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Characterization of macromolecular complexes in red wine: Composition, molecular mass distribution and particle size

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ABSTRACT

Precipitates were prepared from two compositionally different Pinot noir wines with addition of excess ethanol, and contained primarily polysaccharide, tannin and protein. The ethanol-soluble material was further fractionated into polymeric (tannin) and monomeric phenolics. Tannin associated with precipitates was of a higher molecular mass than that remaining in ethanolic solution. Wine fractions were reconstituted at the ratios of the original wine and analyzed using nanoparticle tracking analysis. The average particle size of the tannin fraction was 75–89 nm, and increased when combined with the precipitate (≅200 nm). Addition of the monomeric fraction to the tannin–precipitate complex increased both the incidence and concentration of smaller particles, reducing the average particle size. The formation of aggregates occurred in all fractions and only minor differences in particle size distribution were found between wines. Differences in particle concentration between wines appear to be due to differences in the total concentration of macromolecules rather than compositional differences.

1. Introduction

Wine constitutes a complex matrix of volatile and nonvolatile compounds, which interact to confer the stability and sensory properties of a given wine. In red wines, the major classes of macromolecules are proanthocyanidins (condensed tannins), polysaccharides and proteins. The intrinsic nature of these types of macromolecules is their propensity to interact with one another (Le Bourvellec & Renard, 2012).

The interaction between tannin and protein has been well documented (Le Bourvellec & Renard, 2012), and in wines is understood to contribute to the formation of precipitates (Charlton et al., 2002), hazes (Siebert, Carrasco, & Lynn, 1996; Van Sluyter et al., 2015) and is the mechanism underpinning astringency perception (De Freitas & Mateus, 2012; McRae, Falconer, & Kennedy, 2010). In red wines, nonglycosylated proteins, including haze-forming pathogenesis-resistant proteins found in white wine, typically present a minor component or are absent (Ferreira, Piçarra-Pereira, Monteiro, Loureiro, & Teixeira, 2001; Rowe et al., 2010). It is speculated that these are lost due to precipitation, denaturation and/or proteolysis. As a result, the principle proteins in red wines are those associated as a minor component of a larger polysaccharide molecule, the grape-derived arabinogalactans, or yeast-derived mannoproteins (Ferreira et al., 2001; Rowe et al., 2010). These are thought to be retained due to their high resistance to proteolysis and low pH conditions, and possibly because they form stable colloids in wine. It is important to note that these glycosylated proteins (polysaccharides) typically demonstrate weaker interactions with tannin than observed for other protein types (Rowe et al., 2010; Watrelot, Le Bourvellec, Imberty, & Renard, 2014). Despite these weak associations, wine polysaccharides are thought to interact with tannins in solution (Aron & Kennedy, 2007; Diaz-Rubio & Saura-Calixto, 2006; Guadalupe & Ayestarán, 2008; Poncet-Legrand, Doco, Williams, & Vernhet, 2007; Rodrigues, Ricardo-Da-Silva, Lucas, & Laureano, 2012; Saura-Calixto & Diaz-Rubio, 2007), resulting in the formation of large macromolecular complexes. Key studies have highlighted that under wine-like conditions, a significant proportion of red wine polyphenols remain in a complexed form (Aron & Kennedy, 2007; Diaz-Rubio & Saura-Calixto, 2006; Saura-Calixto & Diaz-Rubio, 2007). However, as discussed previously, these interactions may be limited by polysaccharide composition and tertiary structure, as well as the molecular mass of the tannin itself

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(Le Bourvellec, Watrelot, Ginies, Imberty, & Renard, 2012; Watrelot, Le Bourvellec, Imberty, & Renard, 2013; Watrelot et al., 2014). By inference, complex formation could have implications for sensory perception (Quijada-Morin, Williams, Rivas-Gonzalo, Doco, & Escribano-Bailon, 2014; Vidal et al., 2004) (astringency, viscosity) and possibly the stability of wine in terms of haze or precipitate formation, (Poncet-Legrand et al., 2007; Riou, Vernhet, Doco, & Moutounet, 2002) yet the system remains poorly characterized.

The current study has aimed to provide preliminary data on the concentration, composition and size of macromolecular complexes formed in red wines. To do this, wines were compared which had differences in macromolecules yet otherwise similar composition, achieved through microwave and standard maceration procedures. Three wine fractions were prepared: monomeric phenolics, tannin and 'precipitate' (a component containing polysaccharide, protein and bound tannin prepared by precipitation of wine in excess ethanol). We aimed to determine the proportion of tannin which exists in a complexed form, and to infer the compositional differences in macromolecules which drive this, if any. Secondly, the role of macromolecule concentration and composition on particle size properties was studied on the three wine fractions using nanotracking analysis (NTA). NTA was selected in favor of dynamic light scattering (DLS) due to the high polydispersity index of wine fractions (Tomaszewska et al., 2013). NTA is a technique which combines laser light scattering microscopy with a CCD camera. This approach enables the visualization and recording of nanoparticles in solution and is useful for the assessment of particle size and concentration (Dragovic et al., 2011). This is the first report of NTA use in a wine system.

2. Materials and methods

2.1. Instrumentation

An Agilent model 1100 HPLC (Agilent Technologies Australia Pty Ltd., Melbourne, Australia) was used with Chemstation software for chromatographic analyses. Nanoparticle tracking analysis was performed using a NanoSight NS300 Instrument (NanoSight Ltd., Amesbury, Wiltshire, United Kingdom).

2.2. Grape samples and winemaking treatments

Vitis vinifera L. cv. Pinot noir fruit was obtained from Tasmania, Australia at 22.5 °Brix. Bunches were randomly allocated to 1 kg lots (12 replicate lots) for vinification. For each replicate, grapes were de-stemmed by hand and crushed using a custom-made bench-top crusher. The must was decanted to 1.5 L Bodum™ plunger coffee pots. To each must, 50 mg/L SO_2 was added in the form of potassium metabisulfite solution and four replicate lots were transferred immediately to a 27 °C temperature-controlled room (standard winemaking, control). Microwave maceration was performed after SO_2 addition on the remaining experimental replicates using a previously published method (Carew, Gill, Close, & Dambergs, 2014; Carew, Sparrow, Curtin, Close, & Dambergs, 2014) with the following modifications. Each pot was individually transferred a domestic 1150 W Sharp™ 'Carousel' R-480E microwave and irradiated at full wattage for three time periods of 2 min, 1 min and 15–40 s. Between each irradiation, samples were stirred and the temperature of must using recorded using a solid stem thermometer. The second temperature reading was used to determine the length of time applied for the final irradiation. Each pot reached a peak temperature of 70–71 °C and was held at this temperature by means of a thermal blanket for 1 h. Pots were then placed in an ice bath and stirred periodically, with must temperature in each pot reaching 30 °C in approximately 30 min. The microwave treatment pots were then moved to the 27 °C (±3 °C) constant temperature room. Each pot was inoculated with active dried yeast strain EC1118 (Lallemand Pty Ltd., Australia) that had been rehydrated according to the manufacturer's instructions and fermented at 28 °C for 7 days. The Bodum™ plunger was lowered to leave approximately 1 cm of wine above the plunger surface. The progress of fermentation was monitored daily by the reduction in ferment weight. On a daily basis the plunger was gently lifted to mix the wine, and thereafter re-submerged. After fermentation the plunger was lowered, the wines were decanted and thereafter cold-settled at 4 °C. Wines were not subjected to malolactic fermentation. All wines were stabilized at first racking with 80 mg/L SO_2 and bottled under CO_2 cover in 50 mL amber glassware with wadded polypropylene capping, and stored for 12 months before analysis.

2.3. Small-scale preparation of precipitates for statistical comparison of winemaking replicates

A 1 mL wine aliquot of each of the four treatment replicates was added to 5 mL of absolute ethanol and allowed to precipitate at 4 °C for 18 h. Samples were centrifuged at 8000 *g* for 5 min, the supernatant was discarded and the pellet allowed to air dry briefly to remove excess ethanol. The pellets were reconstituted in Milli Q water and lyophilized. For the respective winemaking treatments, the percentage standard deviation of the mean for the gravimetric recovery of pellets was 8.7% and 4.1% respectively. Pellet recovery was significantly higher for the microwave-macerated treatment in comparison with the control ($P = 0.002$ by *T*-test assuming unequal variance). The treatment replicates were therefore deemed to be sufficiently reproducible to enable wine pooling for subsequent fractionation and analysis. The wines are henceforth referred to as Wine 1 (standard maceration) and Wine 2 (microwave maceration).

2.4. Wine fractionation

A schematic representation of the procedures followed for the preparation of wine fractions and their subsequent analysis is shown in Fig. 1. Wines from the four treatment replicates were pooled in order to obtain sufficient material for fractionation and reconstitution experiments. Four volumes of 96% v/v ethanol were added to 40 mL of wine, and precipitation took place at 4 °C overnight. The sample was centrifuged at 10,000*g* for 20 min, and both supernatant and pellet were retained. The pellets were reconstituted in Milli Q water, lyophilized, recovered gravimetrically as dry powders, and are henceforth referred to as the precipitate fraction. The ethanolic supernatant was recovered, concentrated under reduced pressure at 35 °C and reconstituted in 13% v/v ethanol containing 0.05% v/v TFA, and made up to 40 mL. A 50 mL bed volume Toyopearl TSK HW 40-F (180 × 25 mm) in a glass column (Michel-Miller, Vineland, NJ, USA) was used. The column was equilibrated with 13% v/v ethanol, 0.05% v/v TFA/water containing 0.1% v/v formic acid and thereafter the reconstituted wine sample was applied using a peristaltic pump. The following solvent were applied successively, all containing 0.05% v/v TFA: 150 mL 13% v/v ethanol, 150 mL 50% v/v methanol and 100 mL 70% v/v acetone. The column was re-equilibrated with 13% v/v ethanol, 0.05% v/v TFA between samples. The 50% methanol and 70% acetone fractions were retained, and will henceforth be referred to as methanol and acetone fractions respectively. The eluted fractions were concentrated under reduced pressure at 35 °C. The methanol fraction was dried under a stream of nitrogen. The acetone fraction was frozen at −80 °C and then lyophilized to a dry powder. The gravimetric recovery of all fractions was recorded.