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Phenylalanine and urea foliar applications to grapevine: Effect on wine phenolic content

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ABSTRACT

Phenolic compounds play a key role in the organoleptic properties of wines. Viticultural practices may influence grape and wine phenolic content, thus determining their quality. The objective of this study was to evaluate the effect of foliar applications of phenylalanine and urea, at two different doses, on wine phenolic composition. Grapes were harvested at their optimal technological maturity and their respective wines were elaborated at small scale. Wine detailed phenolic composition was determined. Results revealed that the content of several anthocyanins and flavonols was enhanced by the application of both phenylalanine doses and by the application of the low dose of urea. In contrast, flavanols and non-flavonoid compounds were less affected by the foliar treatments. The findings seem to be related to the time of application, since anthocyanins and flavonols are accumulated after veraison. In conclusion, nitrogen foliar fertilization increased the phenolic content of Tempranillo wines. This could be of interest since anthocyanins and flavonols are associated with wine quality, especially with its color.

1. Introduction

Phenolic compounds are known to be key factor of the wine quality as they determine the wine color, certain mouthfeel properties or the wine aging potential. Moreover, phenolic compounds have been related to some of the health benefits associated with a moderate wine consumption. Wine phenolic composition depends on grape composition, which is in turn affected by many factors, including environmental conditions, viticultural practices or cultivar genetics (Downey, Dokoozlian, & Krstic, 2006; Kennedy, Matthews, & Waterhouse, 2002; Lijavetzky et al., 2006), and on the different techniques applied in the cellar and the numerous reactions occurred during the winemaking process (Sacchi, Bisson, & Adams, 2005; Zimman, Joslin, Lyon, Meier, & Waterhouse, 2002).

Among the viticultural practices, soil fertilization has a strong impact on grape and wine composition (Bell & Henschke, 2005). Previous studies have shown that high nitrogen supply may lead to bunch shading, higher berry size, or metabolic alterations, decreasing the phenolic compound content (Hilbert et al., 2003; Soubeyrand et al., 2014). However, there seems not to be a clear tendency since other works have reported opposite results (Delgado, Martín, Del Álamo, & González, 2004; Martín, Delgado, González, & Gallegos, 2004). The controversial results seem to be related to different experimental designs: nitrogen dose, timing and numbers of applications, besides other external factors like environmental or soil conditions. In an extensive review, Bell and Henschke (2005) suggested that the influence of soil nitrogen fertilization on wine phenolic content is not clear. In general, when grapevines are overfertilized they become excessively vegetative, increasing the competition for carbohydrates between vegetative (shoots) and reproductive (grapes) sinks, resulting in a lower accumulation of secondary metabolites (Bell & Henschke, 2005). In addition, the excessive vine growth alters grapevine canopy leading to changes in vine microclimate (Smart, 1985). However, nitrogen applications under deficit conditions may conduct to higher rates of photosynthesis, allowing then to supply all sinks and metabolic pathways which require carbohydrates and delaying leaf senescence (Bell & Henschke, 2005).

In recent years, there has been a growing interest in foliar fertilization as an alternative or complementary technique to traditional soil nitrogen fertilization. Foliar fertilization entails a quickly and efficient assimilation of the products, allowing the application of lesser amounts of nitrogen than soil fertilization and helping to mitigate the negative effects of stress conditions (Lasa et al., 2012). In this respect, foliar fertilization has been reported to offer considerable advantages. Foliar fertilization may avoid or

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minimize some of the disadvantages that are associated with traditional soil fertilization, such as nitrogen leaching (Fernández & Eichert, 2009). Moreover, foliar sprays can be applied throughout the growing season and nutrient uptake is reported to be faster as compared to soil fertilization (Haytova, 2013). Furthermore, foliar fertilization is considered very effective in crops grown on degraded soil and when soil nutrient availability is low (Kannan, 2010), which are very common in viticulture. Considering fertilization costs, Haytova (2013) stated that fertilizers might be mixed with pesticides, allowing them to be sprayed together and therefore reducing plantation costs. Moreover, Haytova (2013) also reviewed some works which demonstrated the economic viability of foliar application in different crops. However, previous authors have stated that it is difficult to predict the response of plants to the foliar application since its efficacy is affected by a great number of complex factors (Fernández, Sotiropoulos, & Brown, 2013). For instance, Fernández et al. (2013) detailed some of them, including plant species, leaf cuticular composition, plant phenology, environmental aspects, or phloem mobility. Research in viticulture has shown that foliar application of urea may modify the wine aroma profile (Ancín-Azpilicueta, Nieto-Rojo, & Gómez-Cordón, 2013) or improve must nitrogen composition (Garde-Cerdán et al., 2014). In the latter work, the application of phenylalanine led to a similar enhancement of must nitrogen composition in comparison with urea application.

In view of all the foregoing, our hypothesis was that foliar application of nitrogen compounds may lead to differences in wine quality since similar trends have been observed when other compounds, such as fungicides or oak extracts, have been foliar applied to grapevines (Briz-Cid, Figueiredo-González, Rial-Otero, Cancho-Grande, & Simal-Gándara, 2014; Pardo-García et al., 2014). Therefore, the purpose of this work was to study the detailed phenolic composition of red wines made from grapevines which had been treated with two sources of nitrogen, phenylalanine and urea, at two different doses. This study may lead to a better understanding of how foliar applications of nitrogen compounds at veraison might influence wine phenolic quality.

2. Materials and methods

2.1. Reagents and standards

All solvents (methanol, acetonitrile, and formic acid) were of HPLC quality, and all chemicals were analytical grade (>99%) unless otherwise stated, and were purchased from Panreac (Barcelona, Spain). Water was of Milli-Q quality (Millipore, Bedford, USA). Nitrogen compounds (phenylalanine and urea) and Tween 80 were purchased from Sigma–Aldrich (Madrid, Spain). The following commercial standards were purchased from Sigma–Aldrich: (−)-epicatechin, (+)-catechin, (−)-epicatechin-3-gallate, rutin, quercetin, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, kaempferol, myricetin, piceatannol, *trans*-resveratrol, *trans*-piceid, gallic acid, protocatechuic acid, caffeic acid, *p*-coumaric acid, ferulic acid, and caftaric acid. Malvidin-3-*O*-glucoside and procyanidin B2 were purchased from Extrasynthèse (Lyon, France), whereas procyanidin B1 was from Phytolab (Vestenbergsgreuth, Germany).

2.2. Samples, grapevine treatments and vinification

The field experiment was conducted in the experimental *Vitis vinifera* L. cv. Tempranillo vineyard of La Grajera (northern region of La Rioja, Spain) in 2013. Vines were grafted on R-110 rootstock and vineyard was managed under conventional soil tillage management system. The soil was classified as *Typic Calcixerept* according to the American Soil Taxonomy. Apart from the nitrogen foliar treatments, no fertilizer was applied during the project. In 2013, the annual precipitation was 569.3 mm, and the average annual temperature was 17.7 °C. Four nitrogen foliar treatments were carried out using two nitrogen sources at two different doses: 150 and 250 mg N/plant of phenylalanine (Phe1 and Phe2), and 150 and 250 mg N/plant of urea (Ur1 and Ur2). In order to apply the treatments, aqueous solutions were prepared with the corresponding concentration of phenylalanine (Phe), and urea (Ur), and Tween 80 was used as wetting agent (0.1% v/v). Control plants were sprayed with water solution of Tween 80 alone. The treatments were applied to grapevine twice, at veraison and one week later. For each application, 200 mL/plant were sprayed over leaves, so the dose applied in each treatment was 0.9 kg N/ha for Phe1 and Ur1, and 1.5 kg N/ha for Phe2 and Ur2, assuming 3000 plants/ha. Treatments were applied in triplicate and were arranged in a complete randomized block design with 3 vines per replicate.

Grapes were manually harvested when they reached an average probable alcohol of 14%, a common value in wines from our region. Nitrogen treatments had no significant effect on plant production. Grapes from each replicate were elaborated separately. Grapes were destemmed and crushed and the alcoholic fermentation was carried out following the method described by Sampaio, Kennedy, and Vasconcelos (2007). Three kilograms of pomace (must, seed, and skin) were introduced into 4 L glass bottles. Potassium metabisulfite was added to the samples to give a final total SO_2 concentration of 50 mg/L, and then the alcoholic fermentation was induced by inoculation (25 g/hL) with the commercial *Saccharomyces cerevisiae* strain Uvaferm VRB (Lallemand, St. Simon, France). The fermentation was performed at controlled temperature of 25 °C. The alcoholic fermentation finished when reducing sugars were below 2.5 g/L. Once the alcoholic fermentation finished, press was carried out separating wine from seeds and skins. The oenological parameters were then analyzed and aliquots of each wine were frozen at −20 °C until the analyses of phenolic compounds were carried out.

2.3. Oenological parameters of wines

Wines were characterized by determining the alcoholic strength, pH, total acidity, malic acid, lactic acid, volatile acidity, hue, color intensity, and Folin–Ciocalteu index according to ECC methods (1990) and tartaric acid according to Rebelein method (Lipka & Tanner, 1974). Total phenolics were determined as total polyphenol index (TPI) by spectrophotometric absorbance at 280 nm after previous dilution of samples (Ribéreau-Gayon & Stonestreet, 1965). Total anthocyanins were determined by bleaching using sulfur dioxide (Ribéreau-Gayon & Stonestreet, 1965), and total tannins were determined following the method described by Ribéreau-Gayon, Peynaud, Sudraud, and Ribéreau-Gayon (1976). Polymerization index was calculated according to Ruiz (1999).

Since treatments were performed in triplicate and wine was made from each replicate separately, the results of these parameters are the average of three analyses ($n = 3$).

2.4. Analysis of wine phenolic compounds

2.4.1. Sample preparation for the analysis of non-anthocyanin phenolic compounds

Anthocyanins may cause interferences in the chromatographic separation and identification of other phenolic compounds. Therefore, an extraction on PCX SPE cartridges (500 mg, 6 mL; Bond Elut Plexa, Agilent, Palo Alto, CA, USA) containing a mixture of reverse-phase and cation-exchanger materials allowed the isolation of non-anthocyanin phenolic compounds. Cartridges were placed in the extraction system (Vac Elut 20 station from Varian, CA, USA).