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# Interactions between grape skin cell wall material and commercial enological tannins. Practical implications

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ABSTRACT

Commercial enological tannins were used to investigate the role that cell wall material plays in proanthocyanidin adsorption. Insoluble cell wall material, prepared from the skin of *Vitis vinifera* L. cv. Monastrell berries, was combined with solutions containing six different commercial enological tannins (proanthocyanidin-type tannins). Analysis of the proanthocyanidins in the solution, after fining with cell wall material, using phloroglucinolysis and size exclusion chromatography, provided quantitative and qualitative information on the non-adsorbed compounds. Cell wall material showed strong affinity for the proanthocyanidins, one of the commercial tannins being bound up to 61% in the experiment. Comparison of the molecular mass distribution of the commercial enological tannins in solution, before and after fining, suggested that cell walls affinity for proanthocyanidins was more related with the proanthocyanidin molecular mass than with their percentage of galloylation. These interactions may have some enological implications, especially as regards the time of commercial tannins addition to the must/wine.

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## 1. Introduction

Proanthocyanidins, commonly called "tannins" by winemakers, are oligomeric and polymeric flavan-3-ols, linked by C(4)–C(6) or C(4)–C(8) interflavanoid bonds. In the grape, they are located in the skin and seeds and the main flavan-3-ol monomers are (−)-epigallocatechin, (+)-catechin and its isomer (−)-epicatechin, which can also be found in the form of gallic ester (epicatechin-3-O-gallate).

Proanthocyanidins (PAs) or tannins are important for the quality of wines because they are responsible for their bitterness, astringency and colour properties. Their concentration in wines will depend on several parameters, such as grape variety and winemaking practices (Bautista-Ortin et al., 2012; Busse-Valverde et al., 2010). However, previous studies have shown that measuring grape proanthocyanidins will not give an exact idea of the quantity of these compounds that will be detected in the corresponding wines (Adams & Scholz, 2007; Busse-Valverde, Bautista-Ortín, Gómez-Plaza, Fernández-Fernández, & Gil-Muñoz, 2012; Busse-Valverde et al., 2010; Harbertson, Kennedy, & Adams, 2002). Indeed, the quantities found are frequently much lower than expected and show large differences depending on variety (Busse-Valverde et al., 2012). Some authors defend the existence of proanthocyanidin-cell wall interactions as one of the reasons for such observations (Adams & Scholz, 2007; Bindon & Kennedy, 2011; Bindon, Smith, Holt, & Kennedy, 2010a; Hanlin, Hrmova, Harbertson, & Downey, 2010), suggesting that, once the grapes have been crushed to obtain the fermenting must, part of the skin and seed proanthocyanidins could be bound to the pulp and skin cell walls, which are present in large concentrations in the initial must. These proanthocyanidins, therefore, will not form part of the wine proanthocyanidins.

Skin and pulp cell walls may adsorb proanthocyanidins due to their chemical composition. Cell walls are composed of about 90% polysaccharides and 10% structural proteins (McNeill, Darvill, Fry, & Albersheim, 1984). The polysaccharides are generally grouped into three categories: cellulose, pectins and hemicelluloses. Due to their hydroxyl groups, as well as aromatic and glycosidic oxygen atoms, these cell wall polysaccharides have the ability to form hydrogen bonds and hydrophobic interactions with certain molecules, proanthocyanidins among them (Haslam, 1998; Le Bourvellec, Bouchet, & Renard, 2005; McManus et al., 1985). The extent and strength of these interactions will depend on cell wall composition and on the characteristics of the proanthocyanidins, including their molecular mass, degree of galloylation and/or the stereochemistry of their molecules (Le Bourvellec, Guyot, Renard, & Abbal, 2004; McManus et al., 1985; Riou, Vernhet, & Doco, 2002).

In wines, the condensed tannins come from the grape. However, one common practice in enology is the addition of commercial enological tannins to musts and/or wines to enhance colour, flavour and mouthfeel (Bautista-Ortín, Martínez-Cutillas, Ros-García, López-Roca, & Gómez-Plaza, 2005; Crespy & Urban, 2002). The

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results of these exogenous tannin additions might also be affected by interactions with cell walls (Hanlin et al., 2010), since their addition during fermentative maceration (when a large quantity of solid material is present) may result in a large part of these exogenous tannins being bound to the polysaccharides of the cell walls, whereas the additions could be more effective if done following the removal of solid parts at pressing.

Given this, the objective of this study was to assess possible interactions between isolated grape skin cell walls and the proanthocyanidins present in commercial enological tannin preparations, and to ascertanin whether differences existed in the extent of any interactions depending on the composition of the commercial product. The results could provide information of significance for the winemaking process.

## 2. Material and methods

For this study, we used six different enological tannins (proanthocyanidin-type tannins supplied by Agrovin S.A., Spain), two non-comercial purified crude seed tannin extracts (also provided by Agrovin S.A., Spain), and cell wall material (CWM) extracted from Monastrell skins and kindly provided by Dr. Apolinar-Valiente. The composition of these cell walls, isolated according to the method of de Vries, Voragen, Rombouts, and Pilnik (1981) and adapted by Apolinar-Valiente, Romero-Cascales, López-Roca, Gómez-Plaza, and Ros-García (2010), can be found in Apolinar-Valiente (2011).

### 2.1. Binding reactions between tannins and cell wall material

Skin CWM was weighed into 3 mL tubes. Cell wall material (CWM) samples were then combined with the different enological tannins previously dissolved in a model solution (12% ethanol at pH 3.6 adjusted with trifluoroacetic acid) at a concentration of 2 g/L. The reaction volume was 2.5 mL and two different quantities of skin CWM were tested (6 mg/mL and 13 mg/mL). Then, the different samples were shaken at 300 rpm in an orbital shaker at room temperature for 90 min. Each experiment (each tannin solution with each of the two different quantities of CWM) was made in duplicate. For each reaction, a blank without CWM and a CWM blank without tannin was also included, the latter to monitor any possible desorption of CWM-bound tannin. After the binding reaction, samples were centrifuged at 13,000 rpm and the supernatant was transferred to a new tube. Samples were then dried under vacuum at 35 °C. Recovered tannin was reconstituted in 250 μL of methanol and then analysed by phloroglucinolysis and size exclusion chromatography (SEC).

### 2.2. Total phenol content

The total phenol content of the commercial samples was estimated by measurement of optical density at 280 nm (Ribéreau-Gayon, Glories, Meujean, & Dubourdieu, 2006)

### 2.3. Anaylisis of proanthocyanidins by acid hydrolysis

The proanthocyanidin content of a 2 g/L sample of each commercial tannin was estimated spectrofotometrically following the method of Ribéreau-Gayon et al., (2006) after acid hydrolysis of the samples. From the results of this the purity of the samples (the percentage of tannins on the commercial samples) was calculated.

### 2.4. Analysis of proanthocyanidins using the phloroglucinolysis reagent

Proanthocyanidins were determined according to the method of Kennedy and Jones (2001) with some modifications, as follows. A solution of 0.2 N HCl in methanol, containing 100 g/L phloroglucinol and 20 g/L ascorbic acid was prepared (phloroglucinolysis reagent). The methanolic extract was reacted with the phloroglucinolysis reagent (1:1) in a water bath for 20 min at 50 °C and then combined with 2 volumes of 200 mM aqueous sodium acetate to stop the reaction.

HPLC analysis followed the conditions described by Busse-Valverde et al. (2010). The HPLC apparatus was a Waters 2695 system (Waters, Milford, MA) equipped with an autosampler system, a Waters 2996 photodiode array detector. Samples (10 μL injection volume) were injected on an Atlantis dC18 column (250 × 4.6 mm, 5 μm packing) protected with a guard column of the same material (20 mm × 4.6 mm, 5 μm packing) (Waters, Milford, MA). The elution conditions were as follows: 0.8 mL/min flow rate; oven temperature, 30 °C; solvent A, water/formic acid (98:2, v/v), and solvent B, acetonitrile/solvent A (80:20 v/v). Elution began with 0% B for 5 min, linear gradient from 0% to 10% B in 30 min and gradient from 10% to 20% in 30 min, followed by washing and re-equilibration of the column.

Proanthocyanidin cleavage products were estimated using their response factors relative to (+)-catechin, which was used as the quantitative standard. These analyses allowed determination of the recovery by mass of the total proanthocyanidin content, the apparent mean degree of polymerisation (mDP) and the percentage of each constitutive unit. The mDP was calculated as the sum of all subunits (flavan-3-ol monomer and phloroglucinol adducts, in moles) divided by the sum of all flavan-3-ol monomers (in moles).

To determine the naturally-occurring proanthocyanidin monomers (catechin and epicatechin) the methanolic extract was analysed without reaction with the phloroglucinolysis reagent.

### 2.5. Analysis of proanthocyanidins by size exclusion chromatography

An adaptation of the method described by Kennedy and Taylor (2003) was used for size exclusion chromatography (SEC). The method used two PLgel (300 × 7.5 mm, 5 μm, 500 (effective molecular mass range of up to 4000 using polystyrene standards) by 100 Å (effective molecular mass range of 500–30,000 using polystyrene standards) columns connected in series and protected by a guard column containing the same material (50 × 7.5 mm, 5 μm), all purchased from Polymer Labs (Amherst, MA, USA). The amount of sample injection was 40 μg. The isocratic method used a mobile phase consisting of N,N-dimethylformamide containing 1% glacial acetic acid, 5% water and 0.15 M lithium chloride. The flow-rate was maintained at 1 mL/min with a column temperature of 60 °C and elution was monitored at 280 nm.

## 3. Results and discussion

### 3.1. Commercial tannins used in the experiment

For this study, we used six commercial tannins (T1 to T6). Commercially available tannin additives take a number of forms, the most common being referred to as “oenotannins” or “enological tannins” and are mainly in the form of dry powders. Considerable variation exists in enological tannin composition. A recent characterisation of commercial enological tannin products revealed several discrepancies between the labelling of the products and their

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