OENO One
VINE AND WINE OPEN ACCESS JOURNAL
Volume 51 > Number 3 > 2017

Oenological perspective of red wine astringency

Ignacio García-Estévez, Rosa Pérez-Gregorio, Susana Soares, Nuno Mateus, Victor de Freitas*

REQUIMTE/LAQV, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, 687, 4169-007 Porto, Portugal

Abstract

Red wine is a complex matrix and its organoleptic characteristics are affected by a high complexity of wine compounds, namely polyphenols. One of the most important sensations and also a quality attribute of red wine is astringency. Over the years, the main mechanism proposed for astringency perception is the interaction and precipitation of salivary proteins (in particular proline-rich proteins) by red wine polyphenols, mainly tannins. Furthermore, it is also known that this interaction is affected by other red wine compounds such as carbohydrates.

The polyphenolic composition of red wine could be modified by modulation of the several oenological steps. Hence, alterations in the oenological steps could allow the modulation of astringency and/or astringency sub-qualities. Crucial oenological steps include ripening, maceration and fermentation stabilization (fining), filtration and aging. Along this review, the influence of each of these oenological processes on wine polyphenolic composition is systematically reviewed, with a focus on how it affects astringency from an oenological perspective.

Keywords: salivary proteins–wine tannins interaction; proline-rich proteins; wine carbohydrates; ripening; maceration; fermentation; stabilization (fining); filtration; aging

manuscript received 20th January 2017 - accepted 12th May 2017
doi: 10.20870/oeno-one.2017.51.2.1816

*Corresponding author: vfreitas@fc.up.pt

OENO One, 2017, **51**, 3, 237-249

General remarks

Wine is a complex matrix and its taste perception is directly determined by a balance among sensory active compounds such as acids, sugars, ethanol and polyphenols. Thus, wine organoleptic characteristics are affected by a high complexity of wine compounds that could be modulated by different oenological techniques. Oenological technology is supposed to be used to enhance wine quality attributes through the modulation of wine composition and its physicochemical characteristics. Several mouthfeel attributes, such as astringency and bitterness among others, could change depending on the oenological practice. Along this review, the influence of oenological processes on wine composition is comprehensively reviewed focusing mainly on astringency, providing a global view of this organoleptic sensation from an oenological perspective.

Astringency in wines

Astringency is one of the most significant and complex mouthfeel and quality attributes of red wine that is usually well known to most individuals. From a perceptual point of view, there is little or no consensus about whether astringency is a single sensation or a general category made up of multiple sub-qualities. The ASTM on Sensory Evaluation of Materials and Products defined astringency 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, 1989).

Although astringency is often described as an unpleasant sensation, as in the case of unripe fruits, it could be pleasant and desirable in the case of high-quality red wines.

Due to its importance, astringency has been largely studied in wine over the last decades and some important reviews have been published (de Freitas *et al.*, 2012; Upadhyay *et al.*, 2016) Even though astringency could be elicited by different classes of compounds (e.g. metal salts, acids and alcohol) in red wine, it is mainly due to polyphenol compounds, namely tannins.

Several major gaps exist in the literature concerning astringency, and its mechanisms have been thoroughly and controversially discussed for a long time. It is assumed that astringency is a tactile sensation not a taste because it can be perceived in regions of the oral cavity where there exist no taste receptors (Soares *et al.*, 2017).

Currently, the mechanisms proposed for astringency onset include polyphenol–salivary protein interaction leading to a loss of saliva lubricating properties, the interaction of polyphenols with oral cells and/or the activation of mechanoreceptors. The most accepted one is the interaction between polyphenols and salivary proteins proposed by Bate-Smith in 1954. This mechanism has been supported by numerous studies, which have shown good correlations between the perceived astringency and the ability of astringent compounds to interact/precipitate proteins (Sun *et al.*, 2013 ; Rinaldi *et al.*, 2012).

Salivary proteins are usually divided into several major families, including proline-rich proteins (PRPs), statherin, cystatins, P-B peptide, histatins and mucins (Messana *et al.*, 2008) PRPs seem to be the most important family of salivary proteins concerning astringency (Gawel, 1998). PRPs are a heterogeneous family characterized by a high content in proline residues (25-42%), which in turn can be divided in acidic, basic and glycosylated proteins (aPRPs, bPRPs, gPRPs, respectively) (Manconi *et al.*, 2016). The bPRPs family is the most studied family and is reported as having the highest affinity for tannins. In fact, no other specific functional role than binding tannins, preventing the possible deleterious effects of these compounds, has been attributed to these proteins (Bennick, 2002; Lu and Bennick, 1998) However, recent *in vitro* and *in vivo* studies have reported a stronger interaction between polyphenols and aPRPs or P-B peptide than with bPRPs (Quijada-Morín *et al.*, 2016; Brandao *et al.*, 2014) Moreover, it seems that protein glycosylation (gPRPs) prevents the formation of large-size aggregates that would lead to protein precipitation, as a result of a more open conformation of the protein structure (Soares *et al.*, 2011; Sarni-Manchado, 2008). Conversely, it has been also demonstrated that some compounds, although unable to precipitate proteins, are perceived as astringents (Rossetti *et al.*, 2008). Thus, it seems that astringency correlates better with the strength of salivary protein–tannin interaction than with the ability of polyphenols to precipitate proteins (Obreque-Slier *et al.*, 2010). Another salivary protein showing a high content of proline residues (ca. 50% of its sequence) is the P-B peptide. Despite the high content in proline residues, this peptide shows several differences in its structure when comparing to PRPs, so it is usually considered out of the PRPs family (Messana et al., 2008).

Regarding tannins, hydrolysable tannins (gallo- and ellagitannins) and condensed tannins (also known as proanthocyanidins) are the most important polyphenolic compounds present in wines that are

able to interact with proteins and, therefore, the most related to astringency perception. However, other wine phenolic compounds, such as flavonols, phenolic acids or anthocyanins, can also play an important role in astringency development.

Concerning proanthocyanidins, there are key structural characteristics that govern their ability to interact with proteins (Figure 1), namely the molecular weight, the interflavanic bond, the conformation, the presence of galloyl groups (galloylation) and the substitution pattern of the B-ring.

Briefly, with regard to molecular weight, it seems that the more polymerized, the more astringent the proanthocyanidins are (Hufnagel and Hofmann, 2008). In fact, the procyanidin's ability to bind PRPs also seems to increase with molecular weight (de Freitas *et al.*, 2002). Moreover, it has been proposed that astringency is also affected by the type of subunits of the structure, with a remarkable importance of the content of epicatechin subunits in extension positions of the proanthocyanidins (Quijada-Morín *et al.*, 2012). Concerning galloylation, it seems that the presence of galloyl groups increases the affinity towards proteins and, consequently, the astringency (Scharbert *et al.*, 2004). This can be attributed to the ability of galloyl groups for establishing hydrogen bonds with the proteins, thus strengthening and stabilizing the interaction (Charlton *et al.*, 2002; Li and Hagerman, 2014) . Finally, regarding the B-ring substitution pattern, proanthocyanidins could be divided in two groups: dihydroxylated (catechins, which polymerize leading to procyanidins) and trihydroxylated (gallocatechins, present in prodelphinidin structure). It seems that the substitution pattern of the B-ring is decisive for the interaction with salivary proteins. Considering the location in grape, both prodelphinidins and procyanidins are present in skins, whereas only procyanidins are present in seeds. The differences in reactivity and astringency between these two groups of condensed tannins are important, which has to be considered since their levels in wines depend of winemaking and extraction. It seems that catechins have more affinity for proteins than gallocatechins, the former being more astringent, dry, rough, unripe, and persistent and the latter being smoother, more velvety, and viscous (Ferrer-Gallego *et al.*, 2015). Thus, it seems that the astringency elicited by prodelphinidins could be more desirable than the one elicited by procyanidins, which is in accordance with the preference of winemakers for grape skin tannins over grape seed tannins.

Influence of oenological technology in wine astringency

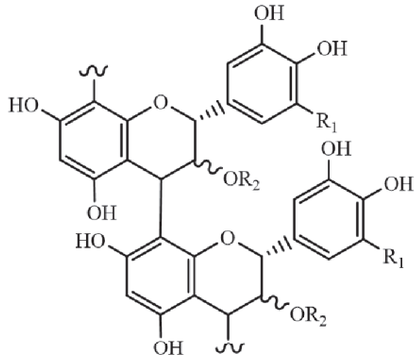
Oenological technology is supposed to be used to enhance wine quality attributes through the modulation of wine composition and its physicochemical characteristics. Several mouthfeel sensations such as bitterness, astringency and alcoholic mouthfeel could change depending on the oenological practice. Moreover, physicochemical changes like colour stability could be induced by oenological practice. Along this section, the influence of oenological technology on wine composition is reviewed focusing on astringency perception.

1. Ripening

The selection of the optimal moment of grape ripeness may be considered as the most crucial decision in winemaking. Optimal ripeness will vary depending on the style of wine that is being produced (sparkling, still, rosé, etc.) (González-Caballero *et al.*, 2012). In viticulture, ripeness constitutes the optimal characteristic of wine grapes to be harvested. Once the grapes are harvested, the physical and chemical components of the grape that will influence wine quality are essentially set.

As the grapes go through veraison, sugars in the grapes will continue to rise as acid levels fall. The balance between sugar (and so the potential alcohol level) and acids is considered one of the most critical aspects of producing quality wine. Thus, both the relative density and "total acidity" of the must, as well as the pH of the grapes, are evaluated to determine ripeness.

Towards the end of the 20th century, winemakers began focusing on the concept of achieving



R_1: H (procyanidins) *or* OH (prodelphinidins)
R_2: H (non-galloylated) *or* galloyl moiety (galloylated)

Figure 1. General structure of wine proanthocyanidins.

OENO One, 2017, **51**, 3, 237-249

"phenolic" ripeness in the grapes. This is described as a more complete ripeness of tannins and other polyphenolic compounds like anthocyanins (coloured compounds). The polyphenolic composition of grape skin, seed and pulp changes during ripening and consequently influences red wine astringency.

There is still a gap in the real influence of ripening on astringency perception because grape tannin composition is affected not only by extrinsic factors like agronomical conditions or grape development stage but also by different intrinsic factors like genotype. Furthermore, the mean degree of polymerization (mDP) decreases after veraison, but it was recently described that the differences observed in tannin mDP could be overestimated by the analytical method (Meléndez *et al.*, 2013). In general, tannin accumulation in grape seed and skin occurs early in berry development; it is completed when ripeness starts and, after that, tannin content remains unchanged or decreases after veraison (Quijada-Morín *et al.*, 2016; Bogs *et al.*, 2007).

Besides total tannin composition, it is important to elucidate if polyphenol extractability also changes during ripening. The extractability of polyphenols is defined as the percentage of total polyphenols extracted during winemaking. This determines the polyphenolic content of wines and is a key element in astringency perception. The extractability of skin and seed tannins during berry development is also different between varieties: tannins become easier to extract during the ripening of Tempranillo berries (Meléndez *et al.*, 2013) but harder during the ripening of Monastrell berries (Fournand *et al.*, 2006). In Sangiovese grapes, total tannin content decreases after veraison but extractable tannins remain unchanged (Allegro *et al.*, 2016). Tannin structure also affects their extractability. Allegro *et al.* (2016) showed that while extractable (epi)catechin decreases after veraison, extractable epigallocatechin and epicatechin gallate increases along ripeness.

Anthocyanin behaviour is opposite to the one observed for tannins. Anthocyanin accumulation in skins starts at veraison and is maximal around harvest (Fournand *et al.*, 2006; Mateus *et al.*, 2002).. Some studies describe a decrease in total anthocyanins just before harvest and/or during over-ripening. In Tempranillo grapes, anthocyanin extractability peaks increase in overripe berries even though the total anthocyanin content has already declined (Fournand *et al.*, 2006). In contrast, no changes in anthocyanin extractability were observed from veraison to harvest in Shiraz berries (Del Llaudy *et al.*, 2008). So, if the polyphenolic composition evolves at ripening, the astringency will also depend on the ripening moment.

As abovementioned, the balance between sugar and acids is considered critical, but the evaluation of the sugar content and acid profile alone does not fully express the real oenological potential of grapes. Indeed, a prediction of the possible astringency intensity could only be estimated by polyphenolic composition. The knowledge of the polyphenolic characteristics of the grapes allows the maceration and winemaking process to be planned in order to fully exploit the potentiality that the grape reaches in the vineyard. Thus, the term "phenolic ripeness" was introduced to describe the concentration of polyphenolic compounds in grapes and the ease with which they are released (Cagnasso *et al.*, 2008).

However, recent researches suggested that astringency perception could be also related to other wine compounds, namely carbohydrates (Wratelot *et al.*, 2017; Boulet *et al.*, 2016). Choosing the optimal ripening date to control wine astringency affects not only phenolic ripeness but also other compounds, namely carbohydrates. However, keeping in mind that there is no balance between phenolic and sugar (carbohydrates) contents during ripeness, the harvest date is difficult to choose.

Presently it could be anticipated that polysaccharides influence astringency through a direct mechanism in which cell wall polysaccharides bind to tannins reducing their extractability or an indirect way through a competition process at a sensory level. In fact, the previously referred differences in anthocyanin extractability among varieties could be correlated with the polysaccharide content of the skin. With regard to different grape varieties, there is no consistent trend in polysaccharide composition associated with ripeness. Polysaccharide composition varies among grape varieties but the individual influence of each polysaccharide is not clear. Some authors have stated that there is no link between polysaccharide composition and tannin binding capacity of cell walls (Hanlin *et al.*, 2010), whereas other studies revealed a different effect in tannin–cell wall binding depending on the polysaccharide tested (Le Bourvellec *et al.*, 2005). However, in general, several polysaccharide families have been described to be able to interact with tannins (Le Bourvellec *et al.*, 2005). Extractability increases when there are low concentrations of pectin, cellulose, arabinoxylan, arabinogalactan and xyloglucan. Polysaccharides would therefore limit the concentration of available proanthocyanidins and, thus, astringency would be reduced.

At a sensory level, it is known that polysaccharides could inhibit the interaction of red wine tannins with proteins, as it has been previously proposed for the loss of astringency in ripening fruits (Ozawa *et al.*, 1987) Several *in vitro* studies have shown that this inhibition could occur by two different mechanisms: (i) polysaccharides could interfere with tannins' ability to bind proteins through encapsulation or (ii) some polysaccharides could inhibit the precipitation process by the formation of ternary complexes (aqueous solubility is enhanced) (Carvalho *et al.*, 2006).

Several structural and chemical changes occur during ripening, leading to the softening of the cellular structure of many fruits. These changes affect especially hemicelluloses, pectins and cellulose microfibrils of the cell wall (Fasoli *et al.*, 2016). Pectin modification is mainly responsible for the progressive loss of firmness during ripening. Fruit softening allows the release of small pectin soluble fragments of the cellular structure during ripening. Furthermore, the xyloglucan content of hemicellulose decreases significantly during berry growth and ripening, whereas cellulose levels are only marginally affected (Fasoli *et al.*, 2016).

Sensory analysis has been also used to study the influence of polysaccharides on astringency perception in model wine solutions, showing that all polysaccharide families reduce the perception of astringency to some degree. These studies revealed that acidic polysaccharides have a greater impact on the reduction of astringency perception. Neutral polysaccharides also tend to decrease the intensity of astringency attributes. Nevertheless, in that study, the differences between model wine and the fraction containing a mixture of mannoproteins and type II arabinogalactan proteins isolated from wine were not statistically significant (Quijada-Morín *et al.*, 2014).

Overall, the effect of ripening time on astringency perception is quite complex. In general, several oenological techniques have been used to enhance the composition deficiencies derived from a trade-off harvest data. Harvesting grapes at different times has been also used to improve wine sensory properties.

2. Maceration and fermentation

Grape seeds and skin tannins are transferred to must/wine during the maceration step of winemaking. The maceration process could be induced by using hydrolytic enzymes, mainly against pectins, cellulose and hemicellulose. Hydrolytic enzymes are supposed to break down the interaction between proanthocyanidins and cell walls. The effective extraction of proanthocyanidins from grapes will depend on having enough knowledge of the nature of these interactions and on the ability to disrupt or manage these associations. Hence, the higher tannin extraction efficiency, the higher tannin content in wines, which, in turn, will participate in the astringency process. As regards the nature of these associations, it has been found that electrostatic or ionic interactions do not seem to play any important role in the association between proanthocyanidins and cell-wall material, rather the adsorption mechanism seems to involve the establishment of weak interactions, more precisely hydrogen bonds and hydrophobic interactions (Castro-López *et al.*, 2016). Enzymes exert their effect by deconstructing the polysaccharide network of the cell wall and by allowing the extraction of the phenolic compounds located inside the vacuoles (Castro-López *et al.*, 2016; Bautista-Ortín *et al., 2016a*). The use of maceration enzyme results in an increase in the proanthocyanidin content of must and wine, not only by favouring the extraction of these compounds from skin cell vacuoles but also by promoting a lower adsorption on cell walls (Castro-López *et al.*, 2016; Bautista-Ortín *et al., 2016a*). It was therefore concluded that the use of enzymes during maceration could promote higher astringent wines. Therefore, it is very important to acquire a deeper knowledge of the nature of cell walls–tannins interactions and the role played by the main enzymatic activities found in the commercial enzymes. This would allow a better understanding of the ability of these enzymes to disrupt or manage these associations, as well as the effect of the polysaccharides released after the action of these enzymes on the quantities of proanthocyanidins in solution.

Anthocyanin content is also affected by maceration. It has been demonstrated that knowing the quantities of anthocyanins in grape skins is not enough for estimating wine anthocyanin concentrations. This lack of correlation has been commonly attributed to the partial retention of these anthocyanins in the skin cells due to the barrier effect of the cell walls (Bautista-Ortín *et al.*, 2016b). Anthocyanins were also described to affect tannin extractability (Bautista-Ortín *et al.*, 2016b), so they could have an indirect influence on wine astringency. Recent studies suggested that the incorporation of anthocyanins in polymeric procyanidins seems to increase the amount of tannin retained in wine, thus explaining the larger quantities of tannins found in red wines and their higher astringency extractability (Bautista-Ortín *et al.*, 2016b). Two mechanisms were proposed: (i) the higher solubility of polymers

OENO One, 2017, **51**, 3, 237-249

formed from anthocyanins and condensed tannins or (ii) the competition between anthocyanins and tannins by cell-wall binding sites.

During winemaking, tannin extractability could considerably vary even under identical fermentation conditions. Furthermore, alcohol and surfactants in food or beverages may increase the solubility of the hydrophobic phenol–protein complexes, decreasing astringent sensations that may depend upon precipitation of salivary proteins (Lee and Lawless, 1991). The ethanol formed during fermentation not only modifies the solubility of must/wine compounds (mainly tannins), but is also directly related to astringency. As the ethanol level increases in model solution, a decrease in perceived astringency and in some astringent sub-qualities was observed (Vidal *et al.*, 2004). In case of red wine, the astringency sensation was reduced by ethanol (Meillon *et al.*, 2009). In fact, tannin–salivary protein interaction, which is the main mechanism in astringency development, depends on solvent composition. In the wine matrix, overall astringency usually decreases with increasing ethanol concentrations (from 0 to 15% ethanol). Moreover, ethanol can disrupt tannin–cell wall binding of apple pulp at concentrations between 20 and 40%, pointing out the influence of ethanol on noncovalent tannin–carbohydrate binding (McRae *et al.*, 2015.

Tannin extractability in wine fermentation was also related to the presence of grape-derived proteins (Springer et al., 2016). Removing proteins through prefermentation bentonite fining can promote two parallel events: (i) reduction of grape-derived protein and (ii) increase of wine tannin concentration. Sensory changes, mainly astringency changes, were observed as a consequence of the increase of phenolic concentration before fermentation.

Wine fermentation is usually promoted by inoculation with specific strains. *Saccharomyces cerevisiae* was the most common yeast used in controlled fermentation. Over the last few years the use of multi-starter inocula has become an attractive biotechnological practice in the search for wine with high flavour complexity or distinctive characters (Lencioni *et al.*, 2016. The use of *Saccharomyces* and non-*Saccharomyces* yeasts influences the ethanol and polysaccharide content in wine. Thus, mixed fermentations with *S. cerevisiae* and a selected strain of *Z. florentina* (formerly *Zygosaccharomyces florentinus*) caused an increase in the production of polysaccharides (Domizio *et al.*, 2011). Non-*Saccharomyces* yeasts have shown potential for producing wines with lower ethanol content. These yeast species, whose main sources are vineyard and grape surface, are present in the early stages of fermentation but, in general, are not capable of completing alcoholic fermentation. Sequential inoculations with *Saccharomyces* and non-*Saccharomyces* at different fermentation steps were proposed to obtain low ethanol concentrations in wines (Contreras *et al.*, 2014).

Given the influence of both ethanol and carbohydrates on astringency, it could be ascertained that the yeast used could also affect wine astringency. Hence, several studies proposed the astringency loose perception in wines obtained after mixed fermentations (Lencioni *et al.*, 2016). Despite the knowledge of the differences in carbohydrate content, ethanol levels and astringency sensation between using single or multi-starter inocula, there is still a gap in our knowledge of how the compositional changes could affect the overall astringency.

Grape skin is also a source of polysaccharides in wine. These compounds are localized in a highly soluble form within the grape intracellular spaces and are extracted into wine during maceration and fermentation. However, polysaccharides could also derive from yeast cell wall like mannoprotein polysaccharides (Pérez-Través *et al.*, 2016). Mannoprotein, a main component of *S. cerevisiae* cell wall (25-50% dry weight), comprising roughly 70-80% polysaccharide and 20-30% protein, may represent as much as 35% of wine polysaccharides (López-Solís *et al.*, 2017). It was already described that mannoproteins decreased the intensity of some astringent attributes and contributed to the fullness of model wine solutions (Vidal *et al.*, 2004). In this case, different mechanisms were proposed in the decrease of astringency modulated by mannoproteins: interaction with wine phenolics, reducing the levels of free reactive proanthocyanidins, or increasing salivary viscosity (Guadalupe *et al.*, 2010).

As mentioned, mannoprotein polysaccharides are extracted from yeast cell walls either during alcoholic fermentation or later by enzymatic action during wine contact with yeast lees (Pérez-Través *et al.*, 2016). Inactive dry yeasts were therefore used as an oenological treatment in the astringency sensation control. Inactive dry yeast products are mostly derived from cultured *S. cerevisiae* strains that are subsequently subjected to thermal or enzymatic inactivation. Interaction of an inactive dry yeast product (CP10) with either salivary protein or a proanthocyanidin-rich extract (binary mixtures) or

with both of them (ternary mixtures) was verified (López-Solís *et al.*, 2017). The use of commercial dry yeast preparations improves some sensory characteristics of white and red wines, probably due to the increase of neutral polysaccharides (Del Barrio-Galán *et al.*, 2012). In red wines, dry yeast preparations mainly reduced *green* tannins, increasing the softness on the palate. Therefore, they could be useful in order to improve the roundness and softness in the mouth, especially in young wines that are more astringent (Del Barrio-Galán *et al.*, 2012). It has also been reported that supplementation with commercial inactive yeasts in grape juice during winemaking and oak aging can significantly decrease the proanthocyanidin content of red wines coinciding with a decrease in high molecular weight mannoproteins (Del Barrio-Galán *et al.*, 2016). This suggests that the co-aggregates mannoprotein–tannin precipitate during treatment (González-Royo *et al.*, 2017). According to Gawel *et al.* (2016), the effect of polysaccharides on mouthfeel was independent of the concentration of phenolic substances. Polysaccharides have a relatively small effect on mouthfeel compared with the effect of wine pH and ethanol. Other authors have confirmed the interaction between mannoproteins and phenolics (Mekoue Nguela *et al.*, 2016). Given this controversy, further studies are required to better establish the influence of these commercial products on sensory attributes, mainly on astringency, according their chemical composition and purity.

3 Stabilization: fining

Wine stabilization and clearness is obtained after winemaking through the precipitation of unstable compounds and the sedimentation of the clouding particles. Wine clarification is naturally produced but this process is slow and may not be enough for proper clarity and stability of the wine. This process is therefore often improved by using different fining agents that will interact with the wine components, reaching a better clearness in less time and improved stability of the wines.

Fining agents could be protein based and, therefore, could induce some organoleptic changes. Astringency and bitterness of wine can decline due to its interaction with tannins. Colour changes could be also promoted. The fining process directly occurs from the precipitation of proanthocyanidins by these fining agents and it is influenced by the chemical characteristics of the protein used. The interactions between proanthocyanidins and fining agents therefore depend of the molecular weight and aminoacid composition of the proteins used (Maury *et al.*, 2001). Different protein types are used for

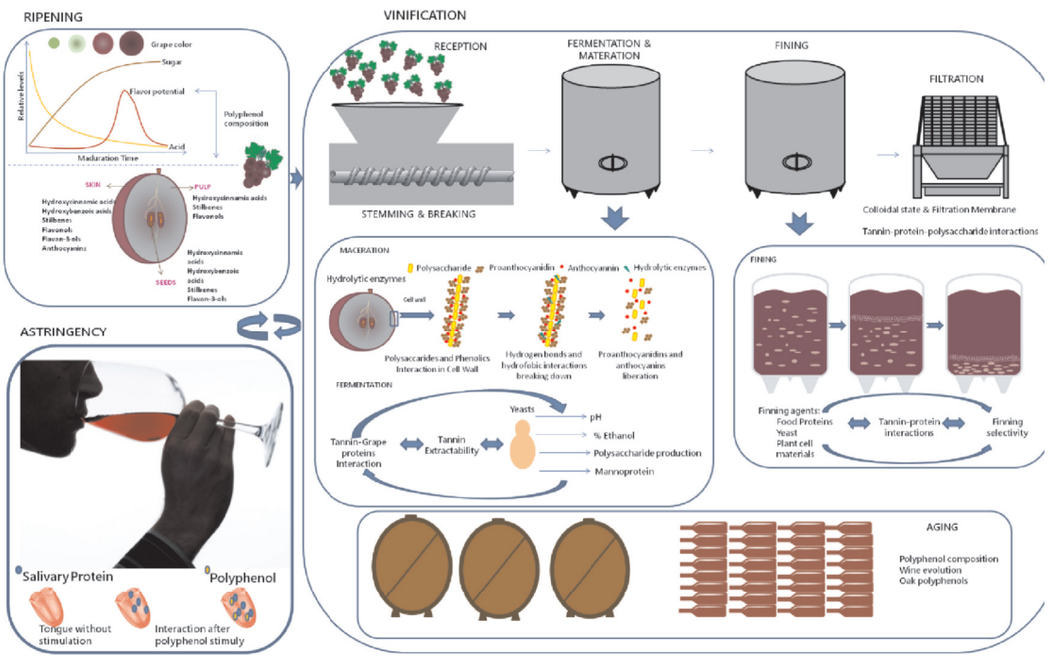


Figure 2. Overview of the main red wine oenological steps and most important influences of each step on wine physicochemical properties.

OENO One, 2017, **51**, 3, 237-249

wine fining. Gelatin, β-lactoglobulin, ovalbumin and casein are the proteins from animal origin most commonly used as fining agents. Different types of gelatin remove different amounts of tannins (9-16%) depending on the wine and gelatin composition (Smith *et al.*, 2015). General observations set that high polymerized tannins as well as high galloylated tannins were preferentially removed (Maury *et al.*, 2001).

Several authors observed that the addition of polysaccharides influenced the clarification process (Maury *et al.*, 2001; Vidal *et al.*, 2004). It was again reflected that other components of the wine matrix, mainly polysaccharides, are also important factors in mouthfeel perception. The effect appears to be dependent on the type of polysaccharide (Cheynier *et al.*, 2006). Polysaccharides influence the fining process since they influence the aggregation between tannins and proteins. The aggregations that can potentially occur may involve proanthocyanidin –proanthocyanidin, proanthocyanidin–protein, proanthocyanidin–polysaccharide and proantho cyanidin–protein–polysaccharide interactions (Scollary *et al.*, 2012).

Plant cell wall materials from grape and apple are also being used as fining agents. Moreover, in oenology, there is another possible technological interest arising from a deeper knowledge of proanthocyanidins–cell wall interactions, that is, using the pomace obtained after maceration as a fining agent to reduce the level of proanthocyanins in wines when they show a high degree of astringency (Guerrero *et al.*, 2013; Bautista-Ortín *et al.*, 2015). Pomace wastes from the winemaking process are widely available and a source of cell wall material. Besides, pomace cell wall material showed a very high degree of affinity for proanthocyanidins. How enzymes may be used to enhance or limit, if necessary, cell wall adsorption properties would be of great interest. Preliminary results suggest that the use of maceration enzymes could affect the retention capacity of the pomace cell walls and the extent to which high or low molecular mass proanthocyanidins are retained, which might have implications for the sensory properties of wine (Bautista-Ortín *et al.*, 2016).

4. Filtration

Keeping clarity and stability in finished wines is essential for winemakers. Filtering prior to bottling contributes to removing residual fining agents as well as providing wine stability both aesthetic and microbial.

Filtering aims to maintain clarity and stability, however, several sensory changes could be induced after this process as well as membrane fouling (Smith *et al.*, 2015). Wine colloidal properties could influence the filtering process. Red wine colloids include tannin–protein and tannin–polysaccharide complexes. As previously argued, these colloids are closely related to astringency perception. However, to our knowledge, no studies have focused on this point.

The concentration of tannins has been found to decrease significantly after fining with cross-flow membranes, suggesting that tannins are involved in membrane fouling. A higher concentration of tannins in wine also significantly decreased the flow rate of wines passing through a ceramic cross-flow membrane, which implies that high concentration of tannins in wine (greater than 1.25 g/L) could potentially foul membranes (Smith *et al.*, 2015). Several model solutions were tested in order to identify the impact of tannins and polysaccharides on membrane fouling (Rayess *et al.*, 2012). The main contribution was attributed to polysaccharides, particularly pectins, which have been shown to form a gel-like coating over cross-flow membranes, and mannoproteins (Rayess *et al.*, 2012).

Different types of filtration process could be used as well as membrane types. The way filtration was developed influences wine composition and, therefore, several sensory changes could occur. Cross-flow filtration showed a decrease in tannin mDP by up to 25% (Ulbricht *et al.*, 2009 ; Oberholster *et al.*, 2013).

There is controversy about the use of polyethersulfone or polypropylene membranes and tannin binding. Model wines filtered with polyethersulfone membranes demonstrated lower fluxes and a greater proportion of adsorbed tannins and polysaccharides than those filtered with polypropylene membranes (Ulbricht *et al.*, 2009). Other studies have demonstrated the opposite, that is, that tannins bind less well to polyethersulfone membranes than to polyvinylpyrrolidone (Schroën *et al.*, 2010). This could be explained by the polarity differences between membranes. Polarity is usually measured in ultrafiltration membranes in terms of relative hydrophobicity, which would influence the way the wine is cleaned. The hydrophobicity of polypropylene and polyethersulfone causes membrane fouling due to the adsorption of hydrophobic and amphoteric solutes in the feed. To be precise, with regard to the filtration of biological solutions, membrane fouling due to pore plugging,

pore narrowing and cake formation by protein is a considerable limitation for its application. Membranes with greater polarity have also greater interaction with phenolic substances because of greater hydrogen bonding (Schroën *et al.*, 2010; Schroën *et al.*, 2016).

The impact of filtration on tannin and polysaccharide concentration and composition was verified (Arriagada-Carrazana *et al.*, 2016), however, there was negligible difference in mouthfeel perception (Buffon *et al.*, 2014). Further research in this area is required to better understand the role of filtration in wine colloid structure and mouthfeel.

5. Aging

The commercial value of aged wines is mainly correlated with their colour stability and mouthfeel perception. These sensory attributes are closely related to phenolic composition (Chira *et al.*, 2011). An optimum balance between tannins and anthocyanins is essential to colour stability during red wine aging. Among sensory properties, wine aging has long been used not only to allow the development of focused flavours but also to get a mellow astringency (Chira *et al.*, 2012). The main reactions occurring during aging and involving colour stabilization are well known, whereas the impact of reactions involving proanthocyanidins and anthocyanins with regard to the decrease of wine astringency is still not well established. Greater astringency intensity is directly associated with higher concentration of tannins with a higher mDP (Soares *et al.*, 2007). During aging, astringency perception becomes mellower, so the reasons for the change in wine astringency could involve a decrease in tannin concentration accompanied by a decrease in tannin size because of subunit cleavage (Chira *et al.*, 2012; Bindon and Smith, 2014).

The aging process could be induced by several ways that could influence wine composition and properties in a different way. Wine could evolve in bottle or in barrels of different age and different wood origin. The cost of maintaining barrels and long-term storage of wine has prompted much research into new technologies to accelerate the aging process. These cheaper alternatives to barrels were the use of oak staves and aging on wine lees. Traditionally, red wines undergo barrel aging in French or American oak prior to bottling. Different favourable flavour compounds as well as tannin changes are induced during wood contact. Oak tannins are extracted from wood to wine, and barrel porous oxygenation also induces tannin changes. Ellagitannins are the most common tannins extracted from oak. This extraction could be oenologically driven by using staves. Extraction of phenolic compounds from oak staves is low, with a concentration up to 250 mg/L compared to around 2000-4000 mg/L of tannin in red wines regardless of oak treatment (Smith *et al.*, 2015). Instead of these compositional changes, a decrease in astringency during aging is supposed to be promoted to a higher extent by oxygenation.

Given the high reactivity of phenolics towards oxygen, many of the complex reactions involving phenolics are affected by oxygen exposure of wine. Thus, a moderate uptake of oxygen during aging can modulate specific reactions that further influence sensory properties, mainly astringency. Different methods of oxygen exposure were tried. Micro-oxygenation and nano-oxygenation were recommended instead of barrel oxygenation. Highly reactive quinones are formed after phenolic oxygen exposure. Tannin polymerization was further developed by direct and indirect condensation reactions between tannins and flavan-3-ols or anthocyanins (Sánchez-Ilárduya *et al.*, 2012). These reactions were favoured with wine aging as a result of low wine pH. The chemical bonds between flavan-3-ol subunits are continually broken and reformed, which also changes the tannin structure in such a way that it reduces water solubility and induces their precipitation in the bottle. The level of precipitation can possibly be mediated by interactions with polysaccharides (Guadalupe and Ayestarán, 2007).

Thus, the combined effects of the structural changes that occur in tannins during wine aging contribute to the softening of astringency during red wine aging.

This thorough literature review focused on the major red wine oenological steps and how each step influences wine compounds, specially polyphenols, carbohydrates and ethanol (Figure 2). In conclusion, the modulation of each step and the combined effect of different oenological practices modulate wine astringency contributing to a final full bodied, flavourful and balanced wine.

Acknowledgments : The authors would like to thank the Fundação para a Ciência e Tecnologia for financial support through two fellowships (SFRH/BPD/88866/2012 and SFRH/BPD/85293/2012) and project PTDC/AGR-TEC/6547/2014. The authors would also like to thank the LAQV (UID/QUI/50006/2013 - POCI/01/0145/FEDER /007265) for financial support from FCT/MEC through national funds and co-financed by FEDER under Partnership Agreement PT2020.

References

Allegro G., Pastore C., Valentini G., Muzzi E. and Filippetti I., 2016. Influence of berry ripeness on accumulation, composition and extractability of skin and seed flavonoids in cv. Sangiovese (*Vitis vinifera* L.). *Journal of the Science of Food and Agriculture. 96,* 4553-4559. doi:10.1002/jsfa.7672

Arriagada-Carrazana J.P., Sáez-Navarr ete C. and Bordeu E., 2005. Membrane filtration effects on aromatic and phenolic quality of Cabernet-Sauvignon wines. *Journal of Food Engineering 68*, 363-368. doi:10.1016/j.jfoodeng.2004.06.011

ASTM, *Standard Terminology to Sensory Evaluation of Materials and Products*, 1989. American Society of Testing and Materials: Philadelphia, PA, Vol. 15.07.

Bate-Smith E.C., 1954. Astringency in foods. *Food, 23*, 124.

Bautista-Ortín A.B, Ruiz-García Y., Marín F., Molero N., Apolinar-Valiente R. and Gómez-Plaza E., 2015. Remarkable proanthocyanidin adsorption properties of Monastrell pomace cell wall material highlight its potential use as an alternative fining agent in red wine production. *Journal of Agricultural and Food Chemistry, 63*, 620-633. doi:10.1021/jf503659y

Bautista-Ortín A., Ben Abdallah R., Castro-López L., Jiménez-Martínez M. and Gómez-Plaza E., 2016a. Technological implications of modifying the extent of cell wall-proanthocyanidin interactions using enzymes. *International Journal of Molecular Sciences, 17*, 123. doi:10.3390/ijms17010123

Bautista-Ortín A.B, Martínez-Hernández A., Ruiz-García, Y., Gil-Muñoz R. and Gómez-Plaza E., 2016b. Anthocyanins influence tannin–cell wall interactions. *Food Chemistry*, 2*06*, 239-248. doi:10.1016/j.foodchem.2016.03.045

Bennick A., 2002. Interaction of plant polyphenols with salivary proteins. *Critical Reviews in Oral Biology & Medicine***,** *13*, 184-196. doi:10.1016/j.foodchem.2016.03.045

Bindon K. A. and Smith P.A., 2013. Comparison of the affinity and selectivity of insoluble fibres and commercial proteins for wine proanthocyanidins. *Food Chemistry, 136*, 917-928. doi:10.1016/j.foodchem.2012.08.016

Bindon K. A., McCarthy M.G. and Smith P.A., 2014. Development of wine colour and non-bleachable pigments during the fermentation and aging of (*Vitis vinifera* L. cv.) Cabernet-Sauvignon wines differing in anthocyanin and tannin concentration. *LWT - Food Science and Technology***,** *59*, 923-932. doi:10.1016/j.lwt.2014.05.051

Bogs J., Jaffé F.W., Takos A. M., Walker A.R. and Robinson S.P., 2007. The grapevine transcription factor VvMYBPA1 regulates proanthocyanidin synthesis during fruit development. *Plant Physiology*, *143*, 1347-1361. doi:10.1104/pp.106.093203

Boulet J.C., Trarieux C., Souquet, J. M., Ducasse, M. A., Caillé S., Samson A., Williams P., Doco T. and Cheynier V., 2016. Models based on ultraviolet spectroscopy, polyphenols, oligosaccharides and polysaccharides for prediction of wine astringency. *Food Chemistry*, *190*, 357-363. doi:10.1016/j.foodchem.2015.05.062

Brandao E., Soares S., Mateus N. and de Freitas V., 2014. *In vivo* interactions between procyanidins and human saliva proteins: effect of repeated exposures to procyanidins solution. *Journal of Agricultural and Food Chemistry, 62*, 9562-9568. doi:10.1021/jf502721c

Buffon P., Heymann H. and Block D.E., 2014. Sensory and chemical effects of cross-flow filtration on white and red wines. *American Journal of Enology and Viticulture, 65*, 305-314. doi:10.5344/ajev.2014.13090

Cagnasso E., Rolle L., Caudana A. and Gerbi V., 2008. Relationship between grape phenolic maturity and red wine phenolic composition. *Italian Journal of Food Science***,** *20*, 365-381.

Carvalho E., Mateus N., Plet, B., Pianet I., Dufourc E. and De Freitas V., 2006. Influence of wine pectic polysaccharides on the interactions between condensed tannins and salivary proteins. *Journal of Agricultural and Food Chemistry***,** *54*, 8936-8944. doi:10.1021/jf061835h

Castro-López L.d.R., Gómez-Plaza E., Ortega-Regules A., Lozada D. and Bautista-Ortín A.B., 2016. Role of cell wall deconstructing enzymes in the proanthocyanidin–cell wall adsorption–desorption phenomena. *Food Chemistry***,** *196*, 526-532. doi:10.1016/j.foodchem.2015.09.080

Charlton A.J., Haslam E., Williamson M.P., 2002. Multiple conformations of the proline-rich protein/epigallocatechin gallate complex determined by time-averaged nuclear Overhauser effects. *Journal of the American Chemical Societ, 124*, 9899-9905. doi:10.1021/ja0126374

Cheynier V., Dueñas-Paton M., Salas E., Maury C., Souquet J. M., Sarni-Manchado P. and Fulcrand H., 2006. Structure and properties of wine pigments and tannins. *American Journal of Enology and Viticulture, 57*, 298-305.

Chira K., Pacella N., Jourdes M. and 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. doi:10.1016/j.foodchem.2010.12.056

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. doi:10.1007/s00217-011-1627-1

Contreras A., Hidalgo C., Henschke P. A., Chambers P. J., Curtin C. and Varela C., 2014. Evaluation of non-

Saccharomyces yeasts for the reduction of alcohol content in wine. *Applied and Environmental Microbiology, 80*, 1670-1678. doi:10.1128/AEM.03780-13

de Freitas V. and Mateus N., 2002. Nephelometric study of salivary protein-tannin aggregates. *Journal of the Science of Food and Agriculture, 82*, 113-119. doi:10.1002/jsfa.1016

de Freitas V. and Mateus N., 2012. Protein/polyphenol interactions: past and present contributions. Mechanisms of astringency perception. *Current Organic Chemistry*, *16*, 724-746. doi:10.2174/138527212799958002

Del Barrio-Galán R., Pérez-Magariño S., Ortega-Heras M., Guadalupe Z. and Ayestarán B., 2012. Polysaccharide characterization of commercial dry yeast preparations and their effect on white and red wine composition. *LWT - Food Science and Technology, 48*, 215-223. doi:10.1016/j.lwt.2012.03.016

Del Barrio-Galán R., Medel-Marabolí M. and Peña-Neira Á., 2016. Effect of different ageing techniques on the polysaccharide and phenolic composition and sensorial characteristics of Chardonnay white wines fermented with different selected *Saccharomyces cerevisiae* yeast strains. *European Food Research and Technology, 242*, 1069-1085. doi:10.1007/s00217-015-2612-x

Del Llaudy M.C., Canals R., Canals, J. M. and Zamora F., 2008. Influence of ripening stage and maceration length on the contribution of grape skins, seeds and stems to phenolic composition and astringency in wine-simulated macerations. *European Food Research and Technology, 226*, 337-344. doi:10.1007/s00217-006-0542-3

Domizio P., Romani, C., Lencioni L., Comitini F., Gobbi M., Mannazzu I. and Ciani M., 2011. Outlining a future for non-*Saccharomyces* yeasts: selection of putative spoilage wine strains to be used in association with *Saccharomyces cerevisiae* for grape juice fermentation. *International Journal of Food Microbiology, 147*, 170-180. doi:10.1016/j.ijfoodmicro.2011.03.020

Fasoli M., Dell'Anna R., Dal Santo S., Balestrini R., Sanson A., Pezzotti M., Monti F. and Zenoni S., 2016. Pectins, hemicelluloses and celluloses show specific dynamics in the internal and external surfaces of grape berry skin during ripening. *Plant and Cell Physiology*, *57*, 1332-1349. doi:10.1093/pcp/pcw080

Ferrer-Gallego R., Quijada-Morín N., Bras, N.F., Gomes P., de Freitas V., Rivas-Gonzalo J.C., Escribano-Bailon M.T., 2015. Characterization of sensory properties of flavanols - A molecular dynamic approach. *Chemical Sense, 40*, 381-390. doi:10.1093/chemse/bjv018

Fournand D., Vicens A., Sidhoum L., Souquet, J.-M., Moutounet M. and Cheynier V., 2006. Accumulation and extractability of grape skin tannins and anthocyanins at different advanced physiological stages. *Journal of Agricultural and Food Chemistry, 54*, 7331-7338. doi:10.1021/jf061467h

Gawel R., 1998. Red wine astringency: a review. *Australian Journal of Grape and Wine Research*, *4*, 74-95. doi:10.1111/j.1755-0238.1998.tb00137.x

Gawel R., Smith, P. A. and Waters E.J., 2016. Influence of polysaccharides on the taste and mouthfeel of white wine. *Australian Journal of Grape and Wine Research, 22*, 350-357. doi:10.1111/ajgw.12222

González-Royo E., Esteruelas M., Kontoudakis N., Fort F., Canals, J. M. and Zamora F., 1997. The effect of supplementation with three commercial inactive dry yeasts on the colour, phenolic compounds, polysaccharides and astringency of a model wine solution and red wine. *Journal of the Science of Food and Agriculture*, *97*, 172-181. doi:10.1002/jsfa.7706

Guadalupe Z. and Ayestarán B., 2007. Polysaccharide profile and content during the vinification and aging of tempranillo red wines. *Journal of Agricultural and Food Chemistry 55*, 10720-10728. doi:10.1021/jf0716782

Guadalupe Z., Martínez L. and Ayestarán B., 2010. Yeast mannoproteins in red winemaking: effect on polysaccharide, polyphenolic, and color composition. *American Journal of Enology and Viticulture*, *61*, 191-200.

Guerrero R.F., Smith P. and Bindon K.A., 2013. Application of insoluble fibers in the fining of wine phenolics. *Journal of Agricultural and Food Chemistry, 61*, 4424-4432. doi:10.1021/jf400172f

González-Caballero V., Sánchez, M.-T., Fernández-Novales J., López, M.-I. and Pérez-Marín D., 2012. On-vine monitoring of grape ripening using near-infrared spectroscopy. *Food Analytical Methods*, *5*, 1377-1385. doi:10.1007/s12161-012-9389-3

Hanlin R., Hrmova M., Harbertson J. and Downey M., 2010. Review: condensed tannin and grape cell wall interactions and their impact on tannin extractability into wine. *Australian Journal of Grape and Wine Research, 16*, 173-188. doi:10.1111/j.1755-0238.2009.00068.x

Hufnagel J. C. and Hofmann T., 2008. Quantitative reconstruction of the nonvolatile sensometabolome of a red wine. *Journal of Agricultural and Food Chemistry*, *56*, 9190-9199. doi:10.1021/jf801742w

Le Bourvellec C., Bouchet B. and Renard C.M.G.C., 2005. Non-covalent interaction between procyanidins and apple cell wall material. Part III: Study on model polysaccharides. *Biochimica et Biophysica Acta (BBA) - General Subject, 1725*, 10-18. doi:10.1016/j.bbagen.2005.06.004

Lee C.B. and Lawless H.T., 1991. Time-course of astringent sensations. *Chemical Senses*, *16*, 225-238. doi:10.1093/chemse/16.3.225

Lencioni L., Romani C., Gobbi M., Comitini F., Ciani M. and Domizio P., 2016. Controlled mixed fermentation at winery scale using *Zygotorulaspora florentina* and *Saccharomyces cerevisiae*. *International Journal of Food Microbiology*, *234*, 36-44. doi:10.1016/j.ijfoodmicro.2016.06.004

Li M. and Hagerman A.E., 2014. Role of the flavan-3-ol and galloyl moieties in the interaction of (-)-epigallocatechin gallate with serum albumin. *Journal of Agricultural and Food Chemistry, 62*, 3768-3775. doi:10.1021/jf500246m

López-Solís R., Duarte-Venegas C., Meza-Candia M., del Barrio-Galán R., Peña-Neira Á., Medel-Marabolí M. and Obreque-Slier E., 2017. Great diversity among commercial inactive dry-yeast based products. *Food Chemistry, 219*, 282-289. doi:10.1021/jf500246m

Lu Y. and Bennick A., 1998. Interaction of tannin with human salivary proline-rich proteins. *Archives of Oral Biology*, *43*, 717-728. doi:10.1016/S0003-9969(98)00040-5

Manconi B., Castagnola M., Cabras T., Olianas A., Vitali A., Desiderio C., Sanna M. T. and Messana I., 2016. The intriguing heterogeneity of human salivary proline-rich proteins Short title: Salivary proline-rich protein species. *Journal of Proteomic, 134*, 47-56. doi:10.1016/j.jprot.2015.09.009

Mateus N., Machado, J. M. and de Freitas V., 2002.Development changes of anthocyanins in *Vitis vinifera* grapes grown in the Douro Valley and concentration in respective wines. *Journal of the Science of Food and Agriculture*, *82*, 1689-1695. doi:10.1002/jsfa.1237

Maury, C., Sarni-Manchado P., Lefebvre S., Cheynier V. and Moutounet M., 2001. Influence of fining with different molecular weight gelatins on proanthocyanidin composition and perception of wines. *American Journal of Enology and Viticulture, 52*, 140-145.

McRae J.M., Ziora Z.M., Kassara S., Cooper M.A. and Smith P.A., 2015. Ethanol concentration influences the mechanisms of wine tannin interactions with poly(l-proline) in model wine. *Journal of Agricultural and Food Chemistry, 63*, 4345-4352. doi:10.1021/acs.jafc.5b00758

Meillon S., Urbano, C. and 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 Preferenc, 20*, 490-499. doi:10.1016/j.foodqual.2009.04.006

Meléndez E., Ortiz M. C., Sarabia L. A., Íñiguez M. and Puras P., 2013. Modelling phenolic and technological maturities of grapes by means of the multivariate relation between organoleptic and physicochemical properties. *Analytica Chimica Acta*, *761*, 53-61. doi:10.1016/j.aca.2012.11.021

Messana I., Inzitari R., Fanali C., Cabras T. and Castagnola M., 2008. Facts and artifacts in proteomics of body fluids. What proteomics of saliva is telling us? *Journal of Separation Science*, *31*, 1948-1963. doi:10.1002/jssc.200800100

Oberholster A., Carstens L. M. and Du Toit W.J., 2013. Investigation of the effect of gelatine, egg albumin and cross-flow microfiltration on the phenolic composition of Pinotage wine. *Food Chemistry*, *138*, 1275-1281. doi:10.1016/j.foodchem.2012.09.128

Obreque-Slier E., Lopez-Solis R., Pena-Neira A. and Zamora-Marin 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 and Technology*, *45*, 2629-2636. doi:10.1111/j.1365-2621.2010.02437.x

Ozawa T., Lilley, T. H. and Haslam E., 1987. Polyphenol interactions: astringency and the loss of astringency in ripening fruit. *Phytochemistry, 26*, 2937-2942. doi:10.1016/S0031-9422(00)84566-5

Pérez-Través L., Querol A. and Pérez-Torrado R., 2016. Increased mannoprotein content in wines produced by *Saccharomyces kudriavzevii* × *Saccharomyces cerevisiae* hybrids. *International Journal of Food Microbiology, 237*, 35-38. doi:10.1016/j.ijfoodmicro.2016.08.014

Quijada-Morín N., Regueiro J., Simal-Gandara J., Tomas E., Rivas-Gonzalo J.C. and Teresa Escribano-Bailon M., 2012. Relationship between the sensory-determined astringency and the flavanolic composition of red wines. *Journal of Agricultural and Food Chemistry*, *60*, 12355-12361. doi:10.1021/jf3044346

Quijada-Morín N., Williams P., Rivas-Gonzalo, J. C., Doco T. and 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. doi:10.1016/j.foodchem.2013.12.101

Quijada-Morín N., Crespo-Expósito C., Rivas-Gonzalo J.C., García-Estévez I and Escribano-Bailón M.T., 2016a. Effect of the addition of flavan-3-ols on the HPLC-DAD salivary-protein profile. *Food Chemistry*, *207*, 272-278. doi:10.1016/j.foodchem.2016.03.118

Quijada-Morín N., García-Estévez I., Nogales-Bueno J., Rodríguez-Pulido, F. J., Heredia, F. J., Rivas-Gonzalo J.C., Escribano-Bailón M.T. and Hernández-Hierro J.M., 2016b. Trying to set up the flavanolic phases during grape seed ripening: a spectral and chemical approach. *Talanta*, *160*, 556-561. ttps://doi.org/10.1016/j.talanta.2016.07.064

Rayess Y.E., Albasi C., Bacchin P., Taillandier P., Mietton-Peuchot M. and Devatine A., 2012. Effect of colloids on critical fouling conditions during cross-flow microfiltration of wine. *Procedia Engineering*, *44*, 27-28. doi:10.1016/j.proeng.2012.08.296

Rinaldi A., Gambuti A. and Moio L., 2012. Application of the SPI (Saliva Precipitation Index) to the evaluation of red wine astringency. *Food Chemistry*, *135*, 2498-2504. doi:10.1016/j.foodchem.2012.07.031

Rossetti D., Yakubov, G. E., Stokes, J. R., Williamson A. M. and Fuller G.G., 2008. Interaction of human whole saliva and astringent dietary compounds investigated by interfacial shear rheology. *Food Hydrocolloids*, *22*, 1068-1078. doi:10.1016/j.foodhyd.2007.05.014

Sánchez-Ilárduya M.B., Sánchez-Fernández C, Viloria-Bernal M., López-Márquez D. M., Berrueta L. A., Gallo B. and Vicente F., 2012 Mass spectrometry fragmentation pattern of coloured flavanol-anthocyanin and anthocyanin-flavanol derivatives in aged red wines of Rioja. *Australian Journal of Grape and Wine Research* **2012,** *18*, 203-214. doi:10.1111/j.1755-0238.2012.00190.x

Sarni-Manchado P., Canals-Bosch, J. M., Mazerolles G. and Cheynier V., 2008. Influence of the glycosylation of human salivary proline-rich proteins on their interactions with condensed tannins. *Journal of Agricultural and Food Chemistry*, *56*, 9563-9569. doi:10.1021/jf801249e

Scharbert S., Jezussek M. and Hofmann T., 2004. Evaluation of the taste contribution of theaflavins in black tea infusions using the taste activity concept. *European Food Research and Technology 218*, 442-447. doi:10.1007/s00217-004-0888-3

Scollary G.R., Pásti G., Kállay M., Blackman J. and Clark A.C., 2012. Astringency response of red wines: potential role of molecular assembly. *Trends in Food Science & Technolog, 27*, 25-36. doi:10.1016/j.tifs.2012.05.002

Schroën C.G.P.H., Roosjen A., Tang K., Norde W. and Boom R.M., 2010. *In situ* quantification of membrane foulant accumulation by reflectometry. *Journal of Membrane Science, 362*, 453-459.

Schroën K., Ferrando M., de Lamo-Castellví S., Sahin S. and Güell C., 2016. Linking findings in microfluidics to membrane emulsification process design: the importance of wettability and component interactions with interfaces. *Membranes, 6,* 26. doi:10.1016/j.memsci.2010.06.066

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. doi:10.1111/ajgw.12188

Soares S., Mateus N. and de Freitas V., 2007. Interaction of different polyphenols with Bovine Serum Albumin (BSA) and Human Salivary α-Amylase (HSA) by fluorescence quenching. *Journal of Agricultural and Food Chemistry*, *55*, 6726-6735. doi:10.1021/jf070905x

Soares S., Vitorino R., Osorio H., Fernandes A., Venancio A., Mateus N., Amado F. and de Freitas V., 2011. Reactivity of human salivary proteins families toward food polyphenols. *Journal of Agricultural and Food Chemistry*, *59*, 5535-5547. doi:10.1021/jf104975d

Soares S., Brandão E., Mateus N., de Freitas V., 2017. Sensorial properties of red wine polyphenols: astringency and bitterness. *Critical Reviews in Food Science and Nutrition, 57*, 937-948. doi:10.1080/10408398.2014.946468

Springer L.F., Chen, L. A., Stahlecker A.C., Cousins P. and Sacks G.L., 2016. Relationship of soluble grape-derived proteins to condensed tannin extractability during red wine fermentation. *Journal of Agricultural and Food Chemistry, 64*, 8191-8199. doi:10.1021/acs.jafc.6b02891

Sun B.S., de Sa M., Leandro C., Caldeira I., Duarte F. L. and Spranger I., 2013. Reactivity of polymeric proanthocyanidins toward salivary proteins and their contribution to young red wine astringency. *Journal of Agricultural and Food Chemistry*, *61*, 939-946. doi:10.1021/jf303704u

Ulbricht M., Ansorge W., Danielzik I., König M. and Schuster O., 2009. Fouling in microfiltration of wine: the influence of the membrane polymer on adsorption of polyphenols and polysaccharides. *Separation and Purification Technology, 68*, 335-342. doi:10.1016/j.seppur.2009.06.004

Upadhyay R., Brossard N. and Chen J., 2016. Mechanisms underlying astringency: introduction to an oral tribology approach. *Journal of Physics D: Applied Physics 49,* 104003. doi:10.1088/0022-3727/49/10/104003

Vidal S., Francis L., Williams P., Kwiatkowski M., Gawel R., Cheynier V. and Waters E., 2004. The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium. *Food Chemistry*, *85*, 519-525. doi:10.1016/S0308-8146(03)00084-0

Watrelot A.A., Schulz, D.L. and Kennedy J.A., 2017. Wine polysaccharides influence tannin-protein interactions. *Food Hydrocolloids*, *63*, 571-579. doi:10.1016/j.foodhyd.2016.10.010