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Short communication

Anthocyanins and polyphenols in Cabernet Franc wines produced with *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* yeast strains: Spectrophotometric analysis and effect on selected sensory attributes

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ABSTRACT

Grapes and wine contain phenolics divided into non-flavonoid and flavonoid classes. Yeast modulates the phenolics of wine by adsorption onto yeast cell walls. This may be advantageous for colour and quality. The effect of *Torulaspora delbrueckii* (654&M2/1) and *Saccharomyces cerevisiae* (VIN13) on phenolics and sensory attributes of Cabernet Franc wines (2012/2013) were evaluated. Spectrophotometric analysis was used to determine the phenolic content. Sensory analysis was conducted by expert tasters to evaluate the wines. ANOVA showed that polyphenols and anthocyanins were higher in M2/1 + VIN13 wines (2012/2013) than 654 + VIN13 wines. Colour, fruitiness, mouthfeel, sweetness, astringency and quality were different between treatments. 654 + VIN13 wines (2012) were higher in polyphenols, acidity, astringency and mouthfeel than M2/1 + VIN13 wines. M2/1 + VIN13 (2012/2013) had increased colour and quality than 654 + VIN13 wines. Two treatments were identified for Cabernet Franc wines; M2/1 + VIN13 and 654 + VIN13, which resulted in wines with increased colour and wines with increased mouthfeel and astringency, respectively.

1. Introduction

Red grapes and resulting wines contain large quantities of phenolic compounds classified as flavonoids and non-flavonoids that play important roles in red wine quality, complexity and structure (Ribéreau-Gayon, Glories, Maujean, & Du Bourdieu, 2006).

Phenolic compounds contribute to the sensory characteristics of red wine, specifically colour, mouthfeel, complexity and astringency, as well as wine stability and antioxidant capacity (Kennedy, 2008; Ivanova, Stefova, & Chinnici, 2010).

Polyphenols, *e.g.* flavanols, which absorb at *ca.* 280 nm and anthocyanins (pigments), which absorb at *ca.* 520 nm, are two major classes of flavonoids that occur in nature and that arise as plant secondary metabolites (Margalit, 2004). Flavanols are located primarily in the seeds and skins of grapes, whereas anthocyanins are mainly located in the grape skin (Cadot, Castello, & Chevalier, 2006). The major monomers are (+)-catechin, (−)-epicatechin and (−)-epicatechin-3-*O*-gallate (Ivanova et al., 2010). Anthocyanins constitute the largest group of water-soluble pigments in the plant kingdom, contributing to the colours displayed by many flowers, fruits and leaves (Clifford, 2000).

In red wine, hydrolysable tannins and anthocyanins are the most important phenolic compounds that are indicators of wine quality (Kennedy, Hayasaka, Vidal, Waters, & Jones, 2001). Tannins contribute to mouthfeel, complexity and astringency of wines, but also form pigmented polymers in association with anthocyanins to provide stable pigments required to give red wine its colour stability (Kennedy, 2008). The most important grape anthocyanins include the 3-*O*-glucoside forms of cyanidin, peonidin, petunidin, delphinidin and malvidin, and their acetylated- and coumaryolated derivatives (Ivanova et al., 2010).

Phenolic compound concentrations in red wine depend on viticultural practices, *terroir*, grape ripeness level (time of harvest) and vinification processes (Andersen & Markham, 2007). Phenolic compounds, which are derived from the skin and seeds of grapes, make up the majority of the phenolic compounds present in wine, while stem-derived phenolic compounds are minor components if included in the vinification process (Kennedy, 2008). One of the most important sensory qualities of red wine is colour, which originates from anthocyanins extracted from the grape skin during maceration (Kumšta, Pavloušek, & Kárnik, 2014).

The colour intensity of red wine can be altered during fermentation, depending on the metabolic characteristics of the yeast used (Morata et al., 2012). Yeast cell walls can adsorb anthocyanins during

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fermentation (Nguela, Vernhet, Sieczkowski, & Brillouet, 2015) and wine mannoproteins and arabinogalactans then interact with these anthocyanins (Gonçalves et al., 2018). The degree of adsorption depends on the yeast strain used (Suárez-Lepe & Morata, 2012). Wine astringency, bitterness and colour are related to proanthocyanidins and polyphenols, resulting from the condensation between flavanols and anthocyanins (Monagas, Gómez-Cordovés, & Bartolomé, 2006).

There are a number of analytical techniques available to quantify phenolic compounds in wine, i.e. tannin precipitation methods (Sarneckis et al, 2006), high speed counter current chromatography and super critical fluid chromatography (Ignat, Volf, & Popa, 2011), high-efficiency performance liquid chromatography (De Villiers, Cabooter, Lynen, Desmet, & Sandra 2011) and tannin assay (Aleixandre-Tudo, Buica, Nieuwoudt, Aleixandre, & du Toit, 2017) but high-performance liquid chromatographic techniques are the preferred tool (Monagas et al., 2006; Downey & Rochfort, 2008; Baiano et al., 2015; Nelson, Kennedy, Zhang, & Kurtural, 2016). However, this technique is not always available for conducting routine analyses in wineries. The alternative analytic tool would be spectrophotometry (Ivanova et al., 2010; Daniel, 2015; Aleixandre-Tudo et al., 2017), which is affordable with less maintenance, usually no reagent consumption and short/rapid measurements. It is used for wine and grape analyses to follow the evolution of phenolic compounds during grape ripening and the winemaking process (Aleixandre-Tudo et al., 2017; Boulet, Du Casse, & Cheynier, 2017).

The aim of this study was to quantify the polyphenols and total anthocyanin content of Cabernet Franc wines produced over two vintages (comparing vintages and treatments) with different *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* strain combinations using a spectrophotometric technique, as well as to determine if this spectrophotometric technique is discriminatory to distinguish between treatments in terms of phenolics. The effect of treatment on selected sensory attributes were also investigated.

2. Materials and methods

2.1. Vinification

Cabernet Franc grapes were grown on a northern slope (southern-hemisphere) in Glenrosa soil on the experimental farm of the Agricultural Research Council (ARC) in Stellenbosch, South Africa (33°54′45.7″S 18°51′47.0″E). The scion was grafted onto CF 213 × Richter 99 (RY 13 C) rootstock. The vineyard received drip-irrigation. Grapes were harvested and the must was prepared at the experimental cellar of the ARC.

The must was co-inoculated with *T. delbrueckii* (strain 654, ARC gene bank collection) + *S. cerevisiae* (strain VIN13, Anchor Wine Yeast, South Africa) and *T. delbrueckii* (strain M2/1, ARC gene bank collection) + *S. cerevisiae* (strain VIN13) at a 1:1 ratio. Yeast starter cultures were cultivated in yeast extract-peptone-dextrose broth in a three-stage procedure (Merck, South Africa).

The two species in each treatment were inoculated one hour apart. *Torulaspora delbrueckii* strains 654 and M2/1 were inoculated as pure wet cultures on day 0 at a concentration of 1.0 × 10 cells/mL. *S. cerevisiae* (0.3 g/L active dry yeast) were added 1 h later to complete AF (Jolly, Augustyn, & Pretorius, 2003). Mixed culture fermentations were conducted in a temperature-controlled room at *ca.* 24 °C. Equal portions of grape skins and grape must were separated, homogenised and collected into 70 L fermentation bins.

The fermentation caps were punched down twice a day and all treatments were subjected to the same grape-pomace contact time. Wines were racked off the lees and the total SO_2 adjusted to *ca.* 85 mg/L. Wines were stored at 15 °C until required for analysis (Minnaar, Ntushelo, Ngqumba, Van Breda, & Jolly, 2015). Wines were made over two consecutive vintages (2012, 2013).

Treatments resulted in twelve wines, i.e. two treatments in triplicate for two vintages. All samples were analysed in triplicate. Selected physicochemical measurements were performed on the base must prior to analyses (Table 1).

Table 1
Oenological parameters measured in Cabernet Franc grape must.

Parameters measured	Vintage	
	2012	2013
	Base must analysis	
Sugar (°Brix)	26.10	23.70
Total titratable acidity (g/L)	4.70	5.30
pH	3.72	3.40

2.2. Reagents and reference standards

Reagents used were methanol, tartaric acid, ethanol and potassium hydroxide (Merck (Pty) Ltd, South Africa). Reference standards used were malvidin 3-*O*-glucoside and (−)-epicatechin (Sigma-Aldrich (Pty) Ltd, South Africa). All reagents and reference standards were of HPLC grade. De-ionised and distilled water were supplied by an in-house Modulab water purification system.

2.3. Preparation of malvidin 3-O-glucoside reference standard stock solution

Malvidin 3-*O*-glucoside stock solution was prepared by accurately weighing off 3.1 mg of malvidin-3-*O*-glucoside reference standard. The 3.1 mg was dissolved in 3.0 mL of de-ionised water in a calibrated glass vial (final conc. 1.033 mg/mL).

2.4. Preparation of (−)-epicatechin reference standard stock solution

Epicatechin (−) stock solution was prepared by accurately weighing off 11.4 mg of (−)-epicatechin reference standard. The 11.4 mg was dissolved in a 1:1 methanol/de-ionised water solution and brought to volume in a 10 mL volumetric flask (final conc. 1.14 mg/mL).

2.5. Model wine solution preparation

A model wine solution, i.e. a solution containing 6 g/L tartaric acid, adjusted to pH 3.30 with 1 N potassium hydroxide, supplemented with 13% ethanol (v/v) was prepared (Lambri, Dordoni, Silva, & De Faveri, 2013).

2.5.1. Preparation of malvidin 3-O-glucoside standard solution (malvidin + model wine)

Standard solutions (n = 4) containing 0.588 mg/mL, 0.294 mg/mL, 0.147 mg/mL and 0.073 mg/mL of malvidin 3-*O*-glucoside were obtained through sequential dilutions. These solutions were made up using model wine and the reference standard, i.e. malvidin 3-*O*-glucoside (stock solution). These standard solutions were used to establish a calibration curve for anthocyanins.

2.5.2. Preparation of (−)-epicatechin standard solution (epicatechin + model wine)

Standard solutions (n = 4) containing 0.565 mg/mL, 0.325 mg/mL, 0.162 mg/mL and 0.081 mg/mL of (−)-epicatechin were obtained through sequential dilutions. The solutions were made up using model wine and the reference standard, i.e. (−)-epicatechin (stock solution). These standard solutions were used to establish a calibration curve for (−)-epicatechin.