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Influence of supplementary irrigation on the amino acid and volatile composition of Godello wines from the Ribeiro Designation of Origin

Yolanda Bouzas-Cid[a,b], Emiliano Trigo-Córdoba[a], Elena Falqué[b], Ignacio Orriols[a,c], José M. Mirás-Avalos[a,d,*]

[a] Estación de Viticultura e Enoloxía de Galicia (EVEGA-INGACAL), Ponte San Clodio s/n, Leiro, Ourense 32428, Spain
[b] Depto. Química Analítica, Facultad de Ciencias, Universidade de Vigo, As Lagoas s/n, Ourense 32004, Spain
[c] Servizo de Prevención e Análise de Riscos, Dirección Xeral de Innovación e Industrias Agrarias e Forestais, Rúa Roma 25-27, Santiago de Compostela 15703, Spain
[d] Proyectos y Planificación, Dpto. Ingeniería Agroforestal, Escola Politécnica Superior de Enxeñaría, Universidad de Santiago de Compostela, GI-1716, Campus Universitario s/n, Lugo 27002, Spain

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ABSTRACT

Concentrations of amino acids and volatile compounds of a given grapevine cultivar might be altered by interannual climate variability and management practices such as irrigation. These compounds determine, in part, aroma and sensory characteristics of wines. The current study aimed at assessing the amino acid profile of musts and wines and the volatile composition of wines from *Vitis vinifera* (L.) cultivar 'Godello' grown in the Ribeiro Designation of Origin (NW Spain) under rain-fed and supplementary irrigation (SI) conditions over three years (2012-2014). Supplementary irrigation increased must titratable acidity. However, must amino acid concentrations were not significantly altered by SI. In contrast, the concentrations of ethyl lactate and geraniol were greater in wines from the SI treatment. Significant correlations between amino acids in musts and volatiles in wines were observed. Our results highlight the low impact of SI on must and wine composition, likely due to the low level of water stress experienced by Godello vines. Understanding the effects of SI on wine properties could aid to adapt management practices in the future.

1. Introduction

Amino acids present in grapes and musts play a relevant role in yeast growth and development during alcoholic fermentation. Their concentrations depend on many factors such as grapevine (*Vitis vinifera* L.) cultivar, soil properties, climate conditions and vineyard management (Garde-Cerdán et al., 2014). Some amino acids are precursors of several volatile compounds that contribute to wine aroma: higher alcohols, aldehydes, ketones and esters (Moreno-Arribas & Polo, 2009), which is proven by significant relationships between amino acids and these compounds (Hernández-Orte, Cacho, & Ferreira, 2002).

Climate change is altering the temporal distribution of rainfall and increasing drought events, which raises a great concern in viticultural regions (Fraga, Malheiro, Moutinho-Pereira, & Santos, 2013). As a consequence, irrigation is increasingly being used to cope with the possible negative effects of climate change on berry composition and to minimize interannual variability in yields, even in cool-humid regions such as Galicia, northwest Spain (Cancela et al., 2016). In fact, the concentrations of volatile compounds in grapes vary during ripening depending on temperature and water availability (Robinson et al., 2014). Therefore, irrigation management is a fundamental tool to control berry growth and composition (Jackson & Lombard, 1993).

Irrigation is usual in the viticulture of New World countries, while supplying water to grapevines for wine production was forbidden by law in Spain until 1996 (Ruiz-Sánchez, Domingo, & Castel, 2010). Since then, research has been devoted to determine the effects of several irrigation protocols on grapevine yield and berry composition on red cultivars under water scarcity conditions (Girona et al., 2006; Intrigliolo, Pérez, Risco, Yeves, & Castel, 2012; Romero et al., 2016). In this scenario, the most convenient strategy was to apply moderate water deficits before veraison and irrigating without considerable restriction afterwards (Ruiz-Sánchez, Domingo, & Castel, 2010).

For these reasons, new research is focused on adapting vineyard management (mainly fertilization and irrigation) to improve the concentrations of amino acids in grapes at harvest (Bouzas-Cid, Falqué, Orriols, & Mirás-Avalos, 2018; Canoura, Kelly, & Ojeda, 2018; Teles Oliveira, de Freitas, & Alves Sousa, 2012). However, contrasting results have been obtained, depending on the cultivar and the colour of the

* Corresponding author at: Estación de Viticultura e Enoloxía de Galicia (EVEGA-INGACAL), Ponte San Clodio s/n, Leiro, Ourense 32428, Spain.
E-mail address: jose.manuel.miras.avalos@xunta.es (J.M. Mirás-Avalos).

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grapes and on the water stress intensity (Canoura, Kelly, & Ojeda, 2018; Deluc et al., 2009), which is usually low in cool-humid areas (Balint & Reynolds, 2017).

In the Northwest of the Iberian Peninsula (Galicia and North of Portugal), white grapevine cultivars are predominantly grown, and their organoleptic characteristics might be altered by increasing temperatures and drought over the growing cycle. Godello is one of the most important cultivars that are traditionally grown in this region and is used to obtain monovarietal wines that are increasingly being recognised worldwide (Blanco, Mirás-Avalos, Pereira, & Orriols, 2013). The volatile composition of Godello wines has been previously described (Losada, Andrés, Cacho, Revilla, & López, 2011; Versini, Orriols, & Dalla Serra, 1994); however, the amino acid profile of this cultivar, as well as the effect that irrigation might exert on its musts and wines have not been determined. A previous work from our group (Bouzas-Cid, Falqué, Orriols, & Mirás-Avalos, 2018) reported that supplementary irrigation under the climate conditions of Galicia altered the concentrations of several amino acids and volatiles of another white grapevine cultivar, Treixadura. However, the field performance of Treixadura and Godello cultivars was different (Trigo-Córdoba, Bouzas-Cid, Orriols-Fernández, & Mirás-Avalos, 2015); therefore, the concentrations of secondary metabolites (amino acids and volatile compounds) might have also been differently affected by supplementary irrigation, as previously reported for French cultivars (Deluc et al., 2009). Therefore, the aim of the current study was to assess the effects of supplementary irrigation on the amino acid composition of musts and wines, and the aromatic profile of wines from the white grapevine (*Vitis vinifera* L.) cultivar Godello during three consecutive years (2012, 2013 and 2014) in Ribeiro Designation of Origin (DO), Northwest Spain. Studies on the effects of irrigation on amino acid concentrations and their relations with wine aroma are very scarce in the literature, and the current work consitutes an attempt to fill this gap.

2. Materials and methods

2.1. Description of the study site

The present study was conducted from 2012 to 2014 in a 0.2 ha Godello vineyard located in Leiro (42° 21.6′ N, 8° 7.02′ W, elevation 115 m), Ourense, Northwest Spain, within the Ribeiro DO. The vineyard was planted in 1998 with vines grafted onto 196-17C rootstock (deep rooting, well adapted to drought and to acid soils, sensitive to carbonates) on a single cordon system. Rows were East-West oriented; vines were spaced 1.25 m between plants and 2.4 m between rows. Soil was sandy textured, slightly acidic and of medium fertility. The study site can be classified as warm-temperate, sub-humid with cold nights (Supplementary Table 1). Increasing temperatures and decreasing rainfall amounts over the growing season were observed from 2012 to 2014 (Table 1).

2.2. Experimental design in the vineyard

Two treatments were applied in a randomized block design with three replications consisting of three rows with 12 vines each (36 vines per experimental unit).

Treatments consisted of a rain-fed control and a supplementary irrigation (SI) to the 40% of the potential evapotranspiration (ET_o). Water was applied through a drip system from fruit set (end June) to approximately two weeks before harvest (mid-August) (Trigo-Córdoba et al., 2015). Irrigation needs were calculated weekly using data recorded by a weather station located 200 m away from the experimental vineyard and corrected by substracting the rainfall amount registered on the previous week. The inter-year variability of ET_o and rainfall caused that the irrigation amount applied differed from year to year. Therefore, total water amounts applied were 50, 79 and 50 mm for 2012, 2013 and 2014, respectively, allowing for a clear differentiation of grapevine water status under both treatments (Trigo-Córdoba et al., 2015).

2.3. Sampling and winemaking

Grapes from the different treatments were manually harvested on the same day. Vinifications were performed separately on samples of approximately 40 kg per replicate (3 vinifications per treatment and year).

Grapes from each treatment were separately destemmed, crushed and pressed in a pneumatic press (yielding, approximately, 50% must). A replicated 250 mL sample from each treatment was collected for analysis. Pectolytic enzyme was added (4 g hL^{-1}) to favour settling and SO_2 (50 mg L^{-1}) was added to avoid oxidation. After 24 h, musts were racked and fermented in 35 L stainless steel tanks. Commercial yeast (Excellence FW, Lamothe-Abiet, Bordeaux, France) was added (20 g hL^{-1}). Density and temperature of fermentations were measured daily. Once alcoholic fermentation finished, wines were racked and sulphited to 35 mg L^{-1} free sulphur dioxide. A natural clarification was carried out at 4 °C for one month. Finally, wines were filtered, bottled and stored.

2.4. Chemical reagents

Ultra-pure water was generated using Milli-Q equipment (Millipore, Bedford, MA, USA). Super-gradient HPLC grade acetonitrile and methanol were purchased from Scharlau (Sentmenat, Spain). Ammonium chloride was acquired from Merck (Darmstadt, Germany). Amino acid solutions were prepared with HCl 0.1 N using standards purchased from Acros Organics (New Jersey, USA).

Dichloromethane, *n*-pentane and anhydrous sodium sulphate (Scharlau, Sentmenat, Spain) were used for the extraction of free terpenes, volatile fatty acids, ethyl esters, acetates of higher alcohols and C6 alcohols. Standards for volatile compounds were purchased from: Merck (Madrid, Spain), Aldrich (Madrid, Spain), Fluka (Seelze, Germany), Alfa Aesar (Barcelona, Spain) and Sigma (Madrid, Spain). The internal standards (Merck, Madrid, Spain) used were 4-methyl-2-pentanol for the determination of major volatile compounds (methanol, 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, acetaldehyde, ethyl acetate, ethyl lactate, 1-hexanol, acetoine, acetol, 2-phenyl-ethanol, 2,3-butanediol *levo*, 2,3-butanediol *meso*); 4-decanol for terpenes and C6 alcohols; 1-heptanol for volatile fatty acids, ethyl esters and acetates of higher alcohols. All the standards were prepared in 50% hydroalcoholic solutions.

2.5. Analytical methods

Basic parameters of musts (including total soluble solids, pH and titratable acidity) and wines (such as alcohol content and pH among others) were determined by Fourier Transform Infrared Spectrometry (FTIR) using a WineScan FT120 analyzer (FOSS Electric, Barcelona, Spain) calibrated according to the official methods (OIV, 2009). Analytical determinations in wines were carried out in triplicate five months after bottling.

2.5.1. Determination of amino acids in musts and wines

The determination of the amino acids present in musts and wines was carried out through high-performance liquid-chromatography (HPLC) using a method based on a reaction of derivatization in a basic methanolic medium (Gómez-Alonso, Hermosín-Gutiérrez, & García-Romero, 2007). The HPLC analysis was performed using an Agilent 1100 series equipment (Agilent Technologies, Palo Alto, CA, USA). Chromatographic separation of amino acids was carried out in a Zorbax Eclipse AAAcolumn (C18), with a particle size of 5 µm (150 mm × 4.6 mm, Agilent Technologies, Palo Alto, CA, USA). A precolumn was also used (Zorbax Eclipse AAA, 12.5 mm × 4.6 mm;