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Research paper

Effect of post-pruning vine-shoots storage on the evolution of high-value compounds

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ABSTRACT

Vine-shoots post-pruning storage time revealed an important stilbenes accumulation, but there are no studies with other minor metabolites. For this reason, the evolution of non-volatile phenolic and volatile compounds of two vine-shoot cultivars (Airén and Cencibel) after 1, 3 and 6 months of post-pruning storage was studied. Significant differences among cultivars were observed, making a total average content of 2 g/kg in Airén and 1.6 g/kg in Cencibel, being the non-volatile phenolic fraction the most abundant. The highest accumulation was observed after 6 months post-pruning storage. Especially significant was the accumulation of vanillin at 6 months, with a 7% increment observed in Airén cultivar and 35% in Cencibel. Such increment was related to the decrement of ferulic acid content with time. Consequently, the storage of vine-shoots post-pruned have a direct impact on the concentration of several metabolites, not only stilbenes, which suggest an alternative new use of such agricultural wastes.

1. Introduction

Viticulture is one of the most important agricultural activities in the world with approximately 7.6 million hectares in 2016. In Spain, Castilla-La Mancha region the vineyard surface is around 443.818,31 ha, which accounts for 13% of European Union (EU) and almost 6% of the world area (OIV, 2017). If it is assumed an average of 1.3 kg of vine-shoots/vine as weight for an annual average production, approximately around $2{\cdot}10^7$ tons of vine-shoots are harvested per year in the world.

Nowadays the concern for the environment has increased in the interest of management and revalorization of wastes. Vine-shoots are the most important wastes in viticulture, for this reason recent studies have been focused on the search for innovative uses to consider them as a resource rather than a waste (Azuara et al., 2017; Briones et al., 2015; Rodríguez-Pazo et al., 2013; Vecino et al., 2015).

Vine-shoots chemical composition is characterized by a holocellulose fraction, whose content is about 55.1% (31.9% α-cellulose and 23.2% hemicellulose) and another lignin fraction, whose content is about 38.5% (Briones et al., 2015). But it is the minor fraction, represented by phenolic and volatile compounds, the ones with a greater importance for the industry, being the stilbenes group the most deeply studied (Rayne, 2013; Piñeiro et al., 2017; Guerrero et al., 2016; Soural et al., 2015; Vergara et al., 2012). The phenolic family of stilbenes is well-known for its beneficial effect on health (Malhotra et al., 2015; Nguyen et al., 2017; Xue et al., 2014), being *trans*-resveratrol the most significant bioactive compound (Aggarwal et al., 2004; Bavaresco et al., 2012). Stilbenes concentration in vine-shoots range from 803 to 7857 mg/kg of dry matter (Gorena et al., 2014), being such differences attributed not only to the cultivar variety, but to the time past between pruning and their processing (Gorena et al., 2014). As well, several analytical factors can also contribute to these differences, including the extraction techniques conditions, solvents and their proportions, in addition to the quantification methods used (Delgado De La Torre et al., 2012; Luque-Rodríguez et al., 2006; Sánchez-Gómez et al., 2014). So, for example, in Pinot Noir variety an important increase of total stilbenoids was observed after 6 months of storage after pruning. Houillé et al. (2015) also pointed out a maximum accumulation of *trans*-resveratrol after 6 weeks of post-pruning, showing that storage temperature and humidity were determining factors for optimal compounds accumulation.

Vine-shoots contain other secondary metabolites, which could be synthesized over post-pruning time in the same way as stilbenes, resulting in other opportunities for new vine-shoot based products. These secondary metabolites include both volatile and non-volatile compounds, many of them with important application properties in the food, health, cosmetic and pharmaceutical industries. For example, vanillin is widely used as flavouring agent (Sánchez-Gómez et al.,

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2016), ferulic and *trans-p*-coumaric acids are known as antioxidant precursors in the biocatalytic production of aromatic natural products, or gallic acid which is used in the manufacture of antibacterial agents (Max et al., 2010; Pérez-Rodríguez et al., 2017).

Then, the aim of this work is to study the effect of post-pruning time storage on vine-shoot minor metabolites (non-volatile phenolic compounds and volatile compounds). Airén and Cencibel, white and red cultivars, have been used for this study, which represents the first and the third cultivars around the world.

2. Materials and methods

2.1. Vine-shoot samples

Two vine-shoot cultivars, Airén and Cencibel, the last one also called Tempranillo in other regions, were pruned in vineyards of D.O. Mancha (Castilla-La Mancha, Spain) after 90 days of grape harvesting. Each cultivar was divided into three fractions and stored intact at dark and room temperature (18 ± 3 °C). Each fraction was processed after one (1 M), three (3 M) and six (6 M) months after pruning, considering these as sampling times. Samples were ground by a hammer miller (LARUS Impianti, Skid Sinte 1000, Zamora, Spain) to a particle size less than 10 mm, and frozen till their characterization. Final humidity was measured for each vine-shoot cultivars and sampling times, resulting 24.22%, 14.26% and 7.98% for Airén and 23.63%, 14.12% and 7.86% for Cencibel at 1, 3 and 6 months respectively.

2.2. Vine-shoot extraction procedures

Vine-shoot samples (20 g) were moisturized with 100 g of an ethanol/water solution (12.5%, pH 3.62) during 8 h at RT. Another 100 g of the same solution was added at this sample and extraction was carried out with a microwave NEOS device (Milestone, Italy). The extraction conditions were set at 75 °C (600W) for 12 min with reflux to prevent dryness. All extracts were centrifuged at 4000 rpm for 10 min and supernatant was separated. Subsequently, the solid sample was extracted another two time until exhaustion using the same volume of ethanolic solution (100 ml). The three consecutive extraction steps reported an 85% yield in terms of total polyphenolic index (Ribéreau-Gayon, 1970) (data not shown). The three extracts were mixed, and kept at 5–7 °C, till their analysis. All extractions were carried out in duplicate for each variety and sampling time. Thereby, the resulting extracts were: Airén vine-shoots at 1 month (AVS–1 M), Airén vine-shoots at 3 months (AVS–3 M), Airén vine-shoots at 6 months (AVS–6 M), Cencibel vine-shoots at 1 month (CVS–1 M), Cencibel vine-shoots at 3 months (CVS–3 M) and Cencibel vine-shoots at 6 months (CVS–6 M), after pruning. Extract pH was about 4.00, no significant differences ($P < 5\%$) among the cultivars nor the post-pruning storage time was observed.

2.3. Extracts analysis

2.3.1. Determination of non-volatile phenolic compounds by HPLC-DAD-MS

This method was based on Sánchez-Gómez et al. (2014). Samples were injected into an Agilent 1200 HPLC chromatograph (Palo Alto, California, USA) equipped with a Diode Array Detector (Agilent G1315D) and atmospheric pressure chemical ionization (MM-ESI/APCI-MS) system, coupled to an Agilent ChemStation (version B.03.01) data-processing station. Separation was performed on a reverse phase Zorbax-Eclipse XDB-C18 (4.6 mm × 150 mm 5 μm particle sizes) and a precolumn Zorbax SB C-18 (2.1 mm × 30 mm 3.5 μm particle size) at 30 °C. The HPLC grade solvents used were water/formic acid/acetonitrile (97.5:1.5:1 v/v/v) as solvent A and acetonitrile/formic acid/solvent A (78.5:1.5:20 v/v/v) as solvent B. The elution gradient was set up for solvent B as: 0 min, 5%; 2 min, 10%; 4 min, 14%; 9 min, 14%; 37 min, 18.5%; 35 min, 20%; 50 min, 25%; 55 min, 50%; 60 min, 5%; 65 min, 5%. The loop volume was 20 μl.

All compounds detection was carried out by means of DAD detector by comparison with the corresponding UV–vis spectra and retention time of their pure standards (Sigma-Aldrich, Steinheim, Germany). Compounds were quantified and identified at different wavelength: (+)-catechin, (−)-catechingallate, (−)-epigallocatechin, (−)-epicatechingallate, (−)-epicatechin, gallic acid, procyanidin (B2), protocatechuic acid, pyrocatechol, pyrogallol and syringic acid at 280 nm; ellagic, ferulic, and vanillic acids at 256 nm; *trans*-caffeic acid, *trans*-caftaric acid, coniferaldehyde, sinapaldehyde, piceannol and *trans*-ε-viniferin at 324 nm; *trans-p*-coumaric acid, *trans-p*-coutaric acid, piceid-*trans*-resveratrol (*t*-resveratrol-3-glucoside) and *trans*-resveratrol at 308 nm; quercetin at 365 nm. Acids *trans*-caftaric and *trans-p*-coutaric were not available, so they were identified with their molecular ion and spectral parameters and quantified as *trans*-caffeic acid and *trans-p*-coumaric acid respectively. Quantification was based on calibration curves of the respective standards at five different concentrations achieved by UV–vis signal (0.70–175 mg/L) (R^2 = 0.92-0.99). All analysis were made in duplicate.

2.3.2. Determination of volatile compounds by HS-SBSE-GC–MS

Volatiles were extracted by means of Headspace Stir Bar Sorptive Extraction (HS-SBSE) according to Sánchez-Gómez et al. (2014) method. The volatile analysis was performed using an automated thermal desorption unit (TDU, Gerstel, Mülheim and der Ruhr, Germany) mounted on an Agilent 7890A gas chromatograph system (GC) coupled to a quadrupole Agilent 5975C electron ionization mass spectrometric detector (MS, Agilent Technologies, Palo Alto, CA, USA) equipped with a fused silica capillary column (BP21 stationary phase, 30-m length, 0.25 mm I.D. and 0.25 μm film thickness) (SGE, Ringwood, Australia). The carrier gas was helium with a constant column pressure of 20.75 psi.

MS data acquisition was carried out at positive scan mode, although to avoid matrix interferences, the MS quantification was performed in the single ion-monitoring mode using their characteristic *m/z* values. The compounds identification was performed using the NIST library and confirmed by comparison with the mass spectra and retention time of their standards. The standards employed to identify and quantify volatile (GC–MS) were purchased in Sigma-Aldrich (Steinheim, Germany) (the numbers in brackets indicates the *m/z* used for quantification): acetovanillone (*m/z* = 151), benzaldehyde (*m/z* = 106), benzylalcohol (*m/z* = 108), citronellol (*m/z* = 69), β-damascenone (*m/z* = 121), ethyl acetate (*m/z* = 43), ethyl butirate (*m/z* = 88), ethyl decanoate (*m/z* = 43), ethyl hexanoate (*m/z* = 101), ethyl octanoate (*m/z* = 101), 4-ethylguaiacol (*m/z* = 137), 4-ethylphenol (*m/z* = 107), ethyl vanillate (*m/z* = 151), eugenol (*m/z* = 164), farnesol (*m/z* = 69), 2-furanmethanol (*m/z* = 98), furfural (*m/z* = 96), geraniol (*m/z* = 69), geranyl acetone (*m/z* = 43), guaiacol (*m/z* = 109), 1-hexanol (*m/z* = 56), 3-hexen-1-ol (Z) (*m/z* = 67), 2-hexen-1-ol (E) (*m/z* = 57), *cis*-3-hexen-1-ol (*m/z* = 67), hexyl acetate (*m/z* = 43), 5-hydroxymethylfurfural (*m/z* = 97), β-ionone (*m/z* = 177), D-limonene (*m/z* = 69), linalool (*m/z* = 71), linalyl acetate (*m/z* = 93), 2-methyl-1-butanol (*m/z* = 57), 3-methyl-1-butanol (*m/z* = 55), 5-methylfurfural (*m/z* = 110), methyl vallinate (*m/z* = 151), nerol (*m/z* = 69), nerolidol (*m/z* = 69), nonanal (*m/z* = 57), 1-nonanol (*m/z* = 56), 1-octen-3-ol (*m/z* = 57), 2-phenylethanol (*m/z* = 91), 2-phenylethyl acetate (*m/z* = 104), syringol (*m/z* = 154), α-terpineol (*m/z* = 59), vanillin (*m/z* = 151), 4-vinylguaiacol (*m/z* = 151), 4-vinylphenol (*m/z* = 91), *trans/cis*-whiskey lactones (*m/z* = 99), 3-methyl-1-pentanol and γ-hexalactone were used as internal standards. Quantification was based on calibration curves of the respective standards at five different concentrations (2 μg/L–15 mg/L) (R^2 = 0.95-0.97). All analysis were made in triplicate.