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Wine polysaccharides influence tannin-protein interactions

Aude A. Watrelot [a, b, *], Delia L. Schulz [a], James A. Kennedy [b, c]

[a] Department of Viticulture and Enology, California State University, 2360 E. Barstow Avenue MS VR89, Fresno, CA, 93740-8003, USA
[b] Department of Viticulture and Enology, University of California at Davis, One Shields Ave., Davis, CA, 95616-5270, USA
[c] Constellation Brands, Inc., 12667 Road 24, Madera, CA, 93637, USA

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ABSTRACT

Tannins are responsible for the astringency of red wine where it is considered to be the perception that follows tannin interaction with salivary protein. In this study, the influence of red wine polysaccharides on the interactions between tannins and proteins has been investigated. The mean degree of polymerization, subunit composition and molecular mass of tannins was determined for tannins isolated from various red wines. In addition, the composition of total soluble polysaccharides was determined for polysaccharides isolated from the same red wines. The influence of polysaccharides on the interaction between tannins and protein were investigated. The ability of tannins to interact with a hydrophobic surface (tannin activity) did not significantly change after the addition of the corresponding polysaccharides. Rhamnogalacturonan II, the proportion of pigmented tannins and the mean degree of polymerization seemed to encourage protein precipitation either through the formation of insoluble tannin-polysaccharide aggregates or because of protein-polysaccharide interactions.

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1. Introduction

One aspect of red wine quality is the sensory perception of mouthfeel. An important mouthfeel component of red wine is astringency which results when extracted condensed tannins interact with salivary proteins leading to aggregate formation and subsequent precipitation (McRae, Falconer, & Kennedy, 2010). Condensed tannins are important macromolecules which influence the mouthfeel perception of red wine (Vidal et al., 2003). They are oligomers/polymers of flavan-3-ols ((−)-epicatechin (EC), (+)-catechin (C), (−)-epigallocatechin (EGC) and (−)-epicatechin-3-*O*-gallate (ECG)), linked mainly by C4−C8 interflavonoid linkages. Depending on the mean degree of polymerization (mDP) which is the average number of constitutive units, condensed tannins can interact more or less with salivary proteins and induce astringency (Sun et al., 2013). This tannin perception can vary with tannin structure such as oxidation products (Poncet-Legrand et al., 2010) as well as interactions with other macromolecules, i.e. polysaccharides (Carvalho, Mateus, et al., 2006; Quijada-Morín, Williams, Rivas-Gonzalo, Doco, & Escribano-Bailón, 2014).

Polysaccharides found in finished red wine originate from grapes and yeasts during the winemaking process. Pectins are among the plant polysaccharides found in wine. They are heteropolysaccharides, are located in the middle lamella of primary cell walls and are mainly composed of a galacturonic acid backbone and chains of several monosaccharides. The smooth region is represented by homogalacturonans (HG) which are galacturonic acid chains more or less methylated/acetylated and the hairy region (high density of side chains) which are known as the rhamnogalacturonans (RG) type I and II (Caffall & Mohnen, 2009). RG I consists of rhamnose and galacturonic acid and represents a very small proportion of grape-based pectins. In red wine, the most abundant pectic polysaccharide is RG II, which is formed in the grape berry during maturation and is extracted during the initial stages of winemaking. Arabinogalactan proteins (AGP) are glycoproteins and are also located in the plant cell walls and extracted during winemaking. They are themselves sidechains of the backbone that arises from the hairy region of pectins and are connected via specific hydroxyproline-rich proteins and together with arabinogalactans, contribute to the so-called “polysaccharides rich in arabinose and galactose” (Guadalupe, Martínez-Pinilla, Garrido, Carrillo, & Ayestarán, 2012).

Polysaccharides originating from yeast cells are released during alcoholic fermentation and wine ageing, when cell walls break

* Corresponding author. Department of Viticulture and Enology, University of California at Davis, One Shields Ave., Davis, CA, 95616-5270, USA. Tel.: +1 530 752 5054; fax: +1 530 752 0382.
E-mail addresses: awatrelot@ucdavis.edu, watrelotaude@yahoo.fr (A.A. Watrelot).

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down. They have been identified as glucans, chitin and mannoproteins (MP) (Escot, Feuillat, Dulau, & Charpentier, 2001), and are located in the outermost layer of yeast cell walls, which represent one third of the dry cell wall material. MP are glycoproteins consisting mainly of mannose (greater than 90%) and glucose connected to the cell matrix (β-1,3 glucan) via covalent bonds. Some MP are released by the action of β-1,3-glucanase during ageing (autolysis), and others are released during alcoholic fermentation of grape must when yeast are actively growing (Doco, Williams, & Cheynier, 2007).

During the winemaking process and once extracted into wine, interactions between polysaccharides and tannins are expected to occur (Bindon, Smith, & Kennedy, 2010; Renard, Baron, Guyot, & Drilleau, 2001). Hydrophobic interactions and hydrogen bonds are the main interactions that occur between tannins and polysaccharides. The strength of the interactions is determined by the structure and conformation of condensed tannins on the one hand, as a higher mDP for example will increase the interaction (Bindon, Smith, & Kennedy, 2010; Le Bourvellec, Guyot, & Renard, 2004; Watrelot, Le Bourvellec, Imberty, & Renard, 2013). On the other hand the structure and composition of polysaccharide cell wall material can facilitate the interaction with tannins. Pectins have been shown to have strong affinities for tannins depending on the degree of methylation of the homogalacturonans (Watrelot et al., 2013), as well as the pectic hairy region monosaccharides structure and linkages (Watrelot, Le Bourvellec, Imberty, & Renard, 2014). RG II does not appear to have a direct influence on tannin aggregation, yet its presence results in an increase in haze particle diameter, possibly the result of co-aggregation between the two distinct hydrophobic zones of the polysaccharide and the tannin (Escot et al., 2001; Riou, Vernhet, Doco, & Moutounet, 2002). AGP has a larger size and can therefore form larger complexes than the smaller RG II. Previously, it has been shown that the choice of yeast strain has an influence on the polyphenolic composition of wine (Escot et al., 2001; Holt et al., 2013). Specifically, macromolecule release from cell walls, especially polysaccharides such as mannoproteins, is yeast strain dependent (Giovani, Rosi, & Bertuccioli, 2012).

Hypothetically, the formation of tannin and polysaccharide complexes influences their association with salivary proteins which then leads to a change in astringency perception. It has been shown that pectic water soluble carbohydrates inhibit tannin-protein aggregation (Carvalho, Mateus, et al., 2006; de Freitas, Carvalho, & Mateus, 2003; Ozawa, Lilley, & Haslam, 1987; Riou et al., 2002). It has been suggested that with regard to tannin interaction, there is a competition between polysaccharides and salivary proteins. Carbohydrates such as xanthan or arabic gum would inhibit protein-tannin interaction (Carvalho, Póvoas, Mateus, & de Freitas, 2006) or inhibit the precipitation of the protein-tannin complexes (de Freitas et al., 2003; Mateus, Carvalho, Luıs, & de Freitas, 2004).

In this study, the effect of polysaccharides on the interaction between tannin and a hydrophobic surface or protein was investigated. To provide a better understanding of the interactions that may influence wine quality, polysaccharides and tannins were extracted from red wine from two grape varieties known to be different in terms of mouthfeel quality and their subsequent interaction was investigated under model conditions.

2. Material and methods

2.1. Chemicals

All reagents were analytical grade (or HPLC grade) unless otherwise stated. Bovine serum albumin, phloroglucinol, (−)-epicatechin (purity ≥ 90%), (+)-catechin hydrate (purity ≥ 98%), trifluoroacetic acid, hexane, L-arabinose, L-fucose, D-galactose, D-galacturonic acid, D-glucose, D-glucuronic acid, L-rhamnose, D-xylose and D-mannose were purchased from Sigma Aldrich (St Louis, MO, USA). Acetone, methanol, acetonitrile, acetic acid, L-(+)-ascorbic acid, lithium chloride, *ortho*-phosphoric acid, *N,N*-dimethylformamide, anhydrous sodium acetate, ethanol, pyridine, hexamethyldisilizane and myo-inositol were from VWR International (Randor, PA, USA) and chlorotrimethylsilane and hydrochloric acid were supplied by Merck (Kenilworth, NJ, USA). Pullulan standards (P-82) were purchased from Shodex, Showa Denko (Japan). All water used was purified using an Ultrapure purification system (Evoqua Corporation, Alpharetta, GA, USA).

2.2. Wine samples

Experiments were carried out on ten red wines of *Vitis vinifera* L. cv. Cabernet Sauvignon and cv. Pinot noir. The wines were mostly from California and were from three vintages (2010, 2012 and 2013) (Table 1). They were stored under cellar conditions in their original bottles and sparged with nitrogen gas after every use to minimize oxidation.

2.3. Instrumentation

For all high performance liquid chromatography (HPLC) measurements the HPLC system used was an Agilent 1260 Infinity (Agilent Technologies, Santa Clara, CA, USA). It consisted of a quaternary pump, column heater, autosampler, diode array detector (DAD) and a refractive index detector (RID) and was controlled by Chemstation software.

The GC-MS system used for polysaccharide characterization was controlled by MSD Chemstation GI701DA software and consisted of a Hewlett Packard HP6890 Series GC maintained with helium of ultra-high purity (uhp 300) at a rate of 1 mL/min and a 5973 Network Mass selective detector from Agilent (Santa Clara, CA, USA).

2.4. Tannin extraction

The method for tannin extraction was adapted from a previously described method (Aron & Kennedy, 2007). Briefly, tannins were extracted from wines using a glass column (bed volume of 206 mL) containing Toyopearl chromatography resin (HW-40C, Supelco, Bellefonte, PA, USA) connected to a peristaltic pump. The resin was activated with 500 mL water containing 0.05% v/v trifluoroacetic acid at a flow rate of 25 mL/min prior to loading 100 mL wine at 18 mL/min. In order to remove sugars and organic acids the column was washed with 1 L water containing 0.05% v/v trifluoroacetic acid, then 1 L methanol:water (1:1, v:v) containing 0.05% v/v trifluoroacetic acid to remove anthocyanins and low molecular weight phenolics. Tannins were eluted with 300 mL acetone:water

Table 1
Grape cultivar, vintage and country of origin of the selected red wines.

Study name	Vitis vinifera L. cv.	Vintage	Country
CS 1	Cabernet Sauvignon	2012	Australia
CS 2	Cabernet Sauvignon	2010	California, USA
CS 3	Cabernet Sauvignon	2012	California, USA
CS 4	Cabernet Sauvignon	2012	California, USA
CS 5	Cabernet Sauvignon	2012	California, USA
PN 1	Pinot noir	2012	California, USA
PN 2	Pinot noir	2013	New Zealand
PN 3	Pinot noir	2012	California, USA
PN 4	Pinot noir	2012	California, USA
PN 5	Pinot noir	2012	California, USA

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