Improvement of grape and wine phenolic content by foliar application to grapevine of three different elicitors: methyl jasmonate, chitosan, and yeast extract

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Abstract

Phenolic compounds play a key role in grape and wine organoleptic properties, being therefore a key parameter in wine quality. Elicitor application constitutes an interesting field of research since it is indirectly involved in the accumulation of phenolic compounds. The aim of this study was to compare the effect of the application of three different elicitors on both grape and wine phenolic content. Methyl jasmonate, chitosan, and a commercial yeast extract were applied to the canopy at veraison and one week later. Results showed that foliar treatments carried out with methyl jasmonate and yeast extract achieved the best results, increasing grape and wine anthocyanin content when compared to the control. Moreover, the application of the yeast elicitor also enhanced grape stilbene content. In contrast, the chitosan treatment did not have a substantial impact on the phenolic compounds. The results of this study indicate that methyl jasmonate and yeast extract applications could be a simple practice to increase grape and wine phenolic content.

Keywords: *Vitis vinifera* L., Tempranillo, foliar application, anthocyanins, flavonols, non-flavonoid, methyl jasmonate, chitosan, yeast extract

1. Introduction

There are a large variety of phenolic compounds that can be found throughout the plant kingdom. These compounds are secondary metabolites mainly involved in the protection of the plant against different abiotic and biotic factors. They are generated in the phenylpropanoid pathway arising from a common precursor: phenylalanine. Phenylalanine ammonia lyase (PAL) is the key enzyme that catalyzes the first step in the phenolic biosynthesis: the transformation of phenylalanine into cinnamic acid. Phenolic compounds have been extensively studied due to their potential for human health. Moreover, in grape and wine research, phenolic compounds are particularly important due to their effect on grape and wine quality as they play key roles in wine colour and mouthfeel properties, as well as its aging potential and stability. In grape and wine, the main classes of phenolic compounds include phenolic acids, stilbenes, and flavonoids (i.e. anthocyanins, flavonols, flavan-3-ol monomers, and proanthocyanidins).

Due to the aforementioned reasons, increasing the wine phenolic content has been a major area of interest in viticultural and enological research. In this context, different tools have been evaluated in recent years. It has been proved that phenolic biosynthesis may be induced in response to different biotic and abiotic elicitors (Goetz et al., 1999; Song, Smart, Wang, Dambergs, Sparrow & Qian, 2015). In this respect, elicitors are molecules able to stimulate plant defense mechanisms which include the activation of secondary biosynthetic pathways such as the one leading to the formation of phenolic compounds (Ferrari, 2010).

Jasmonic acid (JA) and its derivative methyl jasmonate (MeJ) are endogenous plant regulators which act as signaling molecules upon biotic stress and are involved in plant defense mechanisms triggering the synthesis of secondary compounds (Beckers & Spoel, 2006). *In vitro* studies have shown that MeJ treatments may activate the PAL

activity and other enzymes related to phenolic biosynthesis (Belhadj et al., 2006). Moreover, recent field studies have proved that the application of MeJ to the grape bunches may exert a profound effect on the grape and wine phenolic content, particularly in anthocyanins and stilbenes (Fernández-Marín, Puertas, Guerrero, García-Parrilla, & Cantos-Villar, 2014; Ruiz-García, Romero-Cascales, Gil-Muñoz, Fernández-Fernández, López-Roca, & Gómez-Plaza, 2012). Recent evidence suggests that MeJ, when applied to the leaves, may enhance the grape and wine quality too by increasing the content of several phenolic compounds, including anthocyanins, stilbenes and, to a lesser extent, flavonols (Portu, Santamaría, López-Alfaro, López, & Garde-Cerdán, 2015).

Chitosan (CHT) (β-1,4-D-glucosamine) is a polysaccharide obtained from the deacetylation of chitin and is a natural structural compound within the cell wall of several fungi and crustaceous shells. CHT is described as having antimicrobial properties as well as being able to elicit plant defenses reacting to the pathogen challenge by accumulating callose and phenolic compounds (Gozzo, 2003). The application of CHT to control grapevine diseases, such as powdery mildew (Iriti, Vitalini, Di Tommaso, D'Amico, Borgo, & Faoro, 2011) and grey mould, has been widely studied (Romanazzi, Nigro, Ippolito, Di Venere, & Salerno, 2002). Certain studies have reported an induction of PAL activity in CHT-treated bunches (Romanazzi et al., 2002) and leaves (Reglinski, Elmer, Taylor, Wood, & Hoyte, 2010). A previous study had proved that regular CHT applications from spring to harvest may improve grape and wine total polyphenolic content and wine antioxidant activity when compared to conventional fungicide treatments (Iriti et al., 2011). Accumulation of phenolic compounds after CHT treatments has also been reported for other vegetables such as Greek oregano (Yin, Fretté, Christensen, & Grevsen, 2012). However, it has also been

reported that preharvest application of CHT may not influence grape and related wine total phenolic content (Duxbury, Hotter, Reglinski, & Sharpe, 2004; Meng, Li, Liu, & Tian, 2008) nor raspberry pigment content (Tezotto-Uliana, Fargoni, Geerdink, & Kluge, 2014).

On another note, yeast extracts contain several compounds that may act as elicitors. In this respect, yeast cell walls are made up of mannoproteins, β-1,3- and β-1,6-glucans and chitin, while yeast plasmatic membrane comprises 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 PAL following yeast extract applications to plant cell cultures (Peltonen, Mannonen, & Karjalainen, 1997; Yan, Shi, Ng, & Wu, 2006). There are only a few publications about the *in vivo* effect of yeast extracts in field applications. Shehata, Fawzy and El-Ramady (2012) found, on cucumbers, that the treatment with active dry yeast increased plant growth and yield among other parameters. Additionally, a recent study has shown that the exogenous application of a yeast extract to soybean increased the concentration of photosynthetic pigments (i.e. chlorophylls a,b and carotenoids), yield, phenolic content and the antioxidant activity (Dawood, El-Lethy, & Mervat, 2013).

In view of all the foregoing, research has shown the important role that elicitors play in the accumulation of secondary metabolites. However, to our knowledge, there is little information available about the effect of elicitor applications to grapevine under field conditions. What is more, there is an important lack of information about their influence on the detailed phenolic composition of grape and wine. Therefore, the objective of this study was to evaluate the effect of different elicitor foliar applications

to grapevine on the phenolic composition of grape and wine. In this respect, three different elicitors were studied: methyl jasmonate, chitosan, and a yeast extract.

2. Materials and methods

2.1. Reagents and standards

All solvents (methanol, acetonitrile, and formic acid) were of HPLC quality, and all chemicals were of analytical grade (> 99%) unless otherwise stated, they were purchased from Panreac (Barcelona, Spain). Water was of Milli-Q quality (Millipore, Bedford, NY). Methyl jasmonate, chitosan, and Tween 80 were purchased from Sigma-Aldrich (Madrid, Spain). Yeast extract (LalVigne® MATURE) was provided by Lallemand (St. Simon, France). LalVigne® MATURE is a formulation of 100% natural, inactivated wine yeast (*Saccharomyces cerevisiae*) derivatives (specifically designed to be used with the patent foliar application technology WO/2014/024039, Lallemand Inc., Canada). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and DPPH radical (diphenyl-1-picrylhydracyl) were purchased from Fluka Chemie (Buchs, Switzerland). Folin-Ciocalteu reagent was purchased from Merck (Darmstadt, Germany). The following commercial standards were purchased from Sigma-Aldrich: (-)-epicatechin, (+)-catechin, rutin, quercetin, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, kaempferol, myricetin, *trans*-resveratrol, *trans*-piceid, gallic acid, protocatechuic acid, caffeic acid, *p*-coumaric acid, ferulic acid, and caftaric acid. Malvidin-3-*O*-glucoside was purchased from Extrasynthèse (Genay, France).

2.2. Plant material

The experiment was carried out in 2014 on a *Vitis vinifera* cv. Tempranillo commercial vineyard located in Alfaro, Rioja Baja (warmest and driest area of the Rioja

wine region, in northern Spain). Vines had been planted in 1999 at an altitude of 335 m.a.s.l, grafted onto a 1103-Paulsen rootstock and trained to a VSP (vertical shoot positioned) trellis system. Vines were arranged in north-south rows with a between-row and within-row spacing of 2.80 x 1.20 m, respectively. Winter pruning was carried out leaving 12 buds per vine. The vineyard was managed according to the standard viticultural practices for the cultivar and region. Weather conditions were recorded by a meteorological station belonging to the Agroclimatic Information Service of La Rioja (SIAR) installed at about 5 km from the experiment field. Annual rainfall in 2014 was 463.0 mm and the average annual temperature was 14.3 ºC. The soil was classified as *Typic Haplocalcids* according to the American Soil Taxonomy.

2.3. Field treatments

The field trials involved the application of three elicitors: MeJ, CHT, and YE, as well as a control treatment. All solutions were dissolved in water. The MeJ solution was prepared according to Portu et al. (2015) at a concentration of 10 mM; 200 mL per plant were applied. The CHT solution was prepared according to Vitalini, Ruggiero, Rapparini, Neri, Tonni, and Iriti (2014) at a concentration of 0.03% (w/v) (76 kDa molecular weight and 85% deacetylation degree). CHT was dissolved in acetic acid 0.01 M. This solution was sprayed over leaves applying a total amount of 400 mL per plant. The YE solution was prepared, following the manufacturer's instructions (Lallemand), at a concentration of 1.69 g/L; 200 mL were sprayed per plant. In all cases, Tween 80 was used as the wetting agent (0.1% v/v). Control plants were sprayed only with Tween 80 aqueous solution. The treatments were carried out twice, at veraison and one week later. A completely randomized experimental design was set up consisting of three replicates of ten vines per treatment.

2.4. Harvest and vinification

Grapes were harvested when they reached an average ºBrix between 24 and 25. From each replicate, about 150 berries were separated and frozen at -20 °C in order to determine grape monomeric phenolic composition. The remaining grapes were destemmed and crushed and enological parameters were determined in the musts.

In order to evaluate the elicitor's effect on wine quality, grapes were vinified in 25 L vats obtaining three wines for each treatment (one wine per field replicate). Potassium metabisulfite was added to the samples to give a final total SO_2 concentration of 50 mg/L and then musts were inoculated with the commercial *Saccharomyces cerevisiae* strain Uvaferm VRB (Lallemand) (25 g/hL). The must was fermented at a controlled temperature of 25 °C. The end of the alcoholic fermentation was determined by measuring the reducing sugars. Wine enological parameters were then analyzed and aliquots of each wine were frozen and stored at -20 °C until the analyses of monomeric phenolic compounds were carried out.

2.5. Enological parameters of musts and wines

Degree Brix was determined by refractometry. pH, total acidity, malic acid, and potassium were analyzed in musts according to ECC official methods (ECC, 1990), while the tartaric acid was determined following the Rebelein method (Lipka & Tanner, 1974). Wines were characterized by measuring alcoholic degree, pH, total acidity, malic acid, lactic acid, volatile acidity, hue, color intensity (CI), and Folin-Ciocalteu index (FCI) according to ECC official methods (ECC, 1990) and tartaric acid by the Rebelein method (Lipka & Tanner, 1974). Total phenolics were determined as total polyphenol index (TPI) by spectrophotometric absorbance at 280 nm after previous dilution of

177 samples (Lipka & Tanner, 1974). Total anthocyanins were measured by bleaching using
178 sulphur dioxide (Ribéreau-Gayon & Stonestreet, 1965) and total tannins were analyzed
179 following the method described by Ribéreau-Gayon, Peynaud, Ribéreau-Gayon, and
180 Sudraud (1976). Ionised anthocyanins were determined according to Glories (1978) and
181 polymerization index was calculated according to Ruiz (1999).
182 Since treatments were performed in triplicate and a wine was made from each
183 field replicate, the results of these enological parameters are the average of the analyses
184 of three samples (n = 3).
185
186 *2.6. Determination of total antioxidant activity in wines*
187 The total antioxidant activity in wines was determined according to the DPPH
188 method which evaluates the radical-scavenging activity of the sample following the
189 methodology described by Nixdorf and Hermosín-Gutiérrez (2010). Results were
190 compared to a Trolox calibration curve set for the range of 0.10 to 0.80 mM. Results
191 were expressed as millimoles of Trolox equivalents per liter of wine (mmol TE/L).
192
193 *2.7. Determination of grape and wine low molecular weight phenolic compounds*
194 *2.7.1. Extraction of grape phenolics*
195 Grape phenolic compounds were extracted according to the following method:
196 About 50 g of each frozen grape sample were weighed and immersed into 50 mL of a
197 mixture of methanol/water/formic acid (50:48.5:1.5, v/v). The mixture was then
198 homogenized by Ultra-Turrax T-18 (IKA, Staufen, Germany) at high speed (18,000
199 rpm) for 1 min, obtaining a smooth paste. Then, samples were macerated in an
200 ultrasonic bath (JP Selecta, Barcelona, Spain) for 10 min and were centrifuged at 5,000
201 rpm at 10 °C for 10 min. The supernatant was separated and the resulting pellet was

extracted up to three times using the same volume of the solvent mixture (50 mL) each time. The supernatants were then combined and the volume was annotated. Samples were transferred to vials and stored at -20 °C until use.

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

Isolation of non-anthocyanin compounds was carried out based on Castillo-Muñoz, Gómez-Alonso, García-Romero, and Hermosín-Gutiérrez (2007). PCX SPE cartridges (500 mg, 6 mL; Bond Elut Plexa, Agilent, Palo Alto, CA) containing a mixture of reverse-phase and cation-exchanger materials were used. Cartridges were placed in the extraction system (Visiprep^{TM} Vacuum Manifold, Sigma-Aldrich). First, grape phenolic extracts (3 mL) were diluted with 9 mL of 0.1 N HCl. In the case of wine samples, 3 mL of wine were diluted with 3 mL of 0.1 N HCl. The PCX SPE cartridges were conditioned using 5 mL of methanol and 5 mL of water. Then, **the** diluted samples were passed through the PCX SPE cartridges and washing was carried out with 5 mL of 0.1 N HCl and 5 mL of water. The non-anthocyanin phenolic compounds fraction was eluted with 3 x 5 mL of methanol. In order to regenerate the cationic exchange sites for reuse of the cartridges, adsorbed anthocyanins were removed by passing 2 × 5 mL of 2% ammonia in 80% methanol, then 3 × 5 mL of 2% hydrochloric acid in 80% methanol and finally 5 mL of water. The non-anthocyanin phenolic compounds fraction was dried in a centrifugal evaporator (miVac, Genevac Ltd., Suffolk, UK) at 35 ºC and re-solved in 1.5 mL of 20% (v/v) methanol aqueous solution. The anthocyanin-free fraction was used to analyze non-anthocyanin phenolic compounds (flavonols, hydroxycinnamic and hydroxybenzoic acids, stilbenes, and flavan-3-ols).

227 *2.7.3. Analysis of phenolic compounds by HPLC-DAD*

228 Phenolic compounds were analyzed using an Agilent 1260 Infinity
229 chromatograph, equipped with a diode array detector (DAD). Samples were filtered
230 (Chromafil PET 20/25, Machery-Nagel, Düren, Germany) and injected on a
231 Licrospher® 100 RP-18 reversed-phase column (250 x 4.0 mm; 5 μm packing; Agilent)
232 with pre-column Licrospher® 100 RP-18 (4 x 4 mm; 5 μm packing; Agilent), both
233 thermostated at 40 ºC. A flow rate of 0.63 mL/min was established. Chromatographic
234 conditions were based on Castillo-Muñoz, Fernández-González, Gómez-Alonso,
235 García-Romero, and Hermosín-Gutiérrez (2009). For the analysis of anthocyanins, 10
236 μL of sample (grape extract or wine) were injected. Eluents used were (A)
237 acetonitrile/water/formic acid (3:88.5:8.5, v/v/v), and (B) acetonitrile/water/formic acid
238 (50:41.5:8.5, v/v/v). The linear solvents' gradient for anthocyanin analysis was as
239 follows: zero min, 6% B; 15 min, 30% B; 30 min, 50% B; 35 min, 60% B; 38 min, 60%
240 B; 46 min, 6% B. For the analysis of non-anthocyanin phenolic compounds fractions,
241 the injection volume was 20 μL. Eluents were (A) acetonitrile/water/formic acid
242 (3:88.5:8.5, v/v/v), (B) acetonitrile/water/formic acid (50:41.5:8.5, v/v/v), and (C)
243 methanol/water/formic acid (90:1.5:8.5, v/v/v). The linear solvents' gradient for non-
244 anthocyanin analysis was as follows: zero min, 4% B and 0% C; 7 min, 4% B and 0%
245 C; 38 min, 17% B and 13% C; 52 min, 30% B and 20% C; 52.5 min, 40% B and 30%
246 C; 57 min, 50% B and 50% C; 58 min, 50% B and 50% C; 65 min, 4% B and 0% C.

247 Phenolic compounds were identified according to the retention times of available
248 pure compounds and the UV-Vis data obtained from authentic standards and/or
249 published in previous studies (Castillo-Muñoz et al., 2009). For quantification, DAD
250 chromatograms were extracted at 520 nm (anthocyanins), 360 nm (flavonols), 320 nm
251 (hydroxybenzoic and hydroxycinnamic acids and stilbenes), and 280 nm (flavanols) and

252 the calibration graphs of the respective standards (R^2 > 0.999) were used. When a
253 standard was not available, quantification was made according to the calibration graph
254 of the most similar compound. Hence, malvidin-3-*O*-glucoside was used for
255 anthocyanins, quercetin-3-*O*-glucoside was used for flavonols, *trans*-caftaric acid was
256 used for free hydroxycinnamic acids and the corresponding tartaric esters, catechin was
257 used for procyanidins B1 and B2, epicatechin was used for epigallocatechin, and *trans*-
258 piceid and *trans*-resveratrol were used for their respective *cis* isomers. Concentrations in
259 grape samples were expressed as milligrams per weight of grape (mg/kg) while
260 concentrations in wines were expressed as milligrams per liter of wine (mg/L).
261 Since treatments were performed in triplicate and a wine was made from each
262 field replicate, the results for phenolic compounds are the average of the analyses of
263 three samples (n = 3).
264
265 *2.8. Statistical analysis*
266 The statistical procedure was carried out with SPSS Version 21.0 statistical
267 package for Windows (Chicago, IL). The data for the different determinations were
268 processed using the variance analysis (ANOVA). Elicitor treatments and control were
269 compared by a Duncan post hoc test at $p \leq 0.05$.
270
271 **3. Results and discussion**
272 *3.1. Effect of elicitor foliar applications on must and wine enological parameters*
273 Table 1 shows the conventional analysis of control musts and musts from the
274 different foliar treatments. There were only slight differences between the samples.
275 Grapes from CHT and YE treatments showed lower potassium content than control
276 grapes. Moreover, the tartaric acid content was higher in grapes from MeJ treatment

than in grapes from the other elicitor applications. The absence of significant differences between MeJ and control samples agrees with the results obtained by our research group in a previous study (Portu et al., 2015). Moreover, Romanazzi, Murolo, and Feliziani (2013) observed that weekly applications of CHT from May to end of July had no effect on quantitative and qualitative yield parameters in comparison with the control.

Regarding wine enological parameters, most differences were observed between MeJ and the control samples (Table 2). In this respect, a decrease in the pH value was observed together with an increase in tartaric acid content in MeJ wine with respect to the control. Differences on these parameters were not observed in our previous study (Portu et al., 2015). Nonetheless, Ruiz-García et al. (2012) found higher levels of tartaric acid for the MeJ treatment in one of the two years of their study while the pH increased with the application of MeJ in other year. In general, as it has been previously suggested (Ruiz-García et al., 2012), it seems that different climatic conditions may have a strong influence on the grape maturation and its physicochemical parameters. Concerning phenolic-related parameters, color intensity and total anthocyanin content were improved by the MeJ foliar treatment in comparison with the control. This could be an expected outcome since similar results have been reported when MeJ was applied to the leaves (Portu et al., 2015) or directly applied to the grape bunches (Fernández-Marín et al., 2014; Ruiz-García et al., 2012). In contrast to our previous work (Portu et al., 2015), no significant differences were observed regarding Folin-Ciocalteu and polymerization indexes, although both parameters showed a tendency to increase in MeJ wines with respect to control wines. As for YE treatment, significant differences were only found for polymerization index and volatile acidity, which were higher in wines made from grapevines treated with YE than in control wines (Table 2). On the contrary,

no significant differences were observed between CHT and control wines for any of the studied parameters except for the ionization index. This finding is in accordance with the results reported by Tezotto-Uliana et al. (2014). These authors studied the application of CHT at veraison to raspberry and found no effect on color index nor pigment content. In addition, Duxbury et al. (2004) found that preharvest spray application of CHT did not affect Cabernet Sauvignon grape total phenolic and anthocyanin content. Moreover, Iriti et al. (2011) found that wine total polyphenol content was lower in wines from grapevines treated with CHT in comparison with non-treated wines, although CHT treatment improved total phenolic content when compared to grapes treated with conventional fungicides. In the latter study, it was also observed that the CHT treatment improved radical scavenging activity in comparison with conventional fungicides but not with respect to untreated grapes. In contrast, other authors found that both total polyphenolic content and radical-scavenging activity were increased by CHT treatment in comparison with control (Vitalini et al., 2011). In addition, Romanazzi et al. (2002) observed a significant increase of PAL activity in table grapes treated with CHT. Nevertheless, it must be taken into account that most of aforementioned studies evaluated the application of CHT from the time when grape susceptibility to fungal diseases starts (i.e. spring) until veraison or harvest. In general, the elicitors tested in this study seemed to have a slight effect on grape and wine physicochemical parameters. Regarding wine chromatic parameters (i.e. color index and total anthocyanin content), the foliar application of MeJ achieved the best results when compared to the control.

3.2. Elicitors effect on anthocyanins

Results for anthocyanin analysis are shown in Table 3. The monomeric anthocyanins found in grape samples were the 3-*O*-glucosides (3-glc) of delphinidin, cyanidin, petunidin, peonidin and malvidin, together with their acetylated (3-acglc) and *trans*-*p*-coumaroylated (3-cmglc) derivatives. In addition, the *cis*-*p*-coumaroyl (*cis*-3-cmglc) and the caffeoyl (3-cfglc) derivatives of malvidin were also identified. Malvidin derivatives were the most abundant anthocyanin form while 3-cmglc were the most abundant acylated anthocyanins. The same profile was observed in wine samples, although it was also possible to identify two pyranoanthocyanins formed during alcoholic fermentation (vitisins A and B).

As it can be seen in Table 3, elicitors influenced grape anthocyanin composition. Compared to control samples, MeJ increased the content of 3-glc of delphinidin, cyanidin, petunidin, and peonidin, besides peonidin-3-acglc and cyanidin-3-cmglc. YE increased the grape content of malvidin-3-glc and peonidin-3-acglc. However, neither MeJ nor YE treatments showed significant differences regarding total anthocyanin content with respect to control grapes. On the other hand, CHT application did not significantly affect the anthocyanins content compared to the control.

Elicitor treatments also affected anthocyanin content of the wine (Table 3). In general, wines with higher anthocyanin concentrations were obtained from vines which had been treated with MeJ and YE, although only wines from MeJ showed higher total anthocyanin content than control wines, which was well correlated with the spectrophotometrically measure (Table 2). In more detail, MeJ foliar application led to wines with higher content of the 3-glc of petunidin, peonidin and malvidin, and cyanidin-3-acglc, than control wines. Regarding the effect of YE foliar application, malvidin-3-glc and cyanidin-3-acglc concentrations were increased when compared to

350 the control wine. As for CHT treatment, no differences in the anthocyanins content were
351 observed between CHT treated and control wines.
352 Results reported in this study concerning MeJ are in agreement with previous
353 studies. For instance, Portu et al. (2015) found that MeJ foliar application induced
354 anthocyanin synthesis in grapevines, increasing the concentration of several
355 anthocyanins as well as the total anthocyanin content in both grapes and wines. On
356 another note, it has been proved that bunch application of MeJ promotes anthocyanins
357 synthesis. In this respect, Ruiz-García et al. (2012) found that grapes treated with MeJ
358 had higher anthocyanin content than control grapes. This finding was also proved by
359 Ruiz-García et al. (2013) for certain Monastrell clones. However, the latter authors
360 stated that the impact of MeJ is clone-dependent. Regarding YE treatment, there is a
361 lack of information about the effect of YE applications under field conditions.
362 Nonetheless, there is some evidence that YE treatments may elicit plant cell cultures,
363 inducing the accumulation of phenylpropanoid-derived compounds (Peltonen et al.,
364 1997; Yan et al., 2006). Moreover, Dawood et al. (2013) observed that YE application
365 increased soybean photosynthetic pigments content. In our study, malvidin-3-glc, the
366 most abundant anthocyanin, was found in higher concentrations in both grape and wine
367 in the YE treatment when compared to the control samples. Moreover, it is noteworthy
368 to mention that there were not significant differences between MeJ and YE treatments.
369 In contrast, CHT application did not promote the synthesis of anthocyanin compounds
370 in comparison to the control. What is more, grape and wine from this treatment showed
371 lower levels of certain anthocyanins than those from MeJ and YE foliar applications.
372 Although the influence of CHT application on grape and wine detailed wine phenolic
373 composition has not been studied yet, there exists evidence that CHT application may
374 activate key enzymes of the phenylpropanoid pathway, in particular PAL (Reglinski et

al., 2010; Romanazzi et al., 2002). In this sense, certain *in vitro* studies have shown that CHT treatment may lead to an accumulation of anthocyanins (Ferri, Tassoni, Franceschetti, Righetti, Naldrett, & Bagni, 2009). However, previous field studies have shown that CHT application did not affect grape and wine anthocyanin content (Duxbury et al., 2004) or raspberry pigment content (Tezotto-Uliana et al., 2014).

In any case, the improvement of grape and wine anthocyanin composition by foliar application of MeJ and YE is a noteworthy outcome. Anthocyanins play a vital role in the color of red grapes and wine. As wine color is the first feature perceived by the consumer, it has, consequently, a substantial impact on the final wine quality.

3.3. Elicitors effect on flavonols

The HPLC analysis led to identify 11 flavonols in the grape samples (Table 4). It was possibly to identify flavonol glycosides of the six flavonoid structures present in *Vitis vinifera* grapes: myricetin, quercetin, laricitrin, kaempferol, isorhamnetin, and syringetin. As seen in previous studies carried out on Tempranillo grapes from La Rioja (Portu et al., 2015), myricetin-type flavonols were predominant, followed by quercetin-type flavonols, accounting together for around 80% of all total flavonol content. The 3-*O*-glucosides of myricetin and quercetin were the main flavonol glycosides found in the grape samples. Regarding wine samples, it was possible to identify the aglycones corresponding to the glycosides found in the grapes. Flavonol aglycones are released by acid hydrolysis of the flavonol glycosides during the winemaking process (Castillo-Muñoz et al., 2007). Hermosín-Gutiérrez, Castillo-Muñoz, Gómez-Alonso, and García-Romero (2012) stated that the degree of hydrolysis might depend on the flavonoid structure and the kind of glycosylation. In this respect, the latter authors suggested that the 3-*O*-glucuronides and the syringetin-type flavonols might be the most resistant to

hydrolysis. Our results are consistent with this fact since the 3-*O*-glucuronides of myricetin and quercetin, followed by syringetin-3-*O*-glucoside, were the most abundant glycosides in the wine samples.

As it can be seen in Table 4, there were no significant differences in the total flavonol content between the control and the treatments for both grape and wine, except for free-syringetin that was found at significantly lower levels in the wines from MeJ and YE treatments than in the control.

Previous studies showed that MeJ foliar application to Tempranillo grapevines had a stronger effect on anthocyanins than on flavonols (Portu et al., 2015). Results from the present study seem to confirm this previous finding. However, in our previous study (Portu et al., 2015), an improvement in the wine flavonol composition was observed that has not been confirmed in the present study. Different results might be attributed to the different clones used (Ruiz-García, Romero-Cascales, Bautista-Ortín, Gil-Muñoz, Martínez-Cutillas, & Gómez-Plaza, 2013), and the different soil and climate parameters. On account of this, it has been proposed that MeJ application may have a stronger effect in years when pathogen development is more suitable (Gozzo, 2003; Ruiz-García et al., 2012). In particular, the fact that 2014 (463 mm) was considerably less rainy than 2013 (569.3 mm) could explain the small differences observed between the two studies. Nonetheless, in accordance to our results, Ruiz-García et al. (2012) observed that control wines and wines made from bunches treated with MeJ had similar flavonol content. As for YE and CHT treatments, to authors' knowledge no publications can be found that study the effect of these field treatments on grape and wine flavonol composition. Our results suggest that there are only minor differences between the elicitor treatments, although MeJ applications seemed to obtain the best results regarding wine flavonol composition. In general, taking into account the present study

and previous results, it can be suggested that the application of elicitors at veraison usually exerts a limited impact on flavonol synthesis. Despite the fact that anthocyanins and flavonols share a big part of their metabolic pathway, it appears that anthocyanin biosynthesis is preferentially activated in comparison with flavonol's. In any case, it must be taken into account that flavonols are important copigments that contribute to wine color stability (Schwarz, Picazo-Bacete, Winterhalter, & Hermosín-Gutiérrez, 2005).

3.4. Elicitors effect on flavanols.

Results of grape and wine flavanol composition are summarized in Table 5. In grape samples, epicatechin-3-gallate and catechin were the major compounds while, in wine, catechin and epigallocatechin were found in the highest concentrations. If the treatments are compared to the control, only grapes from the CHT application differed to control regarding epicatechin-3-gallate. The flavanol content of wines obtained from treated grapes was similar to that from the untreated ones. Previous studies (Portu et al., 2015) have shown that MeJ foliar application did not have any effect on grape and wine flavanols when compared to control while other authors have stated that MeJ applications may lead to different results according to the grapevine clone (Ruiz-García et al., 2013). In this respect, Ruiz-García et al. (2013) suggested that the enzymes responsible for tannin synthesis might be activated in preference to those responsible for anthocyanin synthesis, being this behavior clone dependent. Regarding the CHT treatment, catechin concentration in berry skin was studied by Romanazzi, Gabler, and Smilanick (2006) after preharvest treatment with CHT. In agreement with our results, CHT application did not increase catechin content in berry skin (Romanazzi et al., 2006). Flavanols have a great importance in wine mouthfeel sensations and color

stability but in the present study, this group of compounds was generally unaffected by the treatments.

3.5. Elicitors effect on non-flavonoid compounds

Results of the HPLC analysis of non-flavonoid compounds in grape and wine are shown in Table 6. Gallic acid was the only hydroxybenzoic acid identified in grape and wine samples. In grape, *trans*-and *cis*-coutaric acids were the most abundant hydroxycinnamic acids. In wine, the hydrolysis of the hyroxycinnamoyl tartaric acids during alcohol fermentation allowed the identification of the corresponding free acids. Regarding stilbenes, *trans*-piceid was identified in the highest concentration in both grape and wine, as it has been shown in previous study (Portu et al., 2015). Moreover, the proportion of *trans*-resveratrol in wine increased when compared to grape samples due to the hydrolysis of both piceid isomers during the alcoholic fermentation. In addition, the hydrolysis of *trans* and *cis*-piceid also allowed us to identify *cis*-resveratrol in the wines.

In agreement with our previous study (Portu et al., 2015), results indicated that MeJ application had no effect on the phenolic acid content when compared to the control. CHT treatment showed a similar pattern and no significant differences were observed with respect to the control in neither grape nor wine samples. Moreover, YE phenolic acid profile in grapes was similar to control and the rest of treatments. Conversely, wines made from grapevines treated with YE differed from control wines in *trans*-caftaric acid content, which was at lower level in YE wines. Additionally, the total hydroxycinnamic acid content was also lower in wines from the YE treatment than in control wines. Hydroxycinnamic acids are known to play a vital role in wine organoleptic characteristics. On the one hand, hydroxycinnamic acids are ethylphenols

475 precursors, volatile compounds responsible for the off-flavors described as animal
476 odors, farm, horse sweat, medicine and animal leather, mainly occurring during wine
477 barrel ageing (Rubio-Bretón, Lorenzo, Salinas, Martínez, & Garde-Cerdán, 2013). On
478 the other hand, hydroxycinnamic acids are as well precursors of
479 hydroxyphenylpyranoanthocyains contributing in a major way to wine color stability
480 (Schwarz et al., 2005). Regarding stilbenes, YE treatment show the strongest effect on
481 these compounds and its grape samples had higher concentrations of *trans*-piceid, *trans*-
482 resveratrol and total stilbene content than control samples. Moreover, MeJ treatment
483 also increased *trans*-resveratrol concentration with respect to the control. However,
484 stilbene content was similar in all the wines, and only wines from MeJ showed highest
485 *cis*-resveratrol content than the other treatments and control. Nonetheless, it has to be
486 taken into account that *cis*-resveratrol content was the lowest of all stilbene compounds.
487 Previous research has shown that bunch (Fernández-Marín et al., 2014) and foliar (Portu
488 et al., 2015) application of MeJ may exert a strong impact on stilbenes. In our previous
489 study (Portu et al., 2015), total stilbene content was significantly higher in both grape
490 and wine from MeJ treatment than control. In the present study, differences have been
491 not as substantial as they were in our previous work. This fact seems to be due to the
492 different climatic conditions (2014 was less rainy than 2013) as abovementioned. In a
493 different way, CHT treatment did not improve stilbene content when compared to
494 control. Ferri et al. (2009) suggested that CHT treatments increase stilbene content in
495 grapevine cell suspensions due to *de novo* biosynthetic activity by the promotion of
496 specific enzymes. However, in agreement with our results, Romanazzi et al. (2006)
497 found that preharvest treatment of table grape berries with CHT did not increase
498 resveratrol concentration in berry skin. In contrast to CHT, the YE treatment obtained
499 the best results in grape samples, although YE wines were similar to control. This fact

has been also observed for other compounds in this study. It seems that YE improved the phenolic potential content in grape, but this observation was not reflected in the corresponding wines. In general, it seems that the accumulation of stilbenes is an expected outcome when the application of the elicitor is effective (Fernández-Marín et al., 2014; Portu et al., 2015). These compounds are considered important phytoalexins with antimicrobial properties that contribute to the plant resistance against pathogen attacks (Cimmino, Andolfi, Abouzeid, & Evidente, 2013).

4. Conclusions

This study has shown that foliar application at veraison of MeJ and a commercial YE may induce grapevine phenolic biosynthesis. In this respect, although grape and wine physicochemical parameters seemed to be only slightly affected by the treatments, MeJ application improved certain wine chromatic parameters. Moreover, the analysis of grape and wine detailed phenolic composition suggested that MeJ and YE treatments improved both grape and wine anthocyanin content. However, the effect on other compounds was less evident although stilbene content was clearly improved by the application of the yeast elicitor. In contrast to the other two elicitors, CHT barely had any effect on either grape or wine phenolic content. Overall, it is noteworthy to mention that the grape and the wine phenolic content were increased by a foliar application, which is simple and accessible to the winegrower. From our results, it could be concluded that the foliar application of methyl jasmonate and the commercial yeast extract seem to be more effective than CHT.

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Table 1. Enological parameters of grape berries from control grapevines and from grapevines treated with methyl jasmonate (MeJ), chitosan (CHT) and yeast extract (YE).

	Control	MeJ-treated	CHT-treated	YE-treated
Yield/vine (kg)	2.21 ± 0.35a	2.19 ± 0.80a	2.72 ± 0.30a	2.49 ± 0.85a
Weight of 100 berries (g)	199.0 ± 23.9a	208.1 ± 13.9a	210.5 ± 9.1a	194.9 ± 16.2a
°Brix	24.7 ± 0.4a	24.4 ± 0.1a	24.3 ± 0.4a	24.3 ± 0.2a
Probable alcohol (%, v/v)	14.7 ± 0.3a	14.5 ± 0.1a	14.3 ± 0.3a	14.4 ± 0.1a
pH	3.44 ± 0.04a	3.43 ± 0.02a	3.41 ± 0.01a	3.48 ± 0.07a
Total acidity (g/L)[a]	5.25 ± 0.07a	5.28 ± 0.16a	5.46 ± 0.17a	5.25 ± 0.17a
Tartaric acid (g/L)	7.49 ± 0.10ab	7.64 ± 0.11b	7.46 ± 0.05a	7.37 ± 0.07a
Malic acid (g/L)	2.26 ± 0.39a	1.93 ± 0.14a	2.11 ± 0.05a	2.13 ± 0.16a
Potassium (mg/L)	1786 ± 111b	1702 ± 39ab	1654 ± 28a	1641 ± 37a

As there were 3 replications per treatment, all parameters are listed with their standard deviation (n = 3). Comparison between treatments is made on each row. For each parameter, values with the same letters are not significantly different between the samples ($p \leq 0.05$). [a]As g/L tartaric acid.

Table 2. Enological parameters of control wine and wines made from grapevines treated with methyl jasmonate (MeJ), chitosan (CHT) and yeast extract (YE).

	Control	MeJ-treated	CHT-treated	YE-treated
Alcoholic degree (%, v/v)	14.4 ± 0.3a	14.3 ± 0.2a	14.5 ± 0.3a	14.6 ± 0.4a
pH	3.82 ± 0.05bc	3.74 ± 0.03a	3.76 ± 0.02ab	3.85 ± 0.01c
Total acidity (g/L)[a]	5.51 ± 0.04a	5.67 ± 0.07a	5.68 ± 0.06a	5.60 ± 0.18a
Tartaric acid (g/L)	1.78 ± 0.08a	2.14 ± 0.04b	1.95 ± 0.15ab	1.97 ± 0.06ab
Malic acid (g/L)	2.44 ± 0.16a	2.21 ± 0.14a	2.46 ± 0.13a	2.40 ± 0.14a
Lactic acid (g/L)	0.02 ± 0.01a	0.03 ± 0.06a	0.00 ± 0.01a	0.00 ± 0.04a
Volatile acidity (g/L)[b]	0.17 ± 0.03a	0.20 ± 0.02ab	0.20 ± 0.02ab	0.25 ± 0.04b
Hue	0.57 ± 0.03a	0.55 ± 0.01a	0.54 ± 0.03a	0.58 ± 0.01a
Color intensity (CI)	12.39 ± 0.77a	15.01 ± 1.82b	12.37 ± 0.98a	12.57 ± 0.15a
Folin-Ciocalteu index	38.1 ± 2.3a	40.2 ± 5.6a	41.0 ± 3.3a	32.9 ± 2.7a
Total polyphenol index (TPI)	48.75 ± 3.37a	48.48 ± 2.99a	48.32 ± 5.36a	48.29 ± 0.52a
Total anthocyanins (mg/L)	816 ± 26a	975 ± 51b	899 ± 76ab	865 ± 49ab
Ionization index	23.97 ± 1.09b	23.67 ± 0.93b	18.69 ± 2.18a	21.52 ± 0.95ab
Polymerization index	1.43 ± 0.12a	1.74 ± 0.25ab	1.50 ± 0.06ab	1.79 ± 0.02b
Total antioxidant activity (mmol TE/L)[d]	6.07 ± 0.32a	6.60 ± 0.47a	6.68 ± 1.29a	6.11 ± 0.34a

As there were 3 replications per treatment, all parameters are listed with their standard deviation (n = 3). Comparison between treatments is made on each row. For each parameter, values with different letters are significantly different between the samples ($p \leq 0.05$). [a]As g/L tartaric acid. [b]As g/L acetic acid. [d]As mmol of Trolox equivalents per liter of wine.

678 Table 3. Anthocyanin content in control samples and samples from grapevines treated with methyl jasmonate (MeJ), chitosan (CHT) and yeast extract (YE).

	Grape berries (mg/kg)				Wines (mg/L)			
	Control	MeJ-treated	CHT-treated	YE-treated	Control	MeJ-treated	CHT-treated	YE-treated
Delphinidin-3-glc	261.60 ± 33.17a	319.98 ± 34.49b	264.77 ± 22.80a	312.30 ± 13.27ab	36.17 ± 4.89a	48.79 ± 7.62a	39.59 ± 6.66a	38.50 ± 5.35a
Cyanidin-3-glc	34.16 ± 7.02a	56.40 ± 14.96b	37.22 ± 3.50ab	50.10 ± 12.96ab	1.84 ± 0.32a	2.70 ± 0.69a	1.96 ± 0.34a	1.81 ± 0.25a
Petunidin-3-glc	190.18 ± 23.30a	225.35 ± 21.40b	190.02 ± 13.43a	222.11 ± 3.61ab	55.47 ± 4.52a	69.72 ± 7.48b	59.30 ± 5.98ab	62.21 ± 4.83ab
Peonidin-3-glc	71.25 ± 11.74a	101.24 ± 22.17b	72.86 ± 2.31ab	93.25 ± 14.26ab	8.93 ± 1.34a	14.37 ± 3.71b	10.16 ± 2.12ab	9.50 ± 1.08ab
Malvidin-3-glc	541.57 ± 61.41a	577.92 ± 13.41ab	535.57 ± 32.98a	618.94 ± 16.37b	280.43 ±	310.57 ± 8.18b	286.58 ± 7.47a	315.54 ± 6.11b
Delphinidin-3-acglc	21.18 ± 2.23ab	22.89 ± 0.67ab	20.93 ± 1.77a	23.89 ± 0.42b	5.82 ± 0.47a	6.37 ± 0.26a	5.83 ± 0.28a	6.29 ± 0.47a
Cyanidin-3-acglc	4.31 ± 0.50a	4.57 ± 0.12a	4.16 ± 0.12a	4.61 ± 0.16a	0.75 ± 0.04a	0.86 ± 0.05b	0.79 ± 0.03ab	0.87 ± 0.01b
Petunidin-3-acglc	13.22 ± 1.63a	13.51 ± 0.64a	12.72 ± 0.96a	13.94 ± 0.58a	4.23 ± 0.27a	4.52 ± 0.14a	4.29 ± 0.18a	4.65 ± 0.28a
Peonidin-3-acglc	3.29 ± 0.22a	3.87 ± 0.30b	3.44 ± 0.03a	3.92 ± 0.27b	1.05 ± 0.07a	1.23 ± 0.12a	1.08 ± 0.09a	1.07 ± 0.04a
Malvidin-3-acglc	35.36 ± 4.92a	33.68 ± 3.09a	33.46 ± 2.42a	36.89 ± 2.95a	16.88 ± 1.76a	17.07 ± 0.76a	16.56 ± 0.17a	18.43 ± 0.59a
Delphinidin-3-cmglc	64.57 ± 7.37a	64.49 ± 3.08a	60.17 ± 3.60a	65.02 ± 7.46a	10.50 ± 1.28a	11.68 ± 0.85a	11.28 ± 1.44a	10.45 ± 1.36a
Cyanidin-3-cmglc	10.40 ± 0.90a	13.25 ± 2.59b	10.31 ± 0.39a	11.96 ± 0.69ab	1.84 ± 0.26a	2.36 ± 0.40a	1.94 ± 0.30a	1.83 ± 0.27a
Petunidin-3-cmglc	52.97 ± 6.38a	51.59 ± 1.73a	49.64 ± 3.34a	53.98 ± 6.66a	9.03 ± 1.03a	10.06 ± 0.94a	9.65 ± 1.13a	9.39 ± 1.27a
Peonidin-3-cmglc	23.17 ± 1.62a	25.63 ± 3.41a	22.33 ± 0.30a	24.95 ± 0.43a	5.94 ± 0.80a	7.39 ± 0.79a	6.63 ± 0.99a	6.29 ± 0.80a
Malvidin-3-*cis*-cmglc	7.11 ± 1.22a	5.70 ± 0.46a	6.03 ± 0.52a	6.47 ± 0.78a	1.78 ± 0.25a	1.55 ± 0.08a	1.65 ± 0.04a	1.70 ± 0.10a
Malvidin-3-*trans*-cmglc	208.91 ± 32.87a	189.25 ± 9.21a	194.08 ± 14.99a	215.44 ± 30.95a	48.15 ± 4.27a	51.81 ± 7.07a	48.72 ± 5.33a	50.40 ± 5.46a
Malvidin-3-cfglc	73.24 ± 21.57a	96.75 ± 21.44a	68.01 ± 8.67a	95.24 ± 13.75a	9.03 ± 1.03a	10.06 ± 0.94a	9.65 ± 1.13a	9.39 ± 1.27a
Total anthocyanins	1616 ± 194ab	1806 ± 124ab	1586 ± 96a	1853 ± 13b	498 ± 32a	571 ± 37b	516 ± 32ab	548 ± 30ab
Vitisin A	n.d.	n.d.	n.d.	n.d.	2.18 ± 0.13b	2.06 ± 0.07ab	1.97 ± 0.06a	2.09 ± 0.05ab
Vitisin B	n.d.	n.d.	n.d.	n.d.	2.22 ± 0.15b	2.18 ± 0.09ab	1.98 ± 0.10a	1.98 ± 0.06a

679 Nomenclature abbreviations: glc, glucoside; acglc, acetylglucoside; cmglc, *trans*-*p*-coumaroylglucoside; cfglc, caffeoylglucoside.
680 As there were 3 replications per treatment, all parameters are listed with their standard deviation (n = 3). Comparison between treatments is made on each row. For each
681 parameter, values with different letters are significantly different between the samples ($p \leq 0.05$). n.d. = not detected.

Table 4. Flavonol content in control samples and samples from grapevines treated with methyl jasmonate (MeJ), chitosan (CHT) and yeast extract (YE).

	Grape berries (mg/kg)				Wines (mg/L)			
	Control	MeJ-treated	CHT-treated	YE-treated	Control	MeJ-treated	CHT-treated	YE-treated
Myricetin-3-glcU	7.98 ± 1.25a	8.44 ± 0.46a	6.76 ± 0.60a	8.24 ± 1.03a	2.12 ± 0.22a	1.85 ± 0.62a	1.57 ± 0.34a	1.68 ± 0.12a
Myricetin-3-gal	7.40 ± 2.12a	7.12 ± 0.88a	6.30 ± 0.76a	7.28 ± 1.08a	1.54 ± 0.28a	1.57 ± 0.24a	1.28 ± 0.33a	1.42 ± 0.02a
Myricetin-3-glc	52.12 ± 9.16a	51.41 ± 5.28a	45.94 ± 3.29a	50.88 ± 5.51a	0.82 ±	1.27 ± 0.13b	0.69 ± 0.33a	0.78 ± 0.11a
Quercetin-3-glcU	17.42± 3.80a	21.29 ± 4.54a	14.86 ± 2.30a	21.10 ± 2.36a	4.95 ± 0.53a	4.34 ± 1.29a	3.29 ± 0.80a	3.85 ± 0.09a
Quercetin-3-glc	23.71± 2.75a	30.99 ± 10.05a	20.11 ± 2.15a	29.72 ± 4.75a	n.d.	n.d.	n.d.	n.d.
Laricitrin-3-glc	7.77± 1.43a	7.43 ± 0.89a	6.74 ± 0.43a	7.31 ± 0.92a	0.50 ±	0.67 ± 0.06b	0.44 ± 0.05a	0.55 ± 0.01ab
Kaempferol-3-glcU	1.02± 0.22a	1.23 ± 0.18a	0.87 ± 0.11a	1.23 ± 0.23a	n.d.	n.d.	n.d.	n.d.
Kaempferol-3-glc	8.49± 1.85a	10.82 ± 4.16a	6.78 ± 1.01a	11.22 ± 2.55a	n.d.	n.d.	n.d.	n.d.
Isorhamnetin-3-gal	0.52± 0.02a	0.55 ± 0.05a	0.51 ± 0.01a	0.55 ± 0.02a	n.d.	n.d.	n.d.	n.d.
Isorhamnetin-3-glc	2.60± 0.18a	3.13 ± 1.03a	2.15 ± 0.17a	2.82 ± 0.43a	n.d.	n.d.	n.d.	n.d.
Syringetin-3-glc	4.81± 0.89a	4.89 ± 0.61a	4.32 ± 0.17a	4.69 ± 0.55a	2.16 ± 0.21a	2.16 ± 0.19a	1.89 ± 0.15a	2.10 ± 0.02a
Free-myricetin	n.d.	n.d.	n.d.	n.d.	4.43 ± 0.97a	3.18 ± 0.88a	3.23 ± 1.48a	3.02 ± 0.34a
Free-quercetin	n.d.	n.d.	n.d.	n.d.	2.96 ± 0.38a	1.95 ± 0.51a	2.01 ± 0.91a	2.01 ± 0.19a
Free-laricitrin	n.d.	n.d.	n.d.	n.d.	0.63 ± 0.10a	0.43 ± 0.03a	0.50 ± 0.20a	0.60 ± 0.25a
Free-kaempferol	n.d.	n.d.	n.d.	n.d.	0.62 ± 0.07a	0.38 ± 0.06a	0.38 ± 0.20a	0.42 ± 0.06a
Free-isorhamnetin	n.d.	n.d.	n.d.	n.d.	0.33 ± 0.03a	0.31 ± 0.08a	0.30 ± 0.06a	0.25 ± 0.03a
Free-syringetin	n.d.	n.d.	n.d.	n.d.	0.26 ± 0.02b	0.20 ± 0.02a	0.22 ± 0.02ab	0.21 ± 0.01a
Total flavonols	133.84 ± 21.86a	147.29 ± 27.38a	115.35 ± 8.97a	145.03 ± 19.10a	21.30 ±	18.30 ± 3.89a	15.80 ± 4.51a	16.90 ± 1.02a

Nomenclature abbreviations: glcU, glucuronide; gal, galactoside; glc, glucoside.
As there were 3 replications per treatment, all parameters are listed with their standard deviation (n = 3). Comparison between treatments is made on each row.
For each parameter, values with different letters are significantly different between the samples ($p \leq 0.05$). n.d. = not detected.

Table 5. Flavanol content in control samples and samples from grapevines treated with methyl jasmonate (MeJ), chitosan (CHT) and yeast extract (YE).

	Grape berries (mg/kg)				Wines (mg/L)			
	Control	MeJ-treated	CHT-treated	YE-treated	Control	MeJ-treated	CHT-treated	YE-treated
Catechin	27.48 ± 10.72a	29.94 ± 1.80a	24.65 ± 5.97a	24.76 ± 3.98a	12.35 ±	12.13 ± 1.24a	11.57 ± 2.57a	10.38 ± 1.27a
Epicatechin	17.36 ± 3.48a	18.48 ± 0.67a	16.08 ± 2.88a	18.28 ± 1.69a	5.90 ±	6.93 ± 0.96b	5.14 ± 0.36a	5.39 ± 1.46ab
Epicatechin-3-gallate	30.11 ± 2.62b	27.60 ± 1.93ab	25.69 ± 1.14a	30.46 ± 2.30b	n.d.	n.d.	n.d.	n.d.
Epigallocatechin	3.33 ± 0.82a	3.33 ± 0.54a	2.93 ± 0.05a	3.52 ± 0.40a	11.53 ±	11.15 ± 0.85a	11.30 ± 1.11a	12.31 ± 0.26a
Procyanidin B1	8.60 ± 0.92a	10.19 ± 0.56a	8.71 ± 1.46a	9.54 ± 0.58a	9.98 ± 1.45a	10.03 ± 4.45a	7.61 ± 0.97a	8.10 ± 0.50a
Procyanidin B2	5.19 ± 0.64a	6.61 ± 0.21a	5.09 ± 0.79a	5.50 ± 1.37a	3.63 ± 0.62a	4.34 ± 1.00a	3.63 ± 0.55a	3.05 ± 1.48a
Total	92.08 ± 16.79a	96.14 ± 4.55a	83.14 ± 11.21a	92.07 ± 7.38a	43.39 ±	44.59 ± 7.55a	39.01 ± 2.77a	39.24 ± 3.97a

As there were 3 replications per treatment, all parameters are listed with their standard deviation (n = 3). Comparison between treatments is made on each row. For each parameter, values with different letters are significantly different between the samples ($p \leq 0.05$). n.d. = not detected.

691 Table 6. non-Flavonoid content in control samples and samples from grapevines treated with methyl jasmonate (MeJ), chitosan (CHT) and yeast extract (YE).

	Grape berries (mg/kg)				Wines (mg/L)			
	Control	MeJ-treated	CHT-treated	YE-treated	Control	MeJ-treated	CHT-treated	YE-treated
Hydroxybenzoic acids								
Gallic acid	9.21 ± 1.28a	9.98 ± 0.66a	8.50 ± 0.69a	8.35 ± 0.65a	11.71 ±	11.11 ± 0.91a	11.82 ± 1.29a	10.82 ± 0.44a
Hydroxycinnamicacids								
trans-Caftaric acid	28.53 ± 4.88a	33.72 ± 4.35a	30.73 ± 4.78a	26.07 ± 4.51a	43.43 ±	38.46 ± 0.92ab	41.34 ± 4.94ab	35.18 ± 1.65a
trans+*cis*-Coutaric	35.55 ± 3.47a	37.35 ± 5.04a	35.54 ± 5.87a	33.34 ± 4.60a	32.69 ±	29.51 ± 1.32a	32.75 ± 5.32a	25.16 ± 2.48a
Caffeic acid	n.d.	n.d.	n.d.	n.d.	4.48 ± 0.14a	4.75 ± 0.19a	4.61 ± 0.57a	4.59 ± 1.09a
p-Coumaric acid	n.d.	n.d.	n.d.	n.d.	1.48 ± 0.06a	1.54 ± 0.07a	1.37 ± 0.17a	1.22 ± 0.55a
Total	64.08 ± 8.35a	71.07 ± 9.37a	66.27 ± 10.60a	59.41 ± 9.06a	82.08 ±	74.26 ± 2.01ab	80.08 ± 10.86ab	66.14 ± 5.78a
Stilbenes								
trans-Piceid	1.47 ± 0.27a	1.89 ± 0.74ab	1.61 ± 0.17ab	2.43 ± 0.45b	0.86 ± 0.15a	0.96 ± 0.14a	0.80 ± 0.20a	0.74 ± 0.19a
cis-Piceid	0.32 ± 0.15a	0.46 ± 0.15a	0.43 ± 0.18a	0.57 ± 0.21a	0.46 ± 0.04a	0.55 ± 0.12a	0.49 ± 0.09a	0.47 ± 0.10a
trans-Resveratrol	0.13 ± 0.08a	0.37 ± 0.16b	0.32 ± 0.02ab	0.39 ± 0.10b	0.32 ± 0.06a	0.27 ± 0.04a	0.32 ± 0.05a	0.25 ± 0.07a
cis-Resveratrol	n.d.	n.d.	n.d.	n.d.	0.03 ± 0.00a	0.05 ± 0.01b	0.03 ± 0.01a	0.03 ± 0.01a
Total	1.92 ± 0.47a	2.72 ± 1.04ab	2.37 ± 0.31ab	3.38 ± 0.71b	1.67 ± 0.25a	1.83 ± 0.30a	1.64 ± 0.34a	1.50 ± 0.36a

692 As there were 3 replications per treatment, all parameters are listed with their standard deviation (n = 3). Comparison between treatments is made on each row. For
693 each parameter, values with different letters are significantly different between the samples ($p \leq 0.05$). n.d. = not detected.