Food Research International 98 (2017) 34–39

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Elicitors used as a tool to increase stilbenes in grapes and wines

Rocío Gil-Muñoz [a,*], José Ignacio Fernández-Fernández [a], Oscar Crespo-Villegas [a], Teresa Garde-Cerdán [b]

[a] Instituto Murciano de Investigación y Desarrollo Agroalimentario, Ctra. La Alberca s/n, 30150 Murcia, Spain
[b] Instituto de Ciencias de la Vid y del Vino (CSIC-CAR-UR), Ctra. de Burgos, km 6, 26007 Logroño, Spain

ARTICLE INFO

Article history:
Received 19 July 2016
Received in revised form 16 November 2016
Accepted 27 November 2016
Available online 22 December 2016

Keywords:
Monastrell
Tempranillo
Grapes
Wines
Stilbenes
Cell wall yeast
Methyl jasmonate

ABSTRACT

Two preharvest treatments (methyl jasmonate or cell wall yeast) of grapevines (Monastrell and Tempranillo) were applied during two vintages (2014 and 2015) to check whether these elicitors enhanced stilbene accumulation in berries at the moment of harvest and in the corresponding wines elaborated with them. The main objective was checking the effect of treatment, variety and year on stilbene composition due to the interesting health-related properties of these compounds in both grapes and wines. The results pointed to inter-varietal and inter-annual differences, and that the treatments generally enhanced the stilbene composition of grapes and, particularly, of wines. The increase was more evident in Monastrell variety than in Tempranillo variety and in their wines more than their grapes during 2014 vintage but not during 2015 vintage.

1. Introduction

Plants produce a wide variety of secondary metabolites through diverse biosynthesis pathways in response to external stimuli, whether in the form of environmental or biotic stress (Paré & Tumlinson, 1999). Among secondary metabolites, phenolic compounds are important for both plants and humans for several reasons. Firstly, they protect plants from biotic and abiotic stress factors. Secondly, most of these metabolites are responsible for the organoleptic and qualitative properties of the foods originating from such plants (Ruiz-García & Gómez-Plaza, 2013). Thirdly, these compounds are unique sources of industrial material in the form of food additives, pharmaceuticals and flavors (Zhao, Davis, & Verpoorte, 2005). Finally, they are considered to be beneficial for health, mainly due to their antioxidant activity.

Stilbenes, a kind of non-flavonoid phenolic compounds, have been reported to be responsible for various beneficial effects. Their biological properties include antibacterial and antifungal effects, as well as cardioprotective, neuroprotective and anticancer actions (Guerrero, García-Parrilla, Puertas, & Cantos-Villar, 2009). They are classified as phytoalexins, *i.e.*, plant metabolites with antimicrobial activity that are synthesized *de novo* and function as the basis of a resistance mechanism (Fumagalli et al., 2006). Resveratrol is the most well-known bioactive stilbene (Espín, García-Conesa, & Tomás-Barberán, 2007). However, their monoglucoside-derived piceid is also bioactive and present in large amounts in grape berries, wines and cell suspensions (Ribeiro de Lima et al., 1999; Gatto et al., 2008; Ferri et al., 2009).

The concentration of stilbenes in grapes and wines depends on many variables including grape variety, growing conditions, climate, harvest year, and winemaking techniques (Bavaresco, Mattivi, de Rosso, & Flamini, 2012). Several techniques have been applied to improve the phenolic content of grapes and wines, the most common of which are related to cultural practices: pruning (González-Neves, Gil, & Ferrer, 2002; Pérez-Lamela, García-Falcón, Simal-Gándara, & Orriols-Fernández, 2007), cluster thinning (Fanzone, Zamora, Jofré, Assof, & Peña-Neira, 2011), leaf removal (Gatti, Bernizzoni, Civardi, & Poni, 2012); shading/bunch exposure (Downey, Dokoozlian, & Krstic, 2005) and deficit irrigation (De la Hera, Romero, Gómez-Plaza, & Martínez, 2007). One novel strategy is to use so-called elicitors, which are regarded as an interesting alternative for obtaining plants with increased polyphenol content in the absence of any external attack and to limit the use of pesticides in vineyards. Elicitors were first used to increase plant resistance to pathogens, although it was later found that the mechanism involved increased polyphenol levels (Ruiz-García & Gómez-Plaza, 2013). This strategy consists of stimulating and/or potentiating the grapevine defense response by applying exogenous molecules often originating from microbes or plants (Delaunois et al., 2014).

Numerous experimental trials have been conducted with different elicitors to promote stilbene synthesis in grapevine berries. These include the use of UV irradiation (Cantos, Espín, Fernández, Oliva, & Tomás-Barberán, 2003) and benzothiadiazole (BTH) (Iriti, Rossoni, Borgo, & Faoro, 2004), and, in cell suspension cultures (for biotechnological purposes), cyclodextrines (Morales, Bru, García-Carmona, Ros-

* Corresponding author at: Avda Ntra Sra Asuncion 24, Jumilla, Murcia, Spain.
E-mail address: mariar.gil2@carm.es (R. Gil-Muñoz).

http://dx.doi.org/10.1016/j.foodres.2016.11.035
0963-9969/

Barcelò, & Pedreño, 1998). Among these studies, some have focused on the effect of the preharvest application of methyl jasmonate (MeJ) or cell wall yeast (CWY) obtaining higher concentration of stilbenes when yeast elicitor was used (Portu, López, Baroja, Santamaría, & Garde-Cerdán, 2016).

The effects of methyl jasmonate (MeJ) applications in fruits (*Fragaria chiloensis*, plums and Fuji apples) are reflected in physical changes such as color, weight, firmness and the amount of bioactive compounds (phenolics, antioxidants) (Concha et al., 2013; Karaman, Ozturk, Genc, & Celik, 2013; Rudell, Fellman, & Mattheis, 2005), as well in grapes (Ruiz-García et al., 2012). Furthermore, MeJ acts as an important intracellular regulator and a diffusible intercellular signal transducer, mediating in diverse developmental processes and defense responses, making it a strong candidate for resistance inference (Seo et al., 2011). Moreover, airborne (gaseous) *in vitro* MeJ application to leaves and berries has been shown to increase stilbene synthesis (Larronde et al., 2003).

Yeast extracts contain several compounds that may act as elicitors. In this respect, yeast cell walls are made up of mannoproteins, *beta*-1,3- and *beta*-1,6-glucans and chitin, while the yeast plasmatic membrane is composed of lipids, sterols and proteins (Kapteyn, Van Den Ende, & Klis, 1999). Most of these compounds are regarded as triggers of various modes of plant defense (Ferrari, 2010). In this way, several *in vitro* studies have reported the accumulation of secondary metabolites and the activation of phenylalanine ammonia lyase (PAL) following yeast extract applications to plant cell cultures (Peltonen, Mannonen, & Karjalainen, 1997; Yan, Shi, Ng, & Wu, 2006).

The present work studies two varieties cultivated in two different zones of Spain: Monastrell, which was cultivated in the south-east of Spain where the climate is dry and hot, and Tempranillo cultivated in the north of Spain with its more humid and colder climate. It is known that phenolic composition is highly affected by differences in grape varieties, environmental conditions and cultural practices (Flamini, Mattivi, De Rosso, Arapitsas, & Bavaresco, 2013).

Monastrell grape variety, also known as Mourvedre in southern France, or Mataró in California and Catalonia, is a native red variety from the Spanish Mediterranean coast. Monastrell grapes exhibit thick-skinned berries, which allow them to thrive vigorously in warm and arid climates. Wines produced from this variety tend to be high in alcohol and tannins and have a distinctive balsamic, blackberry taste, and mineral flavors, especially when young (Moreno-Labanda et al., 2004).

Tempranillo variety is a thick-skinned variety with a high anthocyanin count that makes for deep-colored wines with moderate tannin levels. It is cultivated in La Rioja and Ribera del Duero among other areas, although it has also been successfully adopted in the New World, especially in California, Argentina and Australia.

In the present study, it is discussed the effect of two elicitors (MeJ and CWY) applied at preharvest on the stilbene composition of two grape varieties (Monastrell and Tempranillo) and of their corresponding wines to determine whether there is some significant effect of either treatment. Results will be different because environmental factors, as light/radiation and temperature affected stilbene synthesis, as well physical characteristics of the vineyard as altitude of the cultivation site, heat stress, defoliation, mineral supply or soil type (Teixeira, Eiras-Dias, Castellarin, & Gerós, 2013), and Alfaro and Jumilla are different places with different characteristics.

2. Method

2.1. Reagents and standards

The solvents used (methanol, acetonitrile, and formic acid) were of HPLC grade, and the chemicals (ethyl acetate, and sodium carbonate) were of analytical grade (>99%); all were purchased from Panreac (Barcelona, Spain). Water was of Milli-Q quality (Millipore, Bedford, MA). MeJ and Tween 80 were purchased from Sigma-Aldrich (Madrid, Spain) and CWY (Lavigne® Mature) was provided by Lallemand (St. Simon, France). The standards used, *trans*-resveratrol and *trans*-piceid, were purchased from Sigma-Aldrich.

2.2. Plant material and open field treatments

The treatments were carried out in two consecutive years (2014 and 2015) in commercial vineyards in Jumilla (Murcia, SE Spain; x: 636.099; y: 4.249.299) for Monastrell grapes and in Alfaro (La Rioja, N Spain) for Tempranillo grapes. The study was performed on 6 year old *Vitis vinifera* Monastrell (syn. Mourvedre) red wine grapevines grafted on 1103-Paulsen (clon 249) rootstock. A bilateral cordon training system trellised to a three-wire vertical system was used. Vine rows ran N-NW to S-SE, and the planting density was 3 m between rows and 1.25 m between vines (Six 2- bud spurs (12 nodes) per vine) were retained at pruning. The vineyard was drip-irrigated. Tempranillo vines were planted in 1999 at an altitude of 335 m grafted onto a 1103-Paulsen rootstock and trained to a VSP (vertical shoot positioned) trellis system. Vines were arranged in N-S rows with a between-row and within-row spacing of 2.80 × 1.20 m, respectively (x: 596.519; y: 4.669.003).

All treatments were applied to three replicates and were arranged in a complete randomized block design, with 25 vines for each replication in Monastrell variety and 10 vines in Tempranillo variety. Plants were sprayed at the beginning of veraison and 12 days after the first application for Monastrell grapes and a week later for Tempranillo grapes, with a water suspension at the following concentrations: MeJ solution was prepared at a concentration of 10 mM; 200 mL per plant were applied in clusters; and CWY solution was prepared following the manufacturer's instructions (Lallemand) at a concentration of 1.69 g/L. 200 mL per plant were applied directly in clusters per plant. In all treatments, Tween 80 was used as a wetting agent. Control plants were sprayed with a water suspension of Tween 80 alone. When grapes reached optimum maturity, they were harvested and transported to the winery in 20 kg boxes. For chemical analysis of the stilbenes, five mature clusters per plant were randomly collected at harvest from treated and untreated grapevines. Clusters were immediately transported to the laboratory and frozen at −20 °C until analysis.

The yields were different among years: during 2014 Monastrell variety reached 6173 kg/Ha and Tempranillo variety 7600 kg/Ha and during 2015 Monastrell variety reached 9398 kg/Ha and Tempranillo variety 17,500 kg/Ha.

2.3. Vinifications

The grapes were crushed and destemmed and sulfited (8 g of SO_2/100 kg of grapes in Monastrell and 6.7 g of SO2/100 kg of grapes in Tempranillo). The total acidity was corrected to 5.5 g/L with tartaric acid (expressed as mg/L tartaric acid) only for Monastrell variety, and selected yeasts were added (Uvaferm VRB, Lallemand, 25 g/hL). All of the vinifications were conducted in triplicate, in 50 L tanks (for Monastrell grapes) and in 25 L vats (for Tempranillo grapes) at 25 ± 1 °C. Throughout the fermentative pomace contact period (10 days in both vinifications), the cap was punched down twice a day, and the temperature and must density were recorded. At the end of this period, the wines were pressed at 1.5 bars in a 75 L tank membrane press. Free-run and press wines were combined and stored at room temperature. Wines were analyzed in triplicate at the end of alcoholic fermentation (AF).

2.4. Stilbenes determination by HPLC

The extraction method used was that described by Bavaresco, Pezzutto, Ragga, Ferrari, and Trevisan (2001) with some modifications. Briefly, skin from 20 g of berries was frosted and then lyophilized (Cryodos, Telstar). Samples (0.25 g) of lyophilized skin were extracted twice with 5 mL of sodium carbonate (5%) and 5 mL of ethyl acetate