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A role for anthocyanin in determining wine tannin concentration in Shiraz

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ABSTRACT

Four wines were made to investigate the effect of different anthocyanin and tannin fruit concentrations on wine phenolics and colour. Wines that were made from fruit with high anthocyanin concentration had high tannin concentrations regardless of the concentration of tannin in fruit, while wines made from fruit with low anthocyanin also had low tannin concentration. It was found that fruit anthocyanin concentration correlated with wine tannin concentration, wine colour and polymeric pigment formation. Anthocyanin concentration might be a key component for increasing tannin solubility and extraction into wine and the formation of polymeric pigments. Industry implications include managing tannin and anthocyanin fruit concentration for targeting tannin extraction and polymeric pigment formation in wine.

1. Introduction

In recent years, a substantial body of research has focused on managing quality in the vineyard (Downey, Dokoozlian, & Krstic, 2006; McClymont et al., 2012; Petrie & Clingeleffer, 2006; Petrie, Cooley, & Clingeleffer, 2004). While this research has demonstrated that vineyard management can be used to change levels of compounds associated with wine quality, this research has not always been extended to include winemaking. Because this does not always occur, there are some measures of quality with no direct relationship between grape and wine quality measures.

A long term goal of the Australian wine industry is to develop routine analytical methods that can be used as objective measures of quality (Gishen et al., 2001). Established routine measures such as residual sugar, pH, titratable acidity and total phenolics only capture a portion of the components of wine that contribute to the overall perception of quality. Wine quality also includes flavour, aroma, mouthfeel, and colour (Ferreira, Escudero, Campo, & Cacho, 2007; Gishen et al., 2001; Smith, Mercurio, Dambergs, Francis, & Herderich, 2007), but not all of these parameters can be routinely analysed.

In recent years, the Australian wine industry has widely adopted colour assessment and to a lesser extent tannin analysis as tools for evaluating fruit quality at harvest and in wine. However, the absence of a clear relationship between what is measured in the grape and what is measured in the wine has been a barrier to more widespread adoption of these methods. Generally, where fruit has a high anthocyanin concentration, the wine is highly coloured, but the same relationship is not generally observed for tannin (Harbertson, Kennedy, & Adams, 2002).

To investigate the relationship between grape and wine tannin we sought to make wines from grapes with different levels of tannin. An earlier study, which examined the spatial and temporal variation in anthocyanin and tannin concentration in grapes within vineyards identified a vineyard with a distinct range of different tannin and anthocyanin levels (McClymont et al., 2012). This provided a unique opportunity to make wines with different combinations of anthocyanin and tannin concentrations; within the same vineyard with areas containing all combinations of high, medium and low tannins and anthocyanins.

In response to this, we determined the anthocyanin and tannin level of selected vines within the vineyard to produce parcels of fruit for winemaking that had low–low, low–high, high–low and high–high combinations of anthocyanins and tannin. Here we report the anthocyanin and tannin content of the grape berries used to make wine and the effect of the different combinations of anthocyanin and tannin on wine colour and hue as well as polymeric pigment, wine tannin and anthocyanin concentrations over three years of wine aging.

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2. Materials and methods

2.1. Fruit collection and sample preparation

Fruit samples (100) were collected in 2009 from a *Vitis vinifera* L. Shiraz vineyard located in Sunraysia in southeast Australia (34°27′S,142°14′E) at commercial harvest (24 °Brix). Vines were trained to two lateral cordons (vertically separated) with vine and row spacings 2.4 and 3 m, respectively. Fruit samples were taken from 100 vines that represented the entire range of block variation as previously described (McClymont et al., 2012). For each of the 100 vines, all fruit within 0.5 m each side of the trunk along the row was harvested and weighed. From the harvested fruit sample, a sub-sample of 20 bunches was randomly selected and the remaining fruit sample was kept aside for winemaking. The berries from the 20 bunch sub-sample were removed and a random berry sample of 150 berries was sampled to determine anthocyanin and tannin concentration of the 100 fruit samples kept aside for winemaking. The 150 berry sample was weighed then immediately frozen in liquid nitrogen and ground to a fine powder.

2.2. Tannin and anthocyanin analysis of grape berries

For each berry sample, frozen ground whole berry material was weighed (1 g) and tannin and anthocyanins were extracted by sonicating (20 min) the weighed sample in aqueous ethanol (10 mL, 50% v/v). The sample was then centrifuged (10 min at 4000*g*) and the supernatant was removed for tannin and anthocyanin analysis.

Total anthocyanin concentration and total tannin concentration were determined according to Iland, Bruer, Edwards, Weeks, and Wilkes (2004) and Harbertson, Picciotto, and Adams (2003), respectively.

2.3. Fruit for winemaking

Following analysis of tannin and anthocyanin concentration of each berry sample, the distribution of tannin and anthocyanin for the 100 fruit samples was determined to categorize each sample as containing low levels or high levels of anthocyanin in addition to low and high levels of tannin. Fruit samples were then pooled according to their anthocyanin and tannin content to produce four parcels of fruit for winemaking containing the following combinations of anthocyanin and tannin: Low Tannin/Low Anthocyanin (LT/LA), Low Tannin/High Anthocyanin (LT/HA), High Tannin/Low Anthocyanin (HT/LA), and High Tannin/High Anthocyanin (HT/HA). Fruit that did not fall into any of the four categories was discarded.

The overall anthocyanin and tannin concentration for each of the four parcels of fruit was calculated by first determining the weight proportion each fruit sample contributed to the overall weight of the pooled parcel of fruit. The weight proportion of each fruit sample was then used to calculate the proportion of anthocyanin and tannin each fruit sample contributed to the overall parcel of fruit. The overall anthocyanin and tannin concentration for each fruit parcel was then determined as the average of the proportional contribution of each fruit sample.

2.4. Winemaking

Each parcel of fruit was crushed then subdivided into three winemaking replicates for fermentation (3×25 kg ferments). Crushed grapes were placed in a 20 L polyethylene fermentation container and inoculated with EC 1118 yeast at a rate of 0.2 g/L. Diammonium hydrogen orthophosphate (DAP, 0.15 g/L) and potassium metabisulfite (SO_2, 1 mL/L, 10% v/v) were added to each ferment at crushing. The pH of each ferment was adjusted to 3.5 with tartaric acid then placed in an 18 °C cool room for fermentation. The must was plunged twice daily for cap wetting and phenolic extraction. Pressed must was fermented to dryness (<2.5 g/L residual sugar) in a glass container then racked off lees into another glass container. Wines did not undergo malolactic fermentation and were adjusted to 30 ppm of free SO_2 and a pH of 3.5. Wines were racked into a glass container after 2 weeks of storage at 18 °C for clarification and placed in a 0 °C cool room for 1 month for cold stabilisation. Prior to bottling, wines were adjusted to 30 ppm of free SO_2 and a pH of 3.5. Wine was filtered (0.45 μm) and bottled into 375 mL wine glass bottles with stelvin screw cap closures. Wines were stored at 18 °C for wine aging until analysis.

2.5. Wine aging and analysis

Wines were analysed for tannin, anthocyanin, wine colour and polymeric pigments at pressing, bottling, 2, 6 and 12 months of bottle aging and again after 3 years of bottle aging. Wine samples were centrifuged at 16,000*g* to remove any solid material. Wine colour and wine hue were determined according to Iland et al. (2004). Total tannin, total anthocyanins, non-protein-precipitable polymeric pigments (nPPP) and protein-precipitable polymeric pigments (PPP) were determined according to Harbertson et al. (2003) and Harbertson, Yuan, Mireles, Hanlin, and Downey (2013). Previously, nPPP and PPP were named long (LPP) and short (SPP) pigmented polymers, but the size distribution of the two classes have not been characterised. Thus, Harbertson et al. (2013) revised the terminology to more accurately describe each class as precipitable and non-precipitable pigments.

2.6. Statistical analysis

This experiment was conducted in one season with no field replication. Total tannin and anthocyanin measurements of fruit and wine, wine colour and polymeric pigment data were conducted in triplicate and analysed by analysis of variance (ANOVA) with no blocking using Genstat software (13th edition).

3. Results

3.1. Fruit tannin and anthocyanin concentration

The distribution of total tannin and total anthocyanin concentration for the 100 fruit samples is shown in Fig. 1. The tannin concentration of fruit samples ranged from 1.1 to 2.8 mg/g fresh weight of whole berry with a median value around 2.1 mg/g fresh weight of whole berry, while anthocyanin concentration ranged from 0.5 to 2.3 mg/g fresh weight of whole berry with a median value around 1.5 mg/g fresh weight of whole berry. The ranges used to categorize high and low anthocyanin and high and low tannin for winemaking are shown in Table 1.

For the two wines made from grapes with low tannin, the tannin concentration in the pooled fruit was 1.56 and 1.54 mg/g fresh weight of whole berry for LT/LA and LT/HA, respectively. The two wines made from high tannin grapes had significantly different tannin concentrations of 2.38 and 2.29 mg/g fresh weight of whole berry for HT/LA and HT/HA grapes, respectively ($p < 0.001$, LSD 0.0962) (Table 1).

The concentration of anthocyanins in fruit with low levels of anthocyanin were 0.89 mg/g fresh weight of whole berry for LT/LA and 0.94 mg/g fresh weight of whole berry for HT/LA (Table 1). For fruit with high anthocyanin levels, the concentration of