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Review Article

Wine phenolics: looking for a smooth mouthfeel

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Abstract: Each grape variety has its own phenolic profile. However, the concentration of the phenolic compounds present in wine mainly depends on winemaking processes. Phenolic compounds influence wine sensorial characteristics, namely taste or mouth feel, bitterness, astringency and color. Humans can perceive six basic tastes: sweet, salty; sour; umami; fat-taste and bitter taste. This last basic taste is considered as a defense mechanism against the ingestion of potential poisons. Some of the genes, encoding G-protein-coupled receptors - TAS2Rs, which translate for these distinct bitter compound detectors have been identified. Different phenolic compounds activate distinguished combination of TAS2Rs.Astringency in wine is primarily driven by proanthocyanidins, soluble protein-proanthocyanidins complexes which diminish the protective salivary film and bind to the salivary pellicle; insoluble protein-proanthocyanidins complex and proanthocyanidins are rejected against salivary film and trigger astringency sensation via increasing friction.

Thus, the aim of this review is to expand the knowledge about the role of wine phenolic compounds in wine sensorial properties, namely in bitterness and astringency phenomenon's.

Key words: wine phenolic compounds, proanthocyanidins, bitter taste, astringency, sensorial properties.

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Introduction

Wine is a hydroalcoholic acid solution containing various phenoliccompounds. They are present in seeds, skins and stems of the grapes; therefore their concentration in wine is highly affected by winemaking process such as fermentation/maceration lengthsin which extraction occurred.However, the grape variety used in winemakingis also an important factor that affects the wine phenolic composition, since each grape variety has its own phenolic profile (Jordão et al., 1998; Bautista-Ortin et al., 2007;Jordão and Correia, 2012; Costa et al., 2015).Wine phenolic compounds have an importantinfluence in wine sensorial characteristics. For example, monomeric (+)-catechins give bitter taste to wine, whereas polymers cause astringent Taste (Jackson, 2000; Oliveira et al., 2011).In red wine, phenolic compoundslike, coumaric, caffeic, ferulic and vanillic acids are relatively simple structures while others are complex polymeric structures such as tannins, that can combine with numerous substances including polysaccharides, proteins, and other polyphenols,affectingmouthfeel,bitterness, astringency and color. Anthocyanins and tannins influence the color and color stability of wine besides influencing mouthfeel, depth and astringency (Saint-Cricq de Gaulejac et al., 1998). These complex structures change over time; specifically during the wine aging process,becoming more complex due to the increase ofthe mean degree of polymerization(Suriano et al., 2015).

Wine phenolic composition

Wine contains manyphenolic substances, their major sources being grape stems, seeds and skins (Jordão et al., 2001; Cheynier, 2005). However, the wine phenolic composition is also determined by yeast metabolism, since they can form important wine color components, including anthocyanins adducts and pigmented polymers (Fulcrand et al., 1998; Benabdeljalil et al., 2000;Blazquez Rojas et al., 2012) or by the type of wine aging process, such as the use of oak wood barrels or oak wood fragments (De Coninck et al., 2006; Jordão et al. 2008). According to several authors (Ribéreau-Gayon et al., 2006; Jordão et al. 2012) the levels of polyphenolic compounds in red wine depended from several factors, namely the pomace-contact maceration time and the evolution profile of major polyphenol groups.

Wine phenolic compounds can be classified into two groups: flavonoids and nonflavonoids. The major C_6-C_3-C_6 flavonoids in wine include conjugates of the flavonols, quercetin, and myricetin; the flavan-3-ols (+)-catechin and(-)-epicatechin, and malvidin-3-glucoside and other anthocyanins. The nonflavonoids incorporate the C_6-C_1 hydroxy-benzoic acids, gallic and ellagic acids; the C_6-C_3 hydroxycinnmates caffeic, caftaric, and *p*-coumaric acids, and the C_6-C_2-C_6 stilbenes trans-resveratrol, *cis*-resveratrol, and *trans*-resveratrol glucoside (Waterhouse, 2002;Cosme and Jordão, 2014).

Total phenol content ranged in red wine from 1850-2200 mg/L and in white wine from 220-250 mg/L, being the flavonoid compounds the mainphenols in red wine, extracted from grape skins and seeds during the fermentation/maceration process (Waterhouse and Teissedre, 1997;Cristino et al., 2013).

Non-flavonoid phenolic compounds are present in wine at low concentration, and their origin could be from the grape pulp or oak wood barrels used in wine aging. The three main hydroxycinnamates in grapes and wine are those based on coumaric acid, caffeic acid and Ferulic acid. In grapes hydroxycinnamic acids exist as esters of tartaric acid and are *p*-coutaric acid, caftaric acid, and fertaric acid, respectively(Somers et al., 1987; Waterhouse, 2002).At the concentration found in wines, the hydroxycinnamates seem to have no perceptiblebitterness or astringency, since they are present below their sensory threshold (Verette et al., 1988).Hydroxybenzoic acids comprise *p*-hydroxybenzoic acid, syringic acid, vanillic acid and gallic acid.Gallic acidcould be also originated from the hydrolysisof gallate esters of hydrolyzable tannins and condensed tannin (Waterhouse and Teissedre, 1997; Waterhouse, 2002).

Total monomeric flavan-3-olsin red wine rangedfrom 40–120mg/L, depending on the extraction process during vinification. However, condensed flavan-3-ol units the so calledcondensed tannins or proanthocyanidins (0.5g/L-1.5g/L in red and 10-50mg/L in white wine)are the main phenolic compounds in red wine (Waterhouse, 2002).In terms of sensorial perception, flavan-3-ols ((+)-catechin, (-)-epicatechin, (-)-epicatechin-3-*O*-gallate)can be bothbitter and astringent, however in polymerform bitterness is slight, but astringency remains (Su and Singleton 1969, Robichaud and Noble, 1990). Thus,tannins have an important role inwine astringency and also contribute to impart bitterness sensation.

Monomeric anthocyanins extracted from grapes are the main compounds responsible for the color of young red wines(Boulton, 2001).There are five anthocyanidins: cyanidin, peonidin, delphindin, petunidin and malvidin, which could be at the six-hydroxyl of the glucose, acyl substituted, with ester linkages connecting an acetyl group, a coumaryl group, and a lesser amount of caffeoyl group. There are also derivatives of anthocyanins that result by the interaction of anthocyanins with other molecules such as, vinyl catechol, pyruvic acid, vinyl phenol, acetone, a-ketoglutaric acid, 4-vinylguaiacol or glyoxylic acid (Pinho et al., 2012). For example, pyranoanthocyanins namely, vitisin-A and vitisin-B, are formed by the condensation of anthocyanin, malvidin-3-glucoside with the fermentation by-products pyruvicacid and acetaldehyde, respectively. These compounds are more stable and originate at pH 4.0 deeper colors than monomeric anthocyanins (Morata et al., 2007; Cano-López et al., 2008).During wine aging, polymerization reaction take place and polymeric pigments became responsible for wine color. It was observed that wine color changed from a bright red to a reddish-brown hue.This is associated to the formation of new and more stablepolymeric pigments resulting from reactions between anthocyanins and other phenolic compounds, for example, flavan-3-ol monomers and proanthocyanidins (Somers, 1971, Kantz and Singleton, 1991, Singleton and Trousdale, 1992; He et al., 2012).These reactions are based acetaldehyde mediated condensation, co-pigmentation and self-association reactions(Boulton 2001, Castillo-Sánchez et al., 2008). It is known that anthocyanins do not contribute to mouthfeel sensations; however they are able to contribute to mouthfeel when combined with other species in the form of polymers (Haslam, 1998).

Winemaking technology,including, fermentation temperature and lengths, as well as pH and alcoholconcentrationinfluence the wine phenolic concentration. Also, clarification and stabilization techniques used to achieve wine limpidityand stability result in a potential decrease of phenolic content (Mira et al., 2006; Gonçalves and Jordão, 2009; Lasanta et al., 2013; Guise et al., 2014: Ribeiro et al., 2014; Ibeas et al., 2015). For example, the use of fining agents such asgelatin, egg albumin, isinglass and casein/potassium caseinatealso could reducespecific phenolic compounds in function of the protein fining agent applied and couldlead to changes in color, bitterness and astringency in some wines (Cosme et al., 2007; Braga et al., 2007;Cosme et al., 2008; Cosme et al., 2009; Gonçalves and Jordão, 2009).

Bitterness or astringency?

Phenolic compounds are responsible for bitterness and astringency of many foods and beverages, including wine (Bravo, 1998; Gawel, 1998). Whereas lower-molecular-weight phenolic compounds tend to be bitter, higher-molecular-weight polymers are more likely to be astringent (Noble, 1994). Astringency (drying or puckering mouth feel detectable throughout the oral cavity), may be due to a complexing reaction between polyphenols and proteins of the mouth and saliva (Noble, 1994).

High-molecular-weight polyphenols or tannins have long been regarded as antinutrients because they interfere with protein absorption or reduce iron availability, they complex with proteins, starches, and digestive enzymes and are thought to reduce the nutritional value of foods (Chung et al., 1998).

Phenolic compounds in wine range from low-molecular weight-catechins to high-molecular-weight tannins (Blanco et al., 1998). As referred by Drewnowski and Gomez-Carneros (2000) perceived bitterness and astringency increased as a linear function of concentration for (+)-catechin and for grapeseed tannin. Flavonoid monomers such as (+)-catechin and (-)-epicatechin were rated as more bitter than astringent (Thorngate and Noble, 1995). At higher molecular weights, (+)-catechin polymers became progressively more astringent. Thus, wine polyphenols with molecular weights >500, such as grape-seed tannin, were more astringent than bitter (Peleg et al., 1999).

Kallithraka et al. (1997) realized a sensory study of (+)-catechins in a wine model system similar, in composition, to a dry table wine. The results obtained showed that (-)-epicatechin was significantly more bitter and astringent than (+)-catechin. In this study, tasters associated bitterness and astringency with perceived mouth drying and with mouth roughening, especially in higher concentrations of (-)-epicatechin.

Phenols in wine are largely derived from grape skins (30%) and seeds (70%) that remain in contact with fermenting grape juice from 24 to 36 hours for rosé wines and from 4 to 21 days for red wines. Phenolic content of red wines can thus reach 1000–3.500 mg/L, depending on processing conditions (Chandrashekar et al., 2000; Blanco et al., 1998). However, the bitterness of phenolics is reduced by sucrose and is substantially enhanced by ethanol (Noble, 1994). In fact, Lanier et al. (2005) found that some people experience more bitterness when drinking more alcoholic beverages. This phenomenon is directly related to the genes they've inherited and, individual differences in bitterness and sweetness are predictors of alcohol liking and intake in young adults (Lanier et al., 2005). Actually, as previously reviewed by Jordão et al. (2015),consumers know that wines with high alcohol content can cause a gustatory disequilibrium affecting wine sensory perceptions leading to unbalanced wines. Multiple studies (Wooding et al., 2004; Drayna et al., 2003) have linked variation in TAS2R (taste receptor, type 2) bitter receptor genes, to alcohol intake.

Mechanism of bitter taste perception

The primary organ responsible for the sense of taste is the tongue, which contains the taste receptors to identify non-volatile chemicals in foods and beverages. Taste-stimuli are typically released when food is masticated and dissolved into saliva (pre-digested by oral enzymes, such as amylase, lipase, and proteases (Pedersen et al., 2002)).The taste buds, in the tongue,are located in structures called 'papillae'. These structuresarethe first stage of gustatory signal processing.Cells within a bud communicate with one another, including electric coupling via gap junctions and cell to cell chemical communication via glutamate, serotonin, and ATP (Breslin and Spector,2008; Roper, 2013).

Humans perceive nutrientsand toxins qualitatively as sweet (elicited by sugars); salty (elicited by sodium ion - Na^+, and other ions reflecting mineral content); sour (elicited by free hydrogen ions - H^+); savory or umami (elicited by glutamate and other amino acids), fat taste - elicited by products of fats and fatty acids (Keast and Costanzo, 2015) and bitter tasting - reflecting potential toxins in foods (Breslin and Spector, 2008). This last basic taste modality (bitter taste) may be considered as a defense mechanism against the ingestion of potential poisons, since numerous harmful compounds, including inorganic ions and

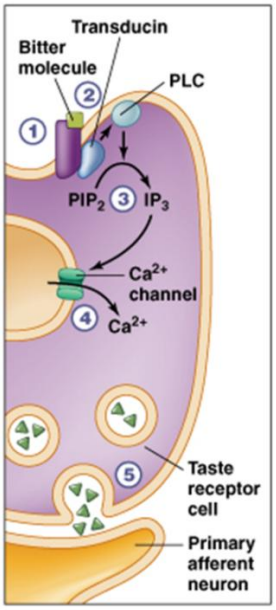
rancid fats, secondary plant metabolites like alkaloids, synthetic chemicals do taste bitter (Meyerhof et al., 2005).

The chemical detectors of the bitter compounds in the tongue canrecognize thousands of different chemicals. Some of the genes that translate for these distinct bitter compounds detectors have been identified (Adler et al., 2000; Bufe et al., 2002). These genes encoding G-protein-coupled receptors, TAS2Rs (previously referred to as T2Rs or TRBs), have been suggested to represent bitter taste receptors and are responsible for bitter taste transduction mechanism. An important gene contributing to PTC (the ability to taste the bitterness of phenylthiocarbamide) TAS2R38—taste receptor, type 2, member 38, perception has been identified. The gene located on chromosome 7q36, is a member of the bitter taste receptor family (Duffy et al., 2004).

Recently, it was evidenced by Soares et al.(2013) that different phenolic compounds activate distinguished combination of TAS2Rs: (-)-epicatechin stimulated threereceptors (TAS2R4, TAS2R5, and TAS2R39) while pentagalloylglucose activated two receptors(TAS2R5 and TAS2R39). Only one receptor was responded to malvidin-3-glucoside and procyanidin trimer.

The bitterness transduction mechanisms is schematized in Figure 1: Initially, bitter ligands activate TAS2Rs causing a conformational change. The active G-protein, transducin,activates enzyme phospholipase C(PLC-b2) to generate from to breakdown of phosphatidylinositol biphosphate (PIP_2)the second messenger - inositol triphosphate (IP_3), initiating the release of $Ca^{2+}$from intracellular stores (vacuoles). TrpM5 is activated by elevated Ca^{2+} to flow in Na^+, resulting in depolarization of receptor cell. The combined action of elevated Ca^{2+} and membrane depolarization opens the pannexin 1 hemichannel torelease transmitters to brain. Adenosine triphosphate (ATP) is secreted to gustatory afferent glossopharyngeal nerve fibers and ultimately generates a nerve signal in the brain recognized as a bitter taste (Ma et al., 2014).

In wines, in contrary to astringency, a gradual reduction of bitterness is perceived as their molecular weight augments (Noble, 1994).In grapesthere are evidences of different proportions of galloyl groupbetween the seed and skin fraction. The seed fraction with a higher proportion of galloyl group and a lower mean degree of polymerization (mDP)seems to be perceived as more bitterthan the skin fraction (Brossaud et al., 2001).



1. A bitter substance like a phenolic compound, binds to its receptor, causing a conformational change.

2. The active G-protein, transducin, activates phospholipase C (PLC).

3. PLC catalyzes the conversion of PIP_2 into de second messenger – IP_3.

4. IP_3 causes the release of Ca^{2+} from intracellular vacuoles.

5. The influx of Ca^{2+} causes neurotransmitter release.

Figure 1 - Bitter taste receptor cell and bitter taste transduction mechanism. Adapted from Moyes and Schulte (2008).

Mechanisms for astringency

Astringency refers to "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 could be stimulated by salts of multivalent metallic cations, dehydrating agents like ethanol, mineral and organic acids, tannins and small polyphenols (Bajec and Pickering, 2008). However, in wine, astringency is primarily driven by proanthocyanidins,also called condensed tannins(Sáenz-Navajas et al., 2012; Brandão et al., 2014).

The mechanism for astringency was first proposed by Bate-Smith(1954) and is believed to be due to the ability of tannins to bind andprecipitate salivary proteins. The loss of lubrication in the oral cavity, including the tongue,occurs when tannins pass by and they bond to salivary proteins forming insoluble tannin–protein precipitates in the mouth, increasing friction which results in the sensation of astringency (Baxter et al. 1997).The general accepted mechanism for protein–tannin interaction was proposed by Siebert et al. (1996). Concerning this mechanism, a protein has a fixed number ofsites to which a tannin can bind. According to the ratio of protein or tannin used, different protein–tannin complexes are formed. According to Charlton et al. (2002), proteins and polyphenols combine to form soluble complexes, but when they grow to colloidal size particles, they become larger, leading to sediment formation.

Charlton et al. in 2002 proposed a 3-stage model of the interaction between tannins and proteins: Initially, hydrophobic associations (π–π) occur between the planar surfaces of the tannin aromatic rings and hydrophobic sites of proteins such as pyrrolidine rings of prolyl residues. Simultaneously, hydrogen bonding effect assists to stabilize the complexes, occurring between the hydroxyl group of tannins and H-acceptor sites (carbonyl and $–NH_2$ groups) of proteins. Next, the protein-tannin complexes self-associate via further hydrogen bonding to produce soluble larger protein-tannin complexes and then aggregate. Finally, the aggregated complexes are large enough to form insoluble sediment and precipitate from solution.

However, several authors supported the idea that "tannin–protein interaction" is more closely associated with astringency than "tannin–protein precipitation" (Obreque-Slier et al., 2010). Recently, Lee et al. (2012) demonstrated that PRPs (proline-rich proteins) precipitated tannins and alum except for hydrochloric acid while mucins mainly consisting the coating of epithelium tissues were able to precipitate acid and alum except for tannins. Thus, a disturbance of oral lubricating coatings may contribute to the increase of astringency. The loss of oral lubricating films/pellicle allows soluble tannin–protein aggregates or free astringent stimuli to interact directly with oral tissue possibly through receptors. The disturbance of the protective salivary film, could also be the explanation for the dry mouth perception usually associated with the astringent mouth-feel (Ma et al., 2014). According to Brandão et al. (2014), salivary proteins families have relative discriminatory functions in rating the perception of astringency depending on the type of astringent stimuli used. They show that repeated stimulations withprocyanidins may differently affect the several families of salivary proteins, suggesting that they could be involved in different stages of the development of astringency. Furlan et al. (2014) recently studied the interactionbetween monomeric flavan-3-ols and lipid liposomes, indicating that astringency sensation may also implicate the binding between red wine tannins and oral cavity membrane. Gibbins and Carpenter (2013) showed a multiple-modal system by which implicates several possible astringency mechanisms. In Figure 2, is a schematic representation of a possible astringency mechanism.

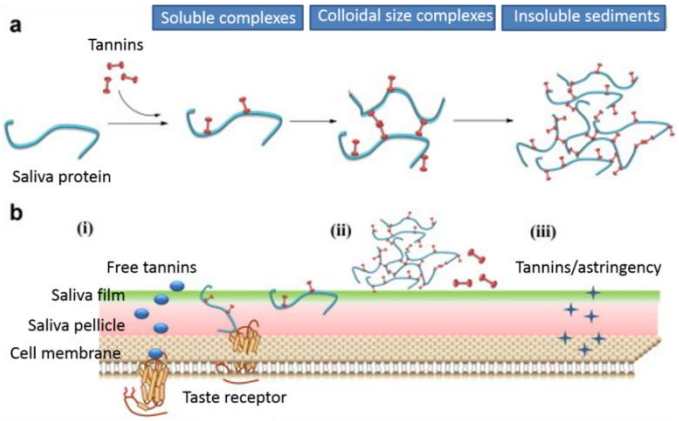


Figure 2 - (a) A 3-stage model of the interaction between tannin and proteins; (b) Astringency stimulation: (i) "Free" tannins and soluble protein–tannin complexes deplete the protective salivary film and eventually bind to the pellicle or even to the receptors exposed; (ii) Insoluble protein–tannin complex and tannins are rejected against salivary film. Insoluble protein–tannin complexes trigger astringency sensation via increasing friction. (iii) Tannins interact with oral cavity membrane causing astringency. Adapted from Ma et al. (2014).

Although it is commonly accepted that interaction between tannins and saliva proteins play an important role in astringency perception in wine (Ma et al., 2014), the physiological and physicochemical mechanisms for this phenomenon are not fully understood and more studies focusing this subject must be done.

Final remarks

This review evidenced the important role of phenolic compounds on the wine sensory characteristics. Therefore, tannin and anthocyanin management during grape-growing by following phenolic maturity of red grapes and during winemaking is avery important factor, for tailoring the wine sensorial characteristics namely taste or mouthfeel, bitterness, astringency and color.

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