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Oak extract application to grapevines as a plant biostimulant to increase wine polyphenols

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ABSTRACT

Oak extract is a mixture of compounds including phenolics (volatiles and non volatiles), which could act as plant biostimulant if they are able to modulate plant physiological response. It is known that it can modify grape volatile composition after the application over grapevines, impacting on wine aroma, but no studies have been carried out on phenolic composition. So, the aim of this work was to evaluate the phenolic composition of wines elaborated from Monastrell grapevines treated with a commercial oak extract in order to study its biostimulant activity. Several families of polyphenols were studied, including phenolic acids, stilbenes, flavanols, flavonols and anthocyanins, which were analyzed by HPLC-DAD-MS. Results showed oak extract could be considered like an important biostimulant of grape polyphenols, since it affected grape composition, producing less alcoholic and acid wines with higher colour intensity, lower shade and so a more stable colour and higher content of polyphenols such as gallic acid, hydroxycynnamoyltartaric acids, acylated anthocyanins, flavanols and stilbenes.

1. Introduction

Polyphenols are plant secondary metabolites, studied by many researches as they are implicated in plant resistance, due to their fundamental function in food process and its nutraceutical characteristics (Cheynier, Sarni-Manchado, & Quideau, 2012). In wines, they are responsible of colour, astringency, bitterness, body and aroma (Santos-Buelga, Escribano-Bailón, & Lattanzio, 2010). Among the healthy benefits attributed to wine, when consumed in moderation, it is important to mention the contribution of polyphenols due to their antioxidant properties related to cardio protector effect (Pezzuto, 2008). These facts justify that several approaches have been proposed to increase the phenolic content of wine grapes.

Besides genetic transformation, forbidden in many countries, there are many factors that can induce or increase phenolic content of grapes, including applications of plant products (Parrado et al., 2007) or commercial products whose active components are also found naturally in plants (Tassoni, Durante, & Ferri, 2012). Recently, plant biostimulants have been defined as substances that are able to alter the plant physiological processes providing benefits for growth, development, response to abiotic stress and improve quality. Among the biostimulant categories, it is included as the one formed by a combination of molecules, which have effect on plant physiological response (Du Jardin, 2012).

Oak is commonly used in winemaking to produce wood tanks, especially barrels, where wines are subjected to the aging process (Pontallier, Salagoïty-August, & Ribéreau-Gayon, 1982). Among its most important components are polyphenols and volatile compounds, which are extracted slowly into the wine, and influence their sensory characteristics such as astringency, body, bitterness, colour and aroma. Some wood components are formed or increased during the process of manufacture of the barrel, particularly in the stages where staves are subject to heat treatment (Fernández de Simón, Sanz, Cadahía, Poveda, & Broto, 2006). In that manufacturing process, important residues are generated, which can be added into the wines in the form of chips, pellets and fragments of different sizes giving new alternatives to aging winemaking. Such applications are well regulated by the CEE 1507/2007 regulation. The remaining oak wastes are often burnt but can be used for obtaining oak extracts of different characteristics

Abbreviations: % AEI, anthocyanin extractability index; % SMI, seed maturity index; D3G, delphinidin 3-O-glucoside; C3G, cyanidin 3-O-glucoside; Pt3G, petunidin 3-O-glucoside; Pe3G, peonidin 3-O-glucoside; M3G, malvidin 3-O-glucoside; ΣG, total glucoside antocyanins; Pe3GAc, peonidin 3-O-(6-acetyl)-glucoside; M3GAc, malvidin 3-O-(6-acetyl)-glucoside; ΣGAc, total acetylated antocyanins; C3GCo, cyanidin 3-(6-p-coumaroyl)-glucoside; Pe3GCo, peonidin 3-(6-p-coumaroyl)-glucoside; M3GCo, malvidin 3-(6-p-coumaroyl)-glucoside; ΣGCo, total coumaroyl antocyanins; M3GCa, malvidin 3-(6-t-caffeoyl)-glucoside; ΣA, total antocyanins; VA, vitisin A malvidin 3-O-glucoside; VB, vitisin B malvidin 3-O-glucoside; ΣV, total vitisins. My3Galact, myricetin 3-O-galactoside; My3Glucur + Glucos, myricetin 3-O-glucuronide + myricetin 3-O-glucoside; ΣMyG, total myricetin glicosides; Qu3Galact, quercetin quercetin 3-O-glucoside; Qu3Glucur + Glucos, quercetin 3-O-glucuronide + quercetin 3-O-glucoside; ΣQuG, total quercetin glicosides; La3Glucs/Galact, laricitrin 3-O-glucoside/galactoside; Sy3Glucos, syringetin 3-O-glucoside.

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which may be an important application for the sustainability of the oak industry.

The most recent works related to the use of aqueous oak extracts showed how their grapevine foliar applications modulated the aroma of the wines produced from these grapes (Martínez-Gil, Garde-Cerdán, Martínez, Alonso, & Salinas, 2011). The main chemical constituents of the aqueous oak extracts are low molecular weight polyphenols, being ellagitannins the most abundant compounds. Recent works (Fernandez & Eichert, 2009) have shown that low molecular weight is needed for absorption of compounds by leaves, either by cuticular pore or by stomata. Due to the high molecular weight of ellagitannins, they could not be absorbed by leaves. The other constituents of the aqueous oak extract are a mixture of a large number of substances which can be assimilated by plants, among which are several compounds derived from shikimic acid pathway, such as the phenylpropanoid eugenol, which is known for its antifungal role and its effect on wine polyphenols (Pardo-García, Martínez-Gil, López-Córcoles, Zalacain, & Salinas, 2013).

It has been reported that grapes can assimilate as glycoside precursors some of the volatile compounds from oak extract foliar applications to grapevines (Martínez-Gil et al., 2013) (Martínez-Gil et al., 2013). Also, wines produced with berries from treated vines had a high content of volatiles present in the applied extract; these volatiles were liberated from their precursors during the winemaking (Martínez-Gil, Garde-Cerdán, Zalacain, Pardo-García, & Salinas, 2012; Martínez-Gil et al., 2011). These treatments impacted on wine aroma at sensory level, giving woody notes as if the wine was aged in oak but, that process did not take place.

As previous works have shown how the aqueous oak extract affects grape and wine aroma, the aim of this work is to expand the knowledge about the oak extracts and their impact on wine polyphenol content when they are applied over grapevines. If the known properties of the oak extracts are expanded with this study, it could be considered as biostimulant with oenological interest.

2. Materials and methods

2.1. Chemicals and standards

The standards employed to identify and quantify phenolic compounds were: (+)-catechin, caffeic acid, *p*-coumaric acid, (−)-epicatechin, ferulic acid, gallic acid, kaempferol, quercetin dihydrate, *trans*-resveratrol, syringic acid and vanillic acid purchase from Sigma-Aldrich (Steinheim, Germany), while malvidin 3-O-glucoside (Mv-3-G), quercetin-3-O-glucoside (Q-3-G) and kaempferol 3-O-glucoside (K-3-G) were obtained from Extrasynthése (Genay, France). HPLC-grade acetonitrile and formic acid were purchased from Panreac (Barcelona, Spain). Water was purified through a Milli-Q system from Millipore (Bedfords, MA, USA). For quantification, different solutions of phenolic standards in ethanol–water (12% v/v, pH = 3.6, 5 g/L tartaric acid) were prepared using concentration ranges for each compound commonly found in red wines.

2.2. Material

2.2.1. Oak extract

The commercial aqueous French oak extract chosen for this study was provided by Protea France S.A.S. (Gensac la Pallue, France) (103 C). This extract was obtained by macerating in water at high temperature French oak chips (*Quercus sessiliflora Salisb*) from natural seasoning toasted at a medium intensity level. The commercial specifications were: pH: 2.8; soluble solids: 1.6°Baumé; total phenols index: 280; % weight: 4.3. This extract is used as food additive in spirits and fruit juices to give its characteristic oak aroma because it is rich in volatile compounds.

2.2.2. Grapevines

Red wine grapes from *Vitis vinifera* variety Monastrell grown during the year 2010 in the experimental field of BSI winery (Jumilla, Southwest of Spain) were used. 8-year-old grapevines were cultivated on a high cordon style trellis system (2.8 × 1.5 m) and the vineyard was equipped with a drip irrigation system to assure the plant water needs. The annual average temperature was 16 °C, with a minimum of −15 °C (January) and a maximum of 40 °C (August).

2.3. Technological methods

2.3.1. Grapevine treatments

Different oak extract treatments were applied to the grapevines during veraison. For all treatments, 0.05% (v/v) of adjuvant Fluvius (BASF, Germany) was added, since this is a superficial wetting agent typically used for treatments with foliar herbicide, constituted by an inert mixture of polymers. The vineyards were treated with different concentrations of the extract. First of all, this extract was diluted with water to four parts. This diluted extract was applied once on the 7 days post-veraison (25%-1 treatment) and also four times, 7, 11, 15, and 18 days post-veraison (25%-4 treatments). Also the undiluted extract was applied once on the 7th day post-veraison (100%-1 treatment) as well. Each treatment was carried out on 16 plants in the same row, leaving other row with untreated plants between the different applications to avoid contamination. 300 mL of each formulation was applied evenly per plant by spraying over leaves. Moreover, an additional row was not treated for control. The treatments were carried out when the environmental temperature was below 20 °C, at approximately 8 a.m. Grapes were harvested on September the 9th at their optimum maturation moment with the °Baumé/titratable acidity ratio around 2.5. Before winemaking some berries were randomly separated in order to measure the oenological parameters.

2.3.2. Winemaking

Grapes of each treatment were destemed, mixed and processed by the winemaking traditional method. Berries were divided and placed into two 30 L stainless steel tanks (2 wines from each treatment were obtained). 80 mg/L of sulphur dioxide were added to each tank. After that, 20 g/hL of yeast UCLM S377 strain (Springer Oenologie, Maisons-Alfort cedex, France) was inoculated to carry out the alcoholic fermentation, which took place at a temperature of 22 ± 1 °C. After 11 days of maceration–fermentation skins and seeds were removed with a traditional vertical hand-press. Then, each wine was put back to its tank to complete the alcoholic fermentation. Malolactic fermentation was induced using 8 g/hL of a commercial bacterium strain, Lall II-4 (Lallemand, Barcelona, Spain). The malolactic fermentation was carried out in 5 L tanks. Once this step finished, the wines were bottled and stored for six months. Three wine sampling times were performed: after alcoholic fermentation (AF), after malolactic fermentation (MLF) and after six months (6 months). After sensory analysis and measuring the chromatic parameters, wine samples were frozen at −20 °C until analysis.

2.4. Analytical methods

2.4.1. Grape and wines oenological parameters

Grape analysis involved the traditional measurements (°Beaumé, pH and titratable acidity) which were determined according the methodology described in ECC (1990). The classical parameters of wines such as alcoholic degree (°A), pH, titratable acidity (TA) and volatile acidity (VA) were analysed by an equipment based in infrared spectroscopy (FT-IR Multispec of TDI) using the methods (ECC, 1990) as reference. The phenolic potential of grapes (anthocyanin extractability index and seed ripeness index) was calculated according to the method described by Saint-Cricq de Gaulejac, Vivas, and Glories (1998), macerating the grapes for 4 h at two pH values (3.6 and 1.0). The pH 3.6 instead of pH