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Modelling phenolic and volatile composition to characterize the effects of pre-fermentative cold soaking in Tempranillo wines

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ABSTRACT

The impact of pre-fermentative cold soak, alone or in combination with dry ice addition, on colour, phenolic and volatile composition of Tempranillo wines at 12 months after bottling was studied. A control wine without cold soak was also evaluated. A sample set consisting of 66 wines was investigated. The results from ANOVA and PCA analysis showed significant treatment-related differences for a number of chemical measurements, as well as overlapping effects. Orthogonal projections to latent structures discriminant analysis (OPLS-DA) of the data showed that the dry ice addition treatment had a major effect on the anthocyanin fraction and on the levels of ethyl decanoate, 2-phenylethyl acetate and decanoic acid. In comparison, the cold soak treatment only had a slight effect on the bisulphite bleaching anthocyanins and volatile composition.

1. Introduction

Polyphenols and volatiles are essential for wine colour, mouth-feel and flavour. The relative proportions of anthocyanins and tannins can be adjusted during skin maceration to achieve a wine capable of undergoing good evolution during aging (Glories & Galvin, 1990). Therefore, when fruity and full bodied red wines are required, pre-fermentative cold maceration appears as an alternative for winemakers (Cai et al., 2014). However, the effects of pre-fermentative techniques are highly dependent on grape sanitary status and phenolic ripeness (Alvarez, Aleixandre, García, & Lizama, 2006), as well as on the aromatic nature of the variety (Moreno-Pérez, Vila-López, & Fernández-Fernández, 2013).

Skin polyphenols and volatile compounds are extracted throughout the pre-fermentative cold soak in the absence of ethanol (Gómez-Míguez, González-Miret, & Heredia, 2007). Dry ice (solid carbon dioxide) addition appears as a common method to obtain the cold temperatures required for this technique (Heredia et al., 2010). After crushing and dry ice addition, grape skin cells are broken and disorganized through freezing, which facilitates aroma and phenolic extraction (Álvarez, Aleixandre, García, Lizama, & Aleixandre-Tudó, 2009).

Contradictory results have been observed in the literature regarding the effect of pre-fermentative cold soak techniques on chemical wine composition. Several studies have shown that cold soak has either no effect, while a decrease in phenolic levels was also observed (De Beer, Joubert, Marais, & Manley, 2006; Heatherbell, Dicey, Goldsworthy, & Vanhanen, 1997; Marais, 2003; Okubo, Goto-Yamamoto, & Okazaki, 2003). Other authors noticed an increased phenolic content and higher sensory scores when wines were cold soaked before fermentation (Gil-Muñoz et al., 2009; Gordillo, López-Infante, Ramírez-Pérez, González-Miret, & Heredia, 2010; Heredia et al., 2010; Koyama, Goto-Yamamoto, & Hashizume, 2007). During the cold soak step numerous reactions between grape phenolics also occur, which may influence wine sensory properties (Parenti, Spugnoli, Calamai, Ferrari, & Gori, 2004). Moreover, other compounds (proteins and polysaccharides) are also extracted, which may participate in condensation reactions (Gómez-Plaza, Gil-Muñoz, López-Roca, Martínez-Cutillas, & Fernández-Fernández, 2001).

Pre-fermentative techniques have been used extensively in the

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production of white and rosé wines and have been recommended as a means for enhancing wine aroma (Sánchez Palomo, González-Viñas, Díaz-Maroto, Soriano-Pérez, & Pérez-Coello, 2007). Cold soak has also been tested in red winemaking (Sacchi, Bisson, & Adams, 2005). However, little is known about its effect on the volatile composition. Moreno-Pérez et al. (2013) cited differences between cold soak treated red wines and conventional winemaking after six months of bottling, although no differences between pre-treatments (cold soak, freezing grapes and dry ice addition) were observed. Moreover, in Monastrell wines increased volatile compound levels were reported for Monastrell wines produced by dry ice addition after 6 months of bottle storage (Alvarez, Aleixandre, García, & Lizama, 2006).

Based on these contradictory reports, the aim of this work was thus to evaluate the impact of cold soak techniques on color, phenolic and volatile composition of red Tempranillo wines at 12 months of bottle storage. Tempranillo is one of the most widely planted grape cultivars in Spain and wines made from this cultivar are increasingly being accepted in new world wine producing countries such as Australia and the United States (Cynkar, Dambergs, Smith, & Cozzolino, 2010; USDA, 2014).

2. Materials and methods

2.1. Wine samples

Tempranillo grapes from a commercial vineyard (Utiel-Requena, Valencia, Spain) were harvested in 2008. At harvest, the grapes had 221.33 ± 9.29 g/L of total sugar content, total acidity of 6.1 ± 0.2 g/L as tartaric acid, pH of 3.34 ± 0.12 and potential alcohol of 13.07 ± 0.64% vol. (N = 3). Wines were produced at an experimental wine production centre (Universitat Politècnica de València (UPV)). 40 kg of grapes were destemmed, crushed, mixed and divided into closed 50 L stainless steel tanks. Potassium bisulphite was added at 100 mg/kg before fermentation. Treatments consisted of traditional vinification wines (T–V) without cold soak (control); cold soaked wines at 6 to 8 °C for four days (C–S); and dry ice addition (0 to 2 °C) followed by cold soak at 6 to 8 °C for four days (D–I). Twenty-two small scale vinifications were made for each treatment. After the cold soak period wines were left to warm up in the fermentation room and commercial yeasts were inoculated at 20 g/hL (*Saccharomyces cerevisiae* strain EP 841, Agrovin, Spain). The highest temperature during fermentation was 25 °C. Manual punching down was carried out twice a day. T–V wines were left on the skins for 15 days to ensure that sugar levels were lower than 2 g/L. Following the same workflow, C–S and D–I wines were also left on the skins for 15 days after the four days cold soak treatment. After fermentation wines were pressed and the first 5 L were mixed with 20 L free-run wine. *Oenococcus oeni* strain OE 104 (Agrovin, Spain) lactic acid bacteria was inoculated and malolactic fermentation (MLF) was conducted at room temperature (~20 °C). Potassium bisulphite was added at 50 mg/L before bottling. Wines were stored at room temperature (15 ± 2 °C) and cork closures were used.

2.2. Analytical methods

A UV–Visible JASCO V-530 spectrophotometer, and a JASCO MD-2010 Plus high-performance liquid chromatography instrument coupled with a diode array detector (DAD) (JASCO LC-Net II/ADC, Tokyo, Japan) were used for phenolic measurements. All the spectrophotometric measurements were performed in triplicate. Using the analytical methods described by Glories (1984) colour intensity, hue, gelatin (astringency) and EtOH (tannin-polysaccharide molecules) indexes were estimated. The Ribéreau-Gayon and Stronestreet (1965) method was used for the determination of bisulphite bleached anthocyanins. Catechins were quantified using the method reported by Sun, Ricardo Da Silva, and Spranger (1998). The modified version of the MCP tannin assay reported by Mercurio, Dambergs, Herderich, and Smith (2007) was used for tannin quantification. The method reported by Boulton (1996) was used to analyze the contribution of the copigmented, free and polymeric anthocyanins to the total wine colour. PVPP (anthocyanin-tannin complexes) and DMACH (tannin degree of polymerization) indexes were calculated according to Vivas and Glories (1995). The Folin-Ciocalteu index was determined using the method developed by Singleton and Rossi (1965).

HPLC was used to quantify individual phenolic compounds using the method reported by Jensen, Blachez, Egebo, and Meyer (2007). Gallic acid, (+)-catechin and (−)-epicatechin were quantified at 280 nm. Flavan-3-ols were defined as the sum of (+)-catechin and (−)-epicatechin. Hydroxycinnamic acids were quantified at 316 nm. Phenolic acids were calculated as the sum of gallic and caffeic, coutaric, *p*-coumaric and caftaric acid. Flavonols (quercetin rutinoside, quercetin glucoside, myricetin, quercetin and kaempherol) were quantified at 365 nm. Delphinidin, cyanidin, peonidin, petunidin and malvidin acetyl and coumaryl glucosides resulted in the derivated anthocyanins. Total anthocyanins were calculated as the sum of anthocyanidin-3-glucosides and derivated anthocyanins. Within each phenolic group, compounds were identified based on their intrinsic spectral features and retention times. Commercially available standards were used to build the calibration curves for phenolics quantifications: gallic acid (Fluka, Milwaukee, WI, USA), (+)-catechin (Fluka, Milwaukee, WI, USA) for flavan-3-ols, caffeic acid (Fluka, Milwaukee, WI, USA) for hydroxycinnamic acids, rutin (Sigma–Aldrich, St Louis, MO) for flavonols and malvidine-3-glucoside (Sigma–Aldrich, St Louis, MO) for anthocyanins. 20 μL of the wine sample were injected twice after centrifugation (5000 rpm) and filtration (0.45 μm membrane Millipore filter). Separation was carried out on a Gemini NX (Phenomenex, Torrance, CA) 5 μm, 250 mm × 4.6 mm i.d. column at 40 °C. Acetonitrile and *o*-phosphoric acid were used as solvents. Solvents composition and the elution gradient were reported elsewhere (Jensen et al., 2007).

An Agilent gas chromatograph (GC) (Agilent Technologies, Waldbronn, Germany) equipped with a split/splitless capillary injection port and flame ionization detector (FID) was used for the analysis of the wine aroma composition. Separations were performed on a ZB- WAX Plus column (50 m × 0.25 mm i.d., 0.25 μm film thickness) from Phenomenex (Aschaffenburg, Germany). Duplicate injections were performed using the following conditions: injector temperature, 250 °C; detector temperature, 300 °C; carrier gas flow (N2), 1 mL/min. Injections were made in split mode (split ratio, 1/60; sample size, 1 μl). The oven temperature was maintained at 40 °C for 7 min, from 40 to 110 °C at 4 °C/min, from 110 to 170 °C at 10 °C/min, and then held for 10 min. The comparison of retention times with those of standard compounds was used to identify volatile compounds. Preparation of the samples was carried out following the method proposed by Hernanz, Heredia, Beltran, and Recamales (1999). Twenty volatile compounds were quantified with 2-octanol as internal standard.

2.3. Statistical analysis

Statgraphics Plus 5.1 software was used for the ANOVA treatment of the data. Principal component analysis (PCA) and orthogonal projections to latent structures discriminant analysis (OPLS-DA) (Trygg & Wold, 2002) was performed using SIMCA version 13.0.3 software (www.umetrics.com). Chemical measurements