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Effect of vegetal ground cover crops on wine anthocyanin content

Y. Bouzas-Cid [a], J. Portu [b], E.P. Pérez-Álvarez [b], A. Gonzalo-Diago [b], T. Garde-Cerdán [b,*]

[a] Viticulture and Enology Research Station of Galicia (EVEGA-INGACAL), Ponte San Clodio s/n, 32428 Leiro, Ourense, Spain
[b] Instituto de Ciencias de la Vid y del Vino (CSIC-CAR-UR), Ctra. de Burgos Km. 6, 26007 Logroño, Spain

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ABSTRACT

Wine colour is a quality index that can provide information about conservation state, age or the presence of defects. Anthocyanin compounds are colour-related molecules and their concentration is affected by several factors such as grape variety, berry maturity degree or cultural practices. The aim of this work was to determine the anthocyanin composition of Mencía wines and how this is affected by the establishment of different cover crops (native vegetation, ryegrass and subterranean clover) respect to soil tillage treatment. This study was carried out during two consecutive seasons in the same vineyard. The use of cover crops significantly affected wine anthocyanin content; even though, their basic attributes were not altered by the treatments. In 2013, the wines from the ryegrass treatment had a significantly greater total anthocyanins concentration and, in 2014, the wines under the native cover had the highest concentration of these compounds. In both years, wines coming from the treatment under subterranean clover had a lower concentration of total anthocyanins when compared with those from the rest of the treatments. Cover crops increased wine anthocyanin concentrations when compared to the tilled treatment. However, the type of cover crop causing the highest increases differed from year to year.

1. Introduction

Phenolic compounds play an important role as constituents of wine colour, thus contributing to the wine sensory quality. Within this family of compounds, anthocyanins are the most important molecules that are found in red grape skins. They are extracted during the winemaking process, providing the bluish-red colour characteristic of red wines. Although anthocyanins are the main compounds responsible for red-wine colour, other phenolic compounds indirectly contribute to it. For instance, flavanols contribute to wine astringency, but they also take part in wine colour stability and in wine ageing capacity (Gómez-Mínguez et al., 2006). Moreover, flavonols and phenolic acids influence wine colour by means of copigmentation reactions (Escribano-Bailón and Santos-Buelga, 2012).

One of the most important factors that influence wine phenolic composition is grape variety, namely genetics (González-Neves et al., 2007). Other factors to consider are the climatic conditions of each growing season, cluster exposure to sunlight (Song et al., 2014), berry maturity degree and cultural practices, such as soil management with cover crops (Pérez-Álvarez et al., 2015) or the foliar application of nitrogen compounds (Portu et al., 2015a).

In the Ribeiro Designation of Origin (DO), Galicia (NW Spain), different traditional grapevine varieties are cultivated. Among the red cultivars, 'Mencía' (*Vitis vinifera* L.) is the most relevant one. This variety has high fertility and productivity, being appropriate for the production of young red wines (Consello Regulador del Ribeiro, 2015), which are traditionally considered as fruity and with high alcoholic degree (12–14% vol.). Several authors (Calleja and Falqué, 2005) described the aromatic profile of this red variety, which is characterised by its high content in different families of varietal aromatic compounds such as linalool and citronellol.

The Galician wine sector is concerned about the excessive vegetative growth of Mencía, which can cause imbalances between vegetative growth and yield, affecting grape quality (Jackson and Lombard, 1993). A cultural practice that may be used to control vine growth is soil management with cover crops, since they compete with vines for water and nutrients, thus limiting vine vegetative growth (Giese et al., 2014; Yuste, 2005). This reduction in vegetative growth caused by cover crops might provide a higher sunlight exposure on the cluster zone through a decrease in shoot secondary growth. This fact may reduce green pruning operations, and of cluster thinning thus improving the polyphenol metabolism in the berries. Therefore, wines would retain more of these compounds, improving their colour properties and structure, hence,

* Corresponding author.
E-mail addresses: teresa.garde@icvv.es, Teresa.GardeCerdan@gmail.com, gardecerdan@hotmail.com (T. Garde-Cerdán).

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having a higher quality. Previous studies reported that the use of cover crops as a soil management strategy in vineyards increased total anthocyanins content in red grapes (Pérez-Álvarez et al., 2013) and, therefore, in wine (Trigo-Córdoba et al., 2015).

However, the influence that the use of cover crops may have on wine anthocyanin detailed composition has not been studied yet. In this context, the aim of this work was to assess the effect of three different cover crops (native vegetation, ryegrass and subterranean clover) respect to a control treatment (tillage) on the anthocyanin composition of Mencía wines. As cover crops increased wine anthocyanins total concentration (Trigo-Córdoba et al., 2015), in the current study, we hypothesised the individual anthocyanins would also be increased by the use of this soil management strategy.

2. Materials and methods

2.1. Experimental design

The experiment was carried out during two consecutive vintages (2013 and 2014) in a Mencía (*Vitis vinifera* L.) vineyard located within the experimental farm of the Viticulture and Enology Research Station of Galicia (EVEGA) in Leiro, Ourense, NW Spain (latitude 42°21.6′N, longitude 8°7.02′W, altitude 115 m above sea level). The vineyard was planted in 2007 on 196-17C rootstock at a spacing of 2.3 × 1.25 m (3478 vines/ha) and vines were trained to a vertical trellis on a single cordon system spur-pruned in winter (10–12 buds/vine) oriented in the East-West direction.

Treatments consisted of four different soil management systems in the inter-rows: i) soil tillage (ST); ii) native vegetation (NV, included grass species such as genus *Bromus* and *Lolium*, and broadleaf species such as *Bellis perennis* L., *Senecio vulgaris* L., *Vicia sativa* L., and genus *Trifolium* and *Conyza*); iii) English ryegrass (*Lolium perenne* L.) sown at 40 kg/ha (ER); and iv) subterranean clover (*Trifolium subterraneum* L.) sown at 30 kg/ha (SC). Treatments with cover crops were mowed three times per year, when vegetation reached 20 cm height. Soil tillage alleys were disked during the growing season, in order to eliminate weeds. The treatments were replicated three times in a complete randomized block design. Each replicate consisted of three rows with seven vines per row. The five vines in the centre of the middle row were used for measurements and the rest acted as buffers (Trigo-Córdoba et al., 2015).

The soil at the site is an Inceptisol (Soil Survey Staff, 2010) of sandy-loam texture (68% sand, 19.4% slit and 12.6% clay), with pH (H2O) of 5.8 and organic matter content of 4%.

The climate in the region is characterised as temperate, humid with cool nights (Fraga et al., 2014). In 2013 and 2014, the annual rainfall was 1283 and 1301 mm and the mean temperature was 13.5 and 14.2 °C for 2013 and 2014, respectively. Data on vegetative growth, yield and must quality from this experiment have been reported elsewhere (Trigo-Córdoba et al., 2015).

2.2. Winemaking and storage

Grapes from the different treatments were manually harvested on the same day, when berries achieved an appropriate balance between probable alcoholic grade and titratable acidity, and were transported to the experimental winery in 20-kg field boxes. Winemaking was performed separately at EVEGA on samples of about 35 kg from each treatment.

Grapes were mechanically destemmed and transferred to 35-L stainless steel containers. During grape processing, 50 mg/L of SO_2 were added to the mass. Fermentations were carried out at room temperature (22–24 °C). Grand rouge XG (Lamothe-Abiet, Bordeaux, France) yeast was added following manufacturer's instructions. The wine lots were punched down once a day until the end of alcoholic fermentation (8 days). Then, they were pressed, racked into new tanks and kept at room temperature for a couple of days. Then, wines were kept at 4 °C in a chamber for a period of approximately one month for cold stabilization. After this period, wines were filtered, bottled and stored at 10 °C.

2.3. Analytical methods

2.3.1. General attributes of wines

Basic attributes of wines (alcohol content, titratable acidity, pH, malic and tartaric acids) were determined by Fourier transform infrared spectrometry (FTIR) using a WineScan FT120 analyser (FOSS Electric, Barcelona, Spain), calibrated according to the official methods (OIV, 2009). Determinations of these attributes were performed in duplicate.

2.3.2. Colour attributes

An ultraviolet-visible spectrophotometer model Thermo Helios Zeta (Thermo Scientific Ltd, Leicester, UK) was used to determine wine colour attributes, using the methodology described by Zamora (2003), and summarized here. Prior to the measurements, wines were kept at room temperature (20 °C) during a period of 24 h, and then they were filtered through a pore size of 0.22 μm.

Colour intensity and tonality were determined through direct reading of wines at three different wavelengths: 420, 520 and 620 nm. Total polyphenol index (TPI) was determined by reading at a wavelength of 280 nm, after diluting the sample to a factor of 100. The concentration of total anthocyanins in wines was quantified according to the discolouration experimented by the addition of metabisulphite to the samples and reading in the spectrophotometer at 520 nm (Zamora, 2003). Results were expressed in mg/L. Total tannins of Mencía wines were determined after an acid hydrolysis of the samples. Then, readings in the spectrophotometer were made at 550 nm (Zamora, 2003). The concentration of these compounds was expressed in g/L. All colour determinations were performed in duplicate.

2.3.3. Determination of anthocyanins compounds in wines

Anthocyanins in Mencía wines were determined in duplicate by high-performance liquid chromatography (HPLC) with direct injection of 10 μL of sample, previously centrifuged (4000 rpm, 10 °C, 10 min) and filtered through a pore size of 0.22 μm (Easyprep, Quebec, Canada). The anthocyanins identified were 3-*O*-glucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin and their corresponding acetyl and *trans*-*p*-coumaroyl derivatives. In addition, four pyranoanthocyanins were determined: vitisins A and B, 10-hydroxyphenyl-pyranomalvidin-3-glucoside (10-HP-pymv-3-glc) and 10-dihydroxyphenyl-pyranomalvidin-3-glucoside (10-DHP-pymv-3-glc).

A HPLC 1260 Infinity (Agilent Technologies, Palo Alto, CA, USA) equipped with a photodiode array detector was used. The separation was carried out in a column Licrosphere® 100 RP18 in reverse phase (4.0 × 250 mm, with particle size of 5 μm, Agilent), with pre-column Licrosphere® 100 RP18 (4 × 4 mm, with particle size of 5 μm, Agilent).

The methodology developed by Castillo-Muñoz et al. (2007) was followed. The analysis temperature was 40 °C with a flow rate of 0.630 mL/min. Mobile phases used in the separation of compounds were: (A) acetonitrile/water/formic acid in proportion 3/88.5/8.5 (v/v/v) and (B) acetonitrile/water/formic acid in proportion 50/41.5/8.5 (v/v/v). The elution gradient was: 0 min, 6% B; 15 min, 30% B; 30 min, 50% B; 35 min, 60% B; 38 min, 60% B; 46 min, 6% B.

Detection was performed at the maximum absorption wavelength of anthocyanins in the ultraviolet-visible region, 520 nm. The compounds were identified according to their retention time