acid	**4 (0-25)**	2 (0-29)	0 (0-3)	0 (0-3)	0 (0-7)	0 (0-1)	0 (0-1)	0 (0-4)	1 (0-17)	0 (0-1)	0 (0-7)	0 (0-1)	0 (0-3)	0 (0-1)	0 (0-5)	0 (0-5)	0 (0-1)
Σphenolic acid	0 (0-5)	0 (0-1)	1 (0-10)	**3 (0-26)**	2 (0-15)	**7 (1-22)**	**19 (6-55)**	**19 (0-47)**	0 (0-3)	1 (0-11)	3 (0-49)	2 (0-10)	0 (0-5)	1 (0-18)	**5 (0-31)**	0 (0-5)	0 (0-14)
Anthocyanins (mg/L)																	
Cyanidin-3-O-glucoside	0 (0-2)	**4 (0-23)**	0 (0-2)	**7 (0-49)**	0 (0-3)	0 (0-14)	1 (0-5)	0 (0-2)	0 (0-2)	2 (0-15)	0 (0-6)	0 (0-2)	0 (0-1)	0 (0-2)	0 (0-1)	0 (0-2)	0 (0-27)
Cyanidin-3-O-acetylglucoside	0	**4 (0-35)**	0	0 (0-5)	0 (0-3)	0 (0-6)	0 (0-3)	0 (0-14)	3 (0-14)	1 (0-10)	0 (0-5)	0 (0-2)	0	0 (0-1)	0	0	0 (0-2)
Malvidin-3-O-acetylglucoside	0 (0-2)	0 (0-3)	1 (0-9)	0 (0-1)	2 (0-25)	1 (0-10)	0 (0-4)	0 (0-5)	1 (0-6)	0 (0-2)	0 (0-2)	**5 (0-23)**	0 (0-1)	0 (0-1)	0 (0-2)	0 (0-16)	1 (0-8)
Petunidin-3-O-(p-cumaroyl)glucoside	0 (0-5)	0 (0-1)	0 (0-7)	0 (0-33)	0 (0-3)	0 (0-2)	0 (0-2)	0 (0-11)	0	0 (0-1)	0 (0-9)	0 (0-2)	0 (0-1)	1 (0-12)	0 (0-1)	**8 (0-29)**	3 (0-14)
Flavonols (mg/L)																	
Laricetrin-3-O-glucoside	3 (0-13)	2 (0-35)	0 (0-3)	0 (0-1)	0 (0-9)	0 (0-3)	0 (0-1)	0 (0-3)	0 (0-1)	0 (0-3)	**12 (1-42)**	0 (0-4)	0 (0-2)	0 (0-1)	4 (0-15)	0 (0-8)	2 (0-19)
Laricetrin aglycone	1 (0-14)	1 (0-9)	0 (0-5)	0 (0-10)	**7 (0-34)**	**6 (0-37)**	1 (0-11)	2 (0-15)	**5 (0-12)**	0 (0-17)	0 (0-4)	0 (0-1)	**8 (0-36)**	0 (0-9)	1 (0-11)	1 (0-4)	0 (0-1)
Myricetin aglycone	0 (0-4)	**5 (0-20)**	**8 (0-20)**	0 (0-7)	1 (0-12)	**9 (0-37)**	**6 (1-17)**	1 (0-9)	0 (0-2)	0 (0-5)	2 (0-30)	0 (0-5)	0 (0-8)	0 (0-5)	0 (0-3)	1 (0-9)	0 (0-1)
Quercetin aglycone	**12 (2-29)**	1 (0-11)	**12 (3-16)**	0 (0-2)	**21 (4-58)**	0 (0-4)	0 (0-1)	0 (0-13)	**13 (1-28)**	0 (0-17)	**5 (0-23)**	**3 (0-20)**	**8 (0-29)**	**9 (0-28)**	2 (0-31)	0 (0-7)	**5 (0-30)**
Σflavonol	0 (0-2)	0 (0-3)	0 (0-4)	0	0 (0-4)	0 (0-16)	0 (0-2)	**4 (0-25)**	0 (0-4)	0 (0-1)	1 (0-22)	0 (0-4)	0 (0-4)	0 (0-1)	0	0 (0-6)	3 (0-20)