Methyl Jasmonate Foliar Application to Tempranillo Vineyard Improved Grape and Wine Phenolic Content

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1 **ABSTRACT**

2 The importance of phenolic compounds on grape and wine quality has drawn attention

3 to study different practices with the aim to increase their content. Cluster application of

4 elicitors is a viticultural practice which has shown promising results in recent years.

5 However, cluster application requires a previous defoliation which is time consuming

6 and expensive. In the present study, methyl jasmonate was foliar applied to Tempranillo

7 grapevines in order to study its effect on grape and wine phenolic composition. Methyl

8 jasmonate foliar application increased anthocyanin and stilbene content in both grape

9 and wine, besides enhancing wine flavonol content. This treatment induced the

10 synthesis of 3-*O*-glucosides of petunidin and peonidin and *trans-p*-coumaroyl

11 derivatives of cyanidin and peonidin. For stilbenes, *trans*-piceid content was

12 considerably increased in both grape and wine. The results obtained suggest that methyl

13 jasmonate foliar application could be a simple and accessible practice that allows to

14 enhance grape and wine quality.

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16 *KEYWORDS: elicitor, Vitis vinifera L. cv. Tempranillo, anthocyanins, stilbenes,*

17 *flavonols, grapevine treatment*

INTRODUCTION

Phenolic compounds are secondary metabolites which have a great importance in plant metabolism. There exists a great diversity of phenolic compounds in grape and wine although all of them share a common structure that consists of a benzene ring with one or more hydroxyl groups attached. These compounds are formed through the phenylpropanoid pathway via phenylalanine ammonia lyase.[1] In grape and wine, phenolic compounds can be divided into two main groups: non-flavonoids (hydroxybenzoic and hydroxycinnamic acids and stilbenes) and flavonoids (anthocyanins, flavonols, and flavanols). On the one hand, hydroxycinnamic acids play a key role in wine color as they participate in copigmentation reactions and in the formation of pyranoanthocyanins.[2] Hydroxybenzoic acids are also important cofactors which contribute to enhance and stabilize red wine color.[3] Moreover, stilbenes are phytoalexins which have been widely studied in recent years due to their health-promoting properties and their role in disease prevention.[4] On the other hand, flavonoids constitute the most abundant phenolic compounds in grape and wine. In this respect, anthocyanins are the main responsible for red wine color; flavonols also contribute to wine color as they act as copigments,[2] as well as being related to wine health benefits;[5] and flavanols (flavan-3-ol monomers or proanthocyanidins) play an important role in wine mouthfeel sensations, such as astringency or bitterness,[6,7] as well as they contribute to wine color stability.[8]

By reasons of the matters aforesaid, phenolic composition is a key parameter of grape and wine quality. Therefore, considerable attention is paid to increase the level of these compounds either in grape or in wine.[9] Different approaches have studied the foliar application to vineyard of hormones or growth regulators in order to improve grape and wine phenolic composition. For example, Balint and Reynolds[10] observed

that pre-harvest foliar application of abscisic acid (ABA) to Cabernet Sauvignon grapevines induced anthocyanin synthesis and Berli et al.[11] observed the same effect when ABA was applied to Malbec grapevines. Moreover, a recent study revealed that foliar application of oak aqueous extract to Monastrell grapevines improved wine phenolic composition.[12]

Quite recently, there has been a growing interest in chemical elicitors as molecules able to improve fruit phenolic content.[13] Elicitors are molecules able to trigger plant defense responses, contributing then to the plant resistance against pathogen attacks.[14] The activation of secondary pathways is one of the inducible defense responses. In regard of this, phenylpropanoid pathway might be activated leading to the accumulation of phenolic compounds. Among chemical elicitors, jasmonic acid (JA) and especially its methyl ester, methyl jasmonate (MeJ), are considered molecules of interest in order to induce plant defenses and therefore improve food quality.[15] In this respect, previous research has shown improvements in anthocyanin and flavonol content when MeJ was applied before harvest to blackberries,[16] raspberries,[17] or apples.[18] Several works have reported an accumulation of diverse phenolic compounds, especially anthocyanins and stilbenes, after MeJ treatments to grapevine cell cultures.[19,20] Moreover, there are a few studies that have evaluated the impact of MeJ application on grape and wine phenolic composition under field conditions. These works have shown that MeJ application to grapevine clusters may lead to an increase in stilbene and anthocyanin content in grape and wine from *Vitis vinifera* L. cvs. Syrah, Monastrell and Barbera.[21-23]

To our knowledge, no publications are found in the literature that address the issue of MeJ foliar application to vineyard. In addition, no studies investigating this treatment on Tempranillo grapevines have been found. Tempranillo is the most

cultivated red grape variety in Spain accounting for around 21.5% of the total wine-growing surface (2011). Moreover, it is the dominant red grape variety in Spanish aged wines. Therefore, improving its phenolic content would be of oenological interest in order to enhance its aging potential. According to the literature, all the experiments conducted under vineyard conditions investigated the effect of direct applications of MeJ to clusters. Cluster application requires a previous defoliation in order to achieve a correct distribution of the applied product. In contrast, foliar application is a costless and easier technique due to the fact that a previous defoliation is not required. Therefore, the aim of this paper was to evaluate the influence of MeJ foliar application to Tempranillo grapevines on grape and wine phenolic composition.

MATERIALS AND METHODS

Reagents and Standards. All solvents (methanol, acetonitrile, and formic acid) were of HPLC quality, and all chemicals were analytical grade (>99%) unless otherwise stated, and were purchased from Panreac (Barcelona, Spain). Water was of Milli-Q quality (Millipore, Bedford, NY). Methyl jasmonate and Tween 80 were purchased from Sigma-Aldrich (Madrid, Spain). 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, piceatannol, *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).

Plant Material, Foliar Treatments and Vinification. The study was conducted in 2013 in the experimental vineyard of La Grajera, located in the northern region of La Rioja (Spain). Vines were grafted onto R-110 rootstock and vineyard was managed under conventional soil tillage management system. The soil was classified as *Typic Calcixerept* according to the American Soil Taxonomy. In 2013, the annual precipitation was 569.3 mm, and the average annual temperature was 17.7 ºC. Methyl jasmonate (MeJ) was applied to Tempranillo (*Vitis vinifera* L.) grapevines. To carry out the treatments, aqueous solutions at a concentration of 10 mM of MeJ were prepared using Tween 80 as wetting agent (0.1% v/v). Control plants were sprayed with water solution of Tween 80 alone. The applications of MeJ and control were carried out twice, at veraison and one week later. For each application, 200 mL/plant were sprayed over leaves. Treatments were carried out in triplicate with three vines for each replication and arranged in a randomized block design.

Grapes were harvested at their optimum technological maturity. From each treatment, about 150 berries were separated and frozen at -20 °C in order to determine their monomeric phenolic composition. Grapes were destemmed and crushed and oenological parameters were determined in the musts.

The alcoholic fermentation was performed following the method described by Sampaio et al.[24] Grapes from each replication (each group of three plants) were elaborated separately. Three kilograms of pomace (must, seed, and skin) were introduced into 4 L glass bottles. Potassium metabisulfite was added to the samples to give a final total SO_2 concentration of 50 mg/L and then must was inoculated with the commercial *Saccharomyces cerevisiae* strain Uvaferm VRB (Lallemand, St. Simon, France) (25 g/hL). The must was fermented at controlled temperature of 25 °C. The end of the alcoholic fermentation was determined by measuring the reducing sugars. Wine

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118 oenological parameters were then analyzed and aliquots of each wine were frozen and

119 stored at -20 °C until the analyses of monomeric phenolic compounds were carried out.

120 **Oenological Parameters of Musts and Wines.** ºBrix was determined by

121 refractometry, pH, total acidity, malic acid, and potassium were determined in musts

122 according to ECC official methods,[25] while tartaric acid was determined following the

123 Rebelein method.[26] Wines were characterized by determining alcoholic degree, pH,

124 total acidity, malic acid, lactic acid, volatile acidity, hue, color intensity (CI), and Folin-

125 Ciocalteu index (FCI) according to ECC official methods[25] and tartaric acid by Rebelein

126 method.[26] Total phenolics were determined as total polyphenol index (TPI) by

127 spectrophotometric absorbance at 280 nm after previous dilution of samples.[27] Total

128 anthocyanins were determined by bleaching using sulphur dioxide[27] and total tannins

129 were determined following the method described by Ribéreau-Gayon et al.[28] Ionised

130 anthocyanins were determined according to Glories[29] and polymerization index was

131 calculated according to Ruiz.[30]

132 Field treatments were performed in triplicate and a wine was made from each

133 replicate, so the results of these oenological parameters are the average of the analyses

134 of three samples (n = 3).

135 **Determination of Total Antioxidant Activity in Wines.** The total antioxidant

136 activity in wines was determined according to the DPPH method which evaluates the

137 radical-scavenging activity of the sample.[31] The analysis was performed following the

138 methodology described by Nixdorf and Hermosín-Gutiérrez.[32] Briefly, 100 μL of wine,

139 previously diluted 5% (v/v) in methanol, were added to 2.9 mL of a 0.06 mM DPPH

140 methanolic solution. The percentage of absorbance decreased was measured after 25

141 min at 515 nm. Results were compared to a Trolox calibration curve set for the range of

142 0.10 to 0.80 mM. Results were expressed as millimoles of Trolox equivalents per liter

143 of wine (mmol TE/L).

144 **Determination of Grape and Wine Low Molecular Weight Phenolic**

145 **Compounds.** *Extraction of Grape Phenolics.* Grape phenolic compounds were

146 extracted according to the following method. About 50 g of each frozen grape sample

147 were weighed and immersed into 50 mL of aqueous methanol solution (50% v/v), pH of

148 the solvent was adjusted at pH 2 with formic acid (>96%). Grapes were then

149 homogenized by Ultra-Turrax T-18 (IKA, Staufen, Germany) at high speed (18,000

150 rpm) for 1 min, obtaining a smooth paste. Then, samples were macerated in an

151 ultrasonic bath (JP Selecta, Barcelona, Spain) for 10 min and were centrifuged at 5,000

152 rpm at 10 °C for 10 min. A second extraction of the resulting pellets was completed

153 using the same volume of the solvent mixture (50 mL). The supernatants were

154 combined and the volume was annotated. Each sample was transferred to vials and

155 stored at -20 °C until the HPLC analyses were carried out.

156 *Sample Preparation for the Analysis of non-Anthocyanin Phenolic Compounds.*

157 Due to the fact that anthocyanins are present in a high quantity in red grape and wine,

158 other phenolic compounds might be masked during the chromatographic separation and

159 identification. On account of this, an extraction on PCX SPE cartridges (500 mg, 6 mL;

160 Bond Elut Plexa, Agilent, Palo Alto, CA) containing a mixture of reverse-phase and

161 cation-exchanger materials allowed the isolation of non-anthocyanin phenolic

162 compounds. Cartridges were placed in the extraction system (Vac Elut 20 station from

163 Varian, Palo Alto, CA). The separation was achieved following the method proposed by

164 Castillo-Muñoz et al.[33] Firstly, 3 mL of grape extracts were diluted with 9 mL of HCl

165 0.1 N. In the case of wine samples, 3 mL of wine were diluted with 3 mL of HCl 0.1 N.

166 The PCX SPE cartridges were previously conditioned using 5 mL of methanol and 5

mL of water. Then, diluted samples were passed through the PCX SPE cartridges and a washing step was carried out with 5 mL of HCl 0.1 N and 5 mL of water. The non-anthocyanin phenolic compounds fraction was eluted with 3 x 5 mL of methanol. Adsorbed anthocyanins were removed by passing methanol with 2% HCl until the eluate was colorless. The latter step also regenerates the cationic exchange sites for a new use of the cartridges. The non-anthocyanin phenolic compounds fraction was dried in a rotary evaporator (35 $^{\circ}$C) 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).

Analysis of Phenolic Compounds by HPLC-DAD. Phenolic compounds were analyzed by reverse-phase HPLC using a chromatograph Agilent 1260 Infinity, equipped with a diode array detector (DAD) coupled to an Agilent Chem Station. Injection of samples was carried out after filtration (0.22 µm, Easyprep, Quebec, Canada). Separation was achieved on a Licrosphere® 100 RP-18 reversed-phase column (250 x 4.0 mm; 5 µm packing; Agilent) with pre-column Licrosphere® 100 RP-18 (4 x 4 mm; 5 µm packing; Agilent), both thermostated at 40 ^{o}C. A flow rate of 0.63 mL/min was established.

Chromatographic conditions were based on Castillo-Muñoz et al.[34] In the case of anthocyanin analysis, 10 µL of sample (grape extract or wine) were injected. Eluents used were (A) acetonitrile/water/formic acid (3:88.5:8.5 v/v/v), and (B) acetonitrile/water/formic acid (50:41.5:8.5 v/v/v). The linear solvents' gradient for anthocyanin analysis was as follows: zero min, 6% B; 15 min, 30% B; 30 min, 50% B; 35 min, 60% B; 38 min, 60% B; 46 min, 6% B. In the case of the analysis of non-anthocyanin phenolic compounds fractions, the injection volume was 20 µL. Eluents

192 were (A) acetonitrile/water/formic acid (3:88.5:8.5 v/v/v), (B) acetonitrile/water/formic

193 acid (50:41.5:8.5 v/v/v), and (C) methanol/water/formic acid (90:1.5:8.5 v/v/v). The

194 linear solvents' gradient for non-anthocyanin analysis was as follows: zero min, 4% B

195 and 0% C; 7 min, 4% B and 0% C; 38 min, 17% B and 13% C; 52 min, 30% B and 20%

196 C; 52.5 min, 40% B and 30% C; 57 min, 50% B and 50% C; 58 min, 50% B and 50%

197 C; 65 min, 4% B and 0% C.

198   Identification of phenolic compounds was carried out according to the retention

199 times of pure compounds and the UV-Vis data obtained from authentic standards and/or

200 published in previous studies.[34,35] Phenolic compounds were quantified according to the

201 DAD chromatograms recorded at 520 nm (anthocyanins), 360 nm (flavonols), 320 nm

202 (hydroxybenzoic and hydroxycinnamic acids and stilbenes), and 280 nm (flavanols) and

203 the calibration graphs of the respective standards (R^2>0.999). When a standard was not

204 available, quantification was made according to the calibration graph of the most similar

205 compound. Hence, malvidin-3-*O*-glucoside was used for anthocyanins, quercetin-3-*O*-

206 glucoside was used for flavonols, *trans*-caftaric acid was used for free hydroxycinnamic

207 acids and the corresponding tartaric esters, catechin was used for procyanidins B1 and

208 B2, epicatechin was used for epigallocatechin, and *trans*-piceid and *trans*-resveratrol

209 were used for their respective *cis* isomers. Concentrations in grape samples were

210 expressed as milligrams per weight of grape (mg/kg) while concentrations in wines

211 were expressed as milligrams per liter of wine (mg/L).

212   Field treatments were performed in triplicate and a wine was made from each

213 replicate, so the results for phenolic compounds correspond to the average of the

214 analyses of three samples (n = 3).

215   **Statistical Analysis.** The statistical procedure was performed using SPSS

216 Version 21.0 statistical package for Windows (Chicago, IL). The data for the different

217 determinations were processed using the variance analysis (ANOVA). Significant

218 differences between means were determined by using the Duncan test at $p \leq 0.05$.

219

220 **RESULTS AND DISCUSSION**

221 **Oenological Parameters.** Results for grape oenological parameters are

222 summarized in Table 1. All samples presented normal values of these parameters for

223 Tempranillo grapes from La Rioja.[36] No significant differences were found between

224 control grapes and grapes from grapevines foliar treated with MeJ. Ruiz-García et al.[37]

225 studied the application of MeJ at the same concentration as our study to clusters from

226 different clones of Monastrell and observed that, in general, grapes from the MeJ

227 treatment were very similar to the control grapes. Moreover, in a two-years study in

228 which Monastrell clusters were sprayed with the same dose of MeJ, authors observed

229 that the effect on total acidity, pH, tartaric and malic acid content varied from one year

230 to another.[22] The latter finding might suggest that the influence of MeJ application on

231 grape physico-chemical parameters could therefore depend on the climatic conditions of

232 the year as well.

233 Physico-chemical parameters of wines are shown in Table 2. All wines

234 presented values within the usual ranges reported for young Tempranillo red wines from

235 La Rioja region.[38] Significant differences between control wines and wines obtained

236 from treated grapevines appeared to be related to phenolic composition. Control wines

237 showed lower color intensity (CI) than wines from MeJ treatment. This result could be

238 explained by a higher proportion of ionised anthocyanins in wines from MeJ treatment

239 than in control ones (Table 2). In this respect, this parameter is correlated with colored

240 anthocyanins content. Moreover, the values obtained for Folin-Ciocalteu index (FCI)

241 were higher in the case of wines elaborated from grapevines treated with MeJ than in

242   control wines, suggesting that those wines had a greater phenolic content. In accordance

243   with our results, Ruiz-García et al.[22] found that the application of MeJ to Monastrell

244   clusters led to wines with better chromatic characteristics (i.e. higher total anthocyanin

245   content, total phenol index (TPI), and CI). The same research group found that the

246   application of MeJ together with another elicitor (benzothiadiazole) led to wines with

247   higher TPI and CI.[39] Moreover, Fernández-Marín et al.[21] showed that the application of

248   MeJ to Syrah clusters resulted in darker wines with higher anthocyanin content at press

249   moment, although no differences were found after bottling. On another note, our results

250   showed that polymerization index in wine increased with the application of MeJ to

251   grapevine (Table 2). This parameter measures the stability of anthocyanins against

252   sulfur dioxide, providing information about anthocyanin polymerization and thus, wine

253   color stability.[30] In this respect, the results suggest that greater color stability could be

254   found in wines made from grapevines treated with MeJ. For the remaining parameters,

255   no significant differences were found between the samples. Indeed, no significant

256   differences were observed regarding total antioxidant activity estimated by the DPPH

257   assay. In contrast, Ruiz-García et al.[39] observed that wine antioxidant capacity,

258   estimated by ABTS assay, increased with the application of a combination of MeJ and

259   benzothiadiazole.

260   **Effect of MeJ Foliar Application on Anthocyanin Compounds.** The HPLC

261   analysis led to identify seventeen anthocyanins in grape samples (Table 3). The 3-*O*-

262   glucosides (3-glc) of delphinidin, cyanidin, petunidin, peonidin, and malvidin were

263   identified together with their acetyl (3-acglc) and *trans-p*-coumaroyl (3-cmglc)

264   derivatives. In addition, *cis-p*-coumaroyl (*cis*-3-cmglc) and caffeoyl derivatives (3-

265   cfglc) of malvidin were also identified. The concentrations of individual anthocyanins

266   found in all the samples were within the ranges described for Tempranillo grapes in

267 previous studies.[40,41] Thus, malvidin-3-*O*-glucoside and its derivatives were the

268 dominant anthocyanin type in all the samples accounting for 40% of all total

269 anthocyanins. non-Acylated anthocyanins represented around 90% of all anthocyanins.

270 Among acylated anthocyanins, *p*-coumaroyl derivatives were the dominant (87% of all

271 acylated anthocyanins), which is typical for this grape variety.[40]

272 The foliar application of MeJ to grapevine led to a significant enhancement of

273 anthocyanin synthesis by the plant. Consequently, grapes from this treatment showed

274 higher content of 3-*O*-glucosides of petunidin and peonidin, and *trans-p*-coumaroyl

275 derivatives of cyanidin and peonidin (Table 3). The total anthocyanin concentration,

276 calculated as the sum of individual anthocyanin contents, was increased in a 23% due to

277 the foliar treatment. The improvement of anthocyanin content seems to be an expected

278 outcome from MeJ application as it has been reported in previous studies. In this

279 respect, Ruiz-García et al.[22] observed that the application of MeJ to Monastrell clusters

280 led to increases around 16% of total anthocyanin content. In a recent study, the

281 application of the same dose of MeJ to different clones of Monastrell also resulted in an

282 increase of anthocyanin content in most of the clones.[37] Similar results were reported by

283 Fernández-Marín et al.[21] who treated clusters of Syrah with MeJ and found that total

284 anthocyanin content was increased in an 11% in comparison with control grapes.

285 Moreover, induction of anthocyanin synthesis has also been observed in cell cultures of

286 *V. vinifera* cv. Gamay Fréaux[20] or in studies in which MeJ was applied in combination

287 with other elicitor, benzothiadiazole,[39] as well as in different works with other fruits

288 such as raspberry[17] or apple.[18] The enhancement of anthocyanin synthesis seems to be

289 explained by the accumulation of different enzymes involved in the phenylpropanoid

290 pathway.[19] In this sense, it has been reported that grapevines may respond to MeJ

291 application by activating enzymes responsible for phenolic biosynthesis such as

292   phenylalanine ammonia lyase, chalcone synthase, stilbene synthase, UDP-glucose:

293   flavonoid-*O*-transferase, proteinase inhibitors and chitinase gene expression, with a

294   subsequent accumulation of anthocyanins and stilbenes in grapevine cell cultures.[20]

295   Regarding wine analysis, in addition to the seventeen anthocyanins found in

296   grape, two pyranoanthocyanins, vitisins A and B, were identified (Table 3). The

297   formation of the latter compounds occurs during the alcoholic fermentation by the

298   reaction of malvidin-3-*O*-glucoside with pyruvic acid (vitisin A) or with acetaldehyde

299   (vitisin B).[42] Similarly to grape analysis, malvidin derivatives were the most abundant

300   type of anthocyanin and *p*-coumaroyl anthocyanins were the dominant acylated form.

301   Higher anthocyanin levels found in grapes from MeJ treatment were reflected in

302   the corresponding wines (Table 3). Thus, wines made from treated grapevines exhibited

303   higher content of 3-*O*-glucosides of delphinidin, petunidin, and peonidin, and higher

304   content of *trans-p*-coumaroyl-3-*O*-glucosides of cyanidin and peonidin, as well as

305   vitisin B. Furthermore, total anthocyanin content in wines from the treatment was

306   increased in a 24% with respect to control wine. The latter finding is partly in contrast

307   with the results obtained with the spectrophotometrically measure of total anthocyanins

308   in wine (Table 2). That measure revealed no significant differences between the samples

309   despite that a higher value (around 13%) was found in MeJ wines. Ruiz-García et al.[22]

310   also observed discrepancies between HPLC and spectrophotometrically analyses

311   regarding anthocyanin concentration. These authors suggested that differences between

312   the two analyses were explained by the analytical method. In any case, other analysis

313   related to wine color (i.e. CI and ionised anthocyanins content) showed higher values

314   for MeJ wines than for control wines. Moreover, the results of the individual analysis of

315   wine anthocyanins are also in accordance with those obtained in the analysis of grapes

316   (Table 3) showing a similar increase in total anthocyanin content. Ruiz-García et al.[22]

317   also found that MeJ application improved wine total anthocyanin content determined by

318   HPLC.

319   Anthocyanins play an important role in grape and wine quality since they are the

320   main responsible compounds for red wine color. Wine color is the first feature

321   perceived by the consumers and consequently, improvements on this parameter may

322   have a substantial impact on wine tasting and quality. Our results suggest that grape and

323   wine anthocyanic composition of Tempranillo variety was improved following the

324   foliar treatment with MeJ, which could be of a great oenological importance.

325   **Effect of MeJ Foliar Application on Flavonol Compounds.** Table 4 shows

326   individual flavonol content of grape and wine samples. The HPLC analysis indicated

327   the presence of fourteen flavonols in all the samples. Derivatives of the two major forms

328   in Tempranillo grape berries, myricetin and quercetin, were identified accounting

329   together for 85% of all total flavonols. The derivatives of the other four flavonol

330   structures naturally present in *V. vinifera* grape berries were also identified: laricitrin,

331   kaempferol, isorhamnetin, and syringetin derivatives. Results indicated the presence of

332   all 3-*O*-glucosides (3-glc) although 3-*O*-glucuronides (3-glcU) and 3-*O*-galactosides (3-

333   gal) of myricetin, quercetin, and kaempferol were detected too. In addition, quercetin-3-

334   *O*-rutinoside (3-rut) and isorhamnetin-3-*O*-galactoside (3-gal) were also identified.

335   There is little research on grape flavonols in comparison with anthocyanins. In this

336   respect, flavonol levels in our study seemed to be in accordance with the scarce

337   literature on this topic, which reports an important proportion of myricetin and

338   quercetin-type flavonols in Tempranillo grape berries.[43]

339   There were not significant differences between control grape samples and those

340   from MeJ foliar treatment except for the content of isorhamnetin-3-*O*-glucoside, which

341   was higher in samples from MeJ treatment (Table 4). Isorhamnetin-type flavonols are

342 formed by gradual methoxylation of quercetin during grape ripening.[33] As can be seen

343 from Table 3, peonidin-3-*O*-glucoside and its coumaroylated form were found in a

344 higher proportion in grapes from MeJ treatment. Therefore, results seem to suggest that

345 MeJ application had a marked effect on the methoxylated forms of disubstituted

346 flavonoids (i.e. peonidin and isorhamnetin). In this respect, flavonols are closely related

347 to anthocyanins since they share a big part of their biosynthetic pathway. However, our

348 results seem to indicate that MeJ application had less influence on flavonol compounds

349 than on anthocyanin composition. In accordance with our study, Ruiz-García et al.[37]

350 observed that flavonol content was normally unaffected by MeJ treatment. As well as

351 this, although Ruiz-García et al.[22] reported an increase in grape anthocyanin content in

352 the two years of their study, total flavonol content was only improved in the second

353 year. Therefore, it appears that the improvement of anthocyanin synthesis by MeJ

354 application does not necessarily imply a significant induction of flavonol synthesis.

355 Based then on the results, it seems as if MeJ application favors the activity of specific

356 enzymes for the anthocyanin synthesis (e.g. dihydroflavonol 4-reductase (DFR) or

357 flavonoid 3-glucosyltransferase (UFGT)) while in contrast, it does not significantly

358 favor the activity of specific enzymes for the flavonol synthesis (i.e. flavonol synthase

359 (FLS)).

360 Regarding wine analysis, during fermentation flavonol glycosides are

361 hydrolyzed releasing their corresponding aglycones.[33,43] In this respect, the aglycones

362 from the six possible structures were identified: myricetin, quercetin, laricitrin,

363 kaempferol, isorhamnetin, and syringetin (Table 4). Moreover, some glycosides that had

364 been quantified in grape were not identified in wine (3-*O*-galactosides of myricetin,

365 quercetin, kaempferol and isorhamnetin, as well as quercetin-3-*O*-rutinoside and

366 kaempferol-3-*O*-glucuronide). These compounds were in a small concentration in grape

samples. Consequently, their hydrolysis during vinification decreased their concentration in wine, making them undetectable. Moreover, Castillo-Muñoz et al.[33] stated that 3-*O*-glucosides of mono and disubstitued flavonols (i.e. kaempferol, quercetin, and isorhamnetin) appeared to be easily hydrolyzed. Furthermore, in our study, syringetin-3-O-glucoside appeared to be the less hydrolyzed compound, which might be an expected outcome according to Hermosín-Gutiérrez et al.[43] As seen in grape analysis, myricetin and quercetin-type flavonols were the dominant type of flavonols in wine samples accounting for around 90% of all total flavonols, followed by laricitrin and syringetin-type flavonols. In agreement previous studies,[43] isorhamnetin and kaempferol-type flavonols were minor compounds in wine.

In general, more significant differences for these compounds were observed between wine samples than between grape samples. Thus, wines obtained from grapevines treated with MeJ showed higher total flavonol content in comparison with control wines (Table 4). This result was probably due to differences in the individual concentration of quercetin-3-*O*-glucoside and free-myricetin, which were quantitatively important in the wine samples. Moreover, as seen in grape analysis, isorhamnetin-3-*O*-glucoside was also found in a higher concentration in wines from the MeJ treatment than in control ones. Since grape samples from control and MeJ treatment had shown similar flavonol levels, the slight differences occurred between wines might be attributed to higher flavonol extractability when MeJ was applied. Schwarz et al.[44] reported that the prefermentation addition to the must of a flavonol compound, rutin, favoured anthocyanin extraction during winemaking due to the formation of copigmentation complexes. On account of this, it could be hypothesized that grapes richer in anthocyanin compounds, as those from MeJ treatment, may as well lead to wines not only richer in anthocyanins but also in flavonols. In contrast to our results,

392   Ruiz-García et al.[22] found that MeJ application to Monastrell clusters had no influence

393   on wine total flavonol content. However, the latter authors found that wine flavonol

394   content increased with the application of another elicitor, benzothiadiazole.[39]

395   **Effect of MeJ Foliar Application on Flavanol Compounds.** Results of grape

396   and wine flavanol analysis are shown in Table 5. Catechin was the major flavanol

397   compound in grapes followed by epicatechin. The results showed that MeJ foliar

398   application did not affect flavanol synthesis since MeJ treated grape samples exhibited

399   similar flavanol levels than those found in control grape samples. There are only a few

400   works in the literature that studied the influence of MeJ application on flavanol

401   composition in grape or in other fruits. Shafiq et al.[18] observed that pre-harvest MeJ

402   application to red blush apples increased skin catechin and epicatechin content, although

403   results depended upon the time of application. Ruiz-García et al.[22] found that skin

404   proanthocyanidin content was increased by MeJ application while, on the other hand,

405   there was barely any difference regarding seed tannin content between control and

406   treated samples. Moreover, the latter research group stated that MeJ application could

407   led to different results depending on the treated clone.[37] In some clones, tannin synthesis

408   might be favored at the expense of anthocyanin synthesis while other clones could show

409   the opposite behavior. In our study, Tempranillo grapes treated with MeJ have shown a

410   significant enhancement of anthocyanin compounds while no differences have been

411   observed in terms of flavanol composition.

412   As seen in grape analysis, catechin and epicatechin were the most abundant

413   compounds in wines accounting together for around 65% of the total flavanols

414   identified (Table 5). Both wines showed a similar flavanol composition suggesting that

415   MeJ foliar application did not influence this group of flavonoids. Therefore, results

416   confirm those obtained from grape analysis which showed no differences between

417   control grape and grapes from the foliar treatment. Similarly, Fernández-Marín et al.[21]

418   did not observe any difference concerning total flavanol content between control wines

419   and wines elaborated from Syrah clusters treated with MeJ. Moreover, Ruiz-García et

420   al.[22] observed that the application of MeJ to Monastrell clusters led to an increase in the

421   total amount of flavanols in the second year of the study, whereas no effect from MeJ

422   application was noticed in the first year.

423   **Effect of MeJ Foliar Application on Grape and Wine non-Flavonoid**

424   **Composition.** non-Flavonoid grape and wine composition is shown in Table 6. Gallic

425   acid was the only hydroxybenzoic acid identified in grape samples. Hydroxycinnamic

426   acids in grapes are esterified with tartaric acid.[45] *trans*-Caftaric and *trans+cis*-coutaric

427   acids were the dominant hydroxycinnamic acids. As for stilbenes, *trans*-piceid was the

428   most abundant stilbene in grape samples representing around 51% of total stilbene

429   content.

430   Hydroxybenzoic and hydroxycinnamic acids content in grapes was independent

431   of the MeJ foliar application since no differences were observed between the

432   concentration found in control grapes and that found in grapes from treated grapevines

433   (Table 6). In contrast, it was observed a markedly increase in stilbene concentration

434   after MeJ foliar treatment. The synthesis of both piceid isomers was enhanced by this

435   application. Thus, *trans*-piceid was 5.6-fold increased while *cis* isomer was 3.3-fold

436   increased. On the other hand, *trans* and *cis*-resveratrol were not affected by MeJ foliar

437   treatment. All the same, total stilbene content was three-fold increased. Induction of

438   stilbene formation seems to be an expected outcome as it has been reported in several

439   works. Ruiz-García et al.[37] observed that MeJ application normally leads to an increase

440   in stilbene concentration (up to five-fold increase depending on the clone). Syrah

441   clusters treated with MeJ were richer in different stilbenes than untreated clusters at the

442   harvest moment.[21] Moreover, *trans*-resveratrol content increased at ripening in Barbera

443   grapes treated by cumulative MeJ applications.[23] Apart from this, Belhadj et al.[20]

444   observed an accumulation of total piceids (*trans* and *cis*-piceids) in Gamay Fréaux cell

445   suspensions treated with MeJ alone or in combination with sucrose. Furthermore, they

446   found that different genes involved in the biosynthesis of polyphenols were up-

447   regulated by MeJ application. In a previous study conducted by the latter research

448   group,[19] Cabernet Sauvignon leaves treated with MeJ showed an accumulation of

449   enzymes responsible for stilbene formation (e.g. stilbene synthase). Therefore, our

450   results confirm those previously reported for other grape varieties and obtained in

451   different conditions. Elicitors may reduce the use of conventional fungicides by

452   preventing the attack of certain pathogens through the induction of plant defense

453   responses.[14,19] Accumulation of phytoalexins (e.g. stilbenes) is one of the plant

454   responses to elicitor perception.[46] Thus, treated grapevines in our study seemed to

455   activate the synthesis and accumulation of stilbene compounds. In contrast, the absence

456   of significant differences regarding phenolic acids seems to be related to the different

457   biosynthetic pathway of these compounds. Results suggest that MeJ application may

458   favor the biosynthetic pathway of anthocyanins (also flavonols in a lesser extent) and

459   stilbenes, while it seemed to have little effect on phenolic acids synthesis.

460   Concerning wine analysis, protocatechuic acid was identified together with

461   gallic acid (Table 6). As expected, the hydrolysis of the hydroxycinnamoyl tartaric acids

462   during alcoholic fermentation released the corresponding free acids, enabling to identify

463   caffeic, *p*-coumaric, and ferulic acids. Still, *trans*-caftaric and *trans*+*cis*-coutaric acids

464   remained as the major hydroxycinnamic acid derivatives in the wine samples. As for

465   stilbenes, like in grapes, *trans*-piceid was the major compound in wine, representing

466   around 60% of total stilbene compounds. Moreover, the average *trans*-piceid/*trans*-

467 resveratrol ratio decreased from 5.74 in grapes to 2.70 in wines, probably due to the

468 hydrolysis of *trans*-piceid to *trans*-resveratrol during the alcoholic fermentation.[46]

469 It was confirmed that samples from MeJ foliar treatment showed a higher

470 concentration of total stilbenes and *trans*-piceid while the effect on other non-flavonoid

471 compounds was less evident. In particular, it could be of interest that wine made from

472 treated grapevines showed a similar concentration of non-sterified hydroxycinnamic

473 acids (caffeic, *p*-coumaric and ferulic acids) to the control wine. These compounds are

474 ethylphenol precursors and therefore they could be involved in ethylphenol formation

475 and subsequent wine alteration, especially during barrel aging.[47] This might be a

476 noteworthy outcome taking into account that Tempranillo grapes are normally used for

477 producing aged wines.[48] Despite this fact, it should be also taken into account that

478 hydroxycinnamic acids are also precursors in the formation of hydroxyphenyl-

479 pyranoanthocyanins, which are anthocyanin-derived pigments that contribute to wine

480 color stability.[49] Regarding stilbenes, *trans*-piceid content was three times higher in

481 wines from MeJ foliar treatment than in control wines. Moreover, total stilbene content

482 was increased in a 163% in wines obtained from grapevines treated with MeJ in

483 comparison with control wines. The increase in wine total stilbene concentration is in

484 good agreement with previous works which have shown that MeJ application in

485 vineyard could result in an improvement of wine stilbene content. In this respect,

486 Fernández-Marín et al.[21] found that total stilbene content was increased in wine, both at

487 press and bottle moments, with the application of MeJ to Syrah clusters. In recent years,

488 research on stilbenes has become very popular due to their importance for human

489 health.[50] Therefore, the foliar application of MeJ to Tempranillo grapevines could be an

490 interesting tool in order to enhance wine health-promoting properties.

491   Our results describe for the first time the effect of MeJ foliar application on the

492   phenolic composition of cv. Tempranillo grape and wine. Grape and wine phenolic

493   content was increased in the samples from MeJ treatment in comparison with control

494   samples. Anthocyanin content was increased in both grape and wine by MeJ

495   application. Similarly, wine color related parameters were also improved as a

496   consequence of MeJ application. Apart from this, stilbene synthesis was enhanced by

497   MeJ application as stilbene content was increased in both grape and wine. The effect of

498   MeJ application was less evident on flavonols, although total flavonol content was

499   higher in wines from MeJ treatment than in control wines. For the remaining

500   parameters, there were not significant differences between control and MeJ samples. It

501   should be taken into account that foliar application does not require a previous

502   defoliation which, in contrast, is necessary when the application is carried out directly

503   on clusters. Therefore, this paper has shown that foliar application of MeJ could be an

504   easy and interesting viticultural practice in order to increase the phenolic content of

505   grape. Furthermore, it could improve as well the wine color and its health-promoting

506   properties.

REFERENCES

1. Jackson, R. S. Grapevine structure and function. In *Wine Science-Principles and Applications*, 3rd ed.; Elsevier: San Diego, CA, 2008; pp. 50-107.

2. Gómez-Míguez, M.; González-Manzano, S.; Escribano-Bailón, M. T.; Heredia, F. J.; Santos-Buelga, C. Influence of different phenolic copigments on the color of malvidin 3-glucoside. *J. Agric. Food Chem.* **2006**, *54*, 5422-5429.

3. Zhang, B.; Liu, R.; He, F.; Zhou, P.-P.; Duan, C.-Q. Copigmentation of malvidin-3-*O*-glucoside with five hydroxybenzoic acids in red wine model solutions: Experimental and theoretical investigations. *Food Chem.* **2015**, *170*, 226-233.

4. Del Rio, D.; Rodriguez-Mateos, A.; Spencer, J. P. E.; Tognolini, M.; Borges, G.; Crozier, A. Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxid. Redox Signaling* **2013**, *18*, 1818-1892.

5. Gómez-Alonso, S.; Collins, V. J.; Vauzour, D.; Rodríguez-Mateos, A.; Corona, G.; Spencer, J. P. E. Inhibition of colon adenocarcinoma cell proliferation by flavonols is linked to a G2/M cell cycle block and reduction in cyclin D1 expression. *Food Chem.* **2012**, *130*, 493-500.

6. Gonzalo-Diago, A.; Dizy, M.; Fernández-Zurbano, P. Taste and mouthfeel properties of red wines proanthocyanidins and their relation to the chemical composition. *J. Agric. Food Chem.* **2013**, *61*, 8861-8870.

7. Gonzalo-Diago, A.; Dizy, M.; Fernández-Zurbano, P. Contribution of low molecular weight phenols to bitter taste and mouthfeel properties in red wines. *Food Chem.* **2014**, *154*, 187-198.

8. Pérez-Magariño, S.; González-San José, M. L. Evolution of flavanols, anthocyanins, and their derivatives during the aging of red wines elaborated from grapes

532 harvested at different stages of ripening. *J. Agric. Food Chem.* **2004***, 52*, 1181-

533   1189.

534 9. Portu, J.; López-Giral, N.; López, R.; González-Arenzana, L.; González-Ferrero, C.;

535   López-Alfaro, I.; Santamaría, P.; Garde-Cerdán, T. Different tools to enhance grape

536   and wine anthocyanin content. In *Handbook of Anthocyanins-Food Sources,*

537   *Chemical Applications and Health Benefits*; Warner, L. M., Ed.; Nova Science

538   Publishers, Inc.: Nueva York, NY, 2015; pp. 51-88.

539 10. Balint, G.; Reynolds, A. G. Impact of exogenous abscisic acid on vine physiology

540   and grape composition of Cabernet Sauvignon. *Am. J. Enol. Vitic.* **2013***, 64*, 74-87.

541 11. Berli, F. J.; Fanzone, M.; Piccoli, P.; Bottini, R. Solar UV-B and ABA are involved

542   in phenol metabolism of *Vitis vinifera* L. Increasing biosynthesis of berry skin

543   polyphenols. *J. Agric. Food Chem.* **2011***, 59*, 4874-4884.

544 12. Pardo-García, A. I.; Martínez-Gil, A. M.; Cadahía, E.; Pardo, F.; Alonso, G. L.;

545   Salinas, M. R. Oak extract application to grapevines as a plant biostimulant to

546   increase wine polyphenols. *Food Res. Int.* **2014**, *55*, 150-160.

547 13. Ruiz-García, Y.; Gómez-Plaza, E. Elicitors: a tool for improving fruit phenolic

548   content. *Agriculture* **2013***, 3*, 33-52.

549 14. Delaunois, B.; Farace, G.; Jeandet, P.; Clément, C.; Baillieul, F.; Dorey, S.;

550   Cordelier, S. Elicitors as alternative strategy to pesticides in grapevine? Current

551   knowledge on their mode of action from controlled conditions to vineyard.

552   *Environ. Sci. Pollut. Res.* **2014***, 21*, 4837-4846.

553 15. Rohwer, C. L.; Erwin, J. E. Horticultural applications of jasmonates: A review. *J.*

554   *Hortic. Sci. Biotechnol.* **2008***, 83*, 283-304.

555   16. Wang, S. Y.; Bowman, L.; Ding, M. Methyl jasmonate enhances antioxidant

556   activity and flavonoid content in blackberries (*Rubus* sp.) and promotes

557   antiprofileration of human cancer cells. *Food Chem.* **2008**, *107*, 1261-1269.

558   17. Wang, S. Y.; Zheng, W. Preharvest application of methyl jasmonate increases fruit

559   quality and antioxidant capacity in raspberries. *Int. J. Food Sci. Technol.* **2005**, *40*,

560   187-195.

561   18. Shafiq, M.; Singh, Z.; Khan, A. S. Time of methyl jasmonate application influences

562   the development of 'Cripps Pink' apple fruit colour. *J. Sci. Food Agric.* **2013**, *93*,

563   611-618.

564   19. Belhadj, A.; Saigne, C.; Telef, N.; Cluzet, S.; Bouscaut, J.; Corio-Costet, M.-F.;

565   Mérillon, J.-M. Methyl jasmonate induces defense responses in grapevine and

566   triggers protection against *Erysiphe necator*. *J. Agric. Food Chem.* **2006**, *54*, 9119-

567   9125.

568   20. Belhadj, A.; Telef, N.; Saigne, C.; Cluzet, S.; Barrieu, F.; Hamdi, S.; Mérillon, J.-M.

569   Effect of methyl jasmonate in combination with carbohydrates on gene expression

570   of PR proteins, stilbene and anthocyanin accumulation in grapevine cell cultures.

571   *Plant Physiol. Biochem.* **2008**, *46*, 493-499.

572   21. Fernández-Marín, M. I.; Puertas, B.; Guerrero, R. F.; García-Parrilla, M. C.; Cantos-

573   Villar, E. Preharvest methyl jasmonate and postharvest UVC treatments: increasing

574   stilbenes in wine. *J. Food Sci.* **2014**, *79*, C310-C317.

575   22. Ruiz-García, Y.; Romero-Cascales, I.; Gil-Muñoz, R.; Fernández-Fernández, J. I.;

576   López-Roca, J. M.; Gómez-Plaza, E. Improving grape phenolic content and wine

577   chromatic characteristics through the use of two different elicitors: methyl

578   jasmonate versus benzothiadiazole. *J. Agric. Food Chem.* **2012**, *60*, 1283-1290.

579   23. Vezzulli, S.; Civardi, S.; Ferrari, F.; Bavaresco, L. Methyl jasmonate treatment as a

580   trigger of resveratrol synthesis in cultivated grapevine. *Am. J. Enol. Vitic.* **2007***, 58*,

581   530-533.

582   24. Sampaio, T. L.; Kennedy, J. A.; Vasconcelos, M. C. Use of microscale

583   fermentations in grape and wine research. *Am. J. Enol. Vitic.* **2007***, 58*, 534-539.

584   25. ECC. Commission Regulation Vo 2676/90 concerning the establishment of common

585   analytical methods in the sector of wine. *Off. J. Eur. Communities* **1990***, L272(3)*,

586   1-192.

587   26. Lipka, Z.; Tanner, H. Une nouvelle méthode de dosage du acide tartarique dans les

588   moûts, les vins et autres boissons (selon Rebelein). *Rev. Suisse Vitic. Arboric.*

589   *Hortic.* **1974***, 6*, 5-10.

590   27. Ribéreau-Gayon, P.; Stonestreet, E. Determination of anthocyanins in red wine.

591   *Bull. Soc. Chim. Fr.* **1965***, 9*, 2649-2652.

592   28. Ribéreau-Gayon, J.; Peynaud, E.; Ribéreau-Gayon, P.; Sudraud, P. *Sciences et*

593   *Techniques du Vin*; Dunod: Paris, France, 1976.

594   29. Glories, Y. *Recherches sur la Matière Colorante des Vins Rouges*. Ph.D. Thesis,

595   Université de Bordeaux II, France, 1978.

596   30. Ruiz, M. *La Crianza del Vino Tinto desde la Perspectiva Vitícola;* AMV Ediciones:

597   Madrid, Spain, 1999.

598   31. Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to

599   evaluate antioxidant activity. *LWT-Food Sci. Technol.* **1995***, 28*, 25-30.

600   32. Nixdorf, S. L.; Hermosín-Gutiérrez, I. Brazilian red wines made from the hybrid

601   grape cultivar Isabel: phenolic composition and antioxidant capacity. *Anal. Chim.*

602   *Acta* **2010***, 659*, 208-215.

603   33. Castillo-Muñoz, N.; Gómez-Alonso, S.; García-Romero, E.; Hermosín-Gutiérrez, I.

604   Flavonol profiles of *Vitis vinifera* red grapes and their single-cultivar wines. *J.*

605   *Agric. Food Chem.* **2007***, 55*, 992-1002.

606   34. Castillo-Muñoz, N.; Fernández-González, M.; Gómez-Alonso, S.; García-Romero,

607   E.; Hermosín-Gutiérrez, I. Red-color related phenolic composition of Garnacha

608   Tintorera (*Vitis vinifera* L.) grapes and red wines. *J. Agric. Food Chem.* **2009***, 57*,

609   7883-7891.

610   35. Lago-Vanzela, E. S.; Da-Silva, R.; Gomes, E.; García-Romero, E.; Hermosín-

611   Gutiérrez, I. Phenolic composition of the edible parts (flesh and skin) of Bordô

612   grape (*Vitis labrusca*) using HPLC-DAD-ESI-MS/MS. *J. Agric. Food Chem.* **2011***,*

613   *59*, 13136-13146.

614   36. López-Alfaro, I.; González-Arenzana, L.; López, N.; Santamaría, P.; López, R.;

615   Garde-Cerdán, T. Pulsed electric field treatment enhanced stilbene content in

616   Graciano, Tempranillo and Grenache grape varieties. *Food Chem.* **2013***, 141*, 3759-

617   3765.

618   37. Ruiz-García, Y.; Romero-Cascales, I.; Bautista-Ortín, A. B.; Gil-Muñoz, R.;

619   Martínez-Cutillas, A.; Gómez-Plaza, E. Increasing bioactive phenolic compounds

620   in grapes: response of six Monastrell grape clones to benzothiadiazole and methyl

621   jasmonate treatments. *Am. J. Enol. Vitic.* **2013***, 64*, 459-465.

622   38. Avizcuri-Inac, J.-M.; Gonzalo-Diago, A.; Sanz-Asensio, J.; Martínez-Soria, M.-T.;

623   López-Alonso, M.; Dizy-Soto, M.; Echávarri-Granado, J.-F.; Vaquero-Fernández,

624   L.; Fernández-Zurbano, P. Effect of cluster thinning and prohexadione calcium

625   applications on phenolic composition and sensory properties of red wines. *J. Agric.*

626   *Food Chem.* **2013***, 61*, 1124-1137.

627 39. Ruiz-García, Y.; Gil-Muñoz, R.; López-Roca, J. M.; Martínez-Cutillas, A.; Romero-
628   Cascales, I.; Gómez-Plaza, E. Increasing the phenolic compound content of grapes
629   by preharvest application of abcisic acid and a combination of methyl jasmonate
630   and benzothiadiazole. *J. Agric. Food Chem.* **2013**, *61*, 3978-3983.

631 40. Gómez-Alonso, S.; García-Romero, E.; Hermosín-Gutiérrez, I. HPLC analysis of
632   diverse grape and wine phenolics using direct injection and multidetection by DAD
633   and fluorescence. *J. Food Compos. Anal.* **2007**, *20*, 618-626.

634 41. Diago, M. P.; Ayestarán, B.; Guadalupe, Z.; Poni, S.; Tardáguila, J. Impact of
635   prebloom and fruit set basal leaf removal on the flavonol and anthocyanin
636   composition of Tempranillo grapes. *Am. J. Enol. Vitic.* **2012**, 63, 367-376.

637 42. Bakker, J.; Timberlake, C. F. Isolation, identification, and characterization of new
638   color-stable anthocyanins occurring in some red wines. *J. Agric. Food Chem.* **1997**,
639   *45*, 35-43.

640 43. Hermosín-Gutiérrez, I.; Castillo-Muñoz, N.; Gómez-Alonso, S.; García-Romero, E.
641   Flavonol profiles for grape and wine authentication. In *Progress in Authentication*
642   *of Food and Wine*; Ebeler, S. E., Takeoka, G. R., Winterhalter, P., Eds.; ACS
643   Symposium Series; American Chemical Society: Washington, DC, 2011; pp. 113-
644   129.

645 44. Schwarz, M.; Picazo-Bacete, J. J.; Winterhalter, P.; Hermosín-Gutiérrez, I. Effect of
646   copigments and grape cultivar on the color of red wines fermented after the
647   addition of copigments. *J. Agric. Food Chem.* **2005**, *53*, 8372-8381.

648 45. Rentzsch, M.; Wilkens, A.; Winterhalter, P. Non-flavonoid phenolic compounds. In
649   *Wine Chemistry and Biochemistry*; Moreno-Arribas, M. V., Polo, M. C., Eds.;
650   Springer: New York, NY, 2009; pp. 509-527.

651 46. Bavaresco, L.; Mattivi, F.; de Rosso, M.; Flamini, R. Effects of elicitors, viticultural

652   factors, and enological practices on resveratrol and stilbenes in grapevine and wine.

653   *Mini-Rev. Med. Chem.* **2012***, 12*, 1366-1381.

654 47. Schopp, L. M.; Lee, J.; Osborne, J. P.; Chescheir, S. C.; Edwards, C. G. Metabolism

655   of nonesterified and esterified hydroxycinnamic acids in red wines by

656   *Brettanomyces bruxellensis*. *J. Agric. Food Chem.* **2013***, 61*, 11610-11617.

657 48. Garde-Cerdán, T.; Lorenzo, C.; Carot, J. M.; Jabaloyes, J. M.; Esteve, M. D.;

658   Salinas, M. R. Statistical differentiation of wines of different geographic origin and

659   aged in barrel according to some volatile components and ethylphenols. *Food*

660   *Chem.* **2008**, *111*, 1025-1031.

661 49. Rentzsch, M.; Schwarz, M.; Winterhalter, P.; Hermosín-Gutiérrez, I. Formation of

662   hydroxyphenyl-pyranoanthocyanins in Grenache wines: precursor levels and

663   evolution during aging. *J. Agric. Food Chem.* **2007***, 55*, 4883-4888.

664 50. Guerrero, R. F.; García-Parrilla, M. F.; Puertas, B.; Cantos-Villar, E. Wine

665   resveratrol and health: a review. *Nat. Prod. Commun.* **2009***, 4*, 635-658.

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Table 1. Oenological Parameters of Grape Berries from Control Grapevines and from Grapevines Treated with Methyl Jasmonate (MeJ)[a]

	control	MeJ
weight of 100 berries (g)	232 ± 25 a	225 ± 5 a
ºBrix	23.2 ± 0.8 a	22.3 ± 0.3 a
pH	3.30 ± 0.02 a	3.33 ± 0.04 a
total acidity (g/L)[b]	8.42 ± 0.19 a	8.53 ± 0.40 a
tartaric acid (g/L)	6.16 ± 0.11 a	6.23 ± 0.03 a
malic acid (g/L)	4.26 ± 0.36 a	4.40 ± 0.28 a
potassium (mg/L)	1635 ± 110 a	1583 ± 58 a

[a]Since the treatments were performed in triplicate, all parameters are listed with their standard deviation (n = 3). For each parameter, values with the same letters are not significantly different between the samples ($p \leq 0.05$). [b]As g/L tartaric acid.

Table 2. Oenological Parameters and Total Antioxidant Activity of Control Wine and Wine Made from Grapevines Treated with Methyl Jasmonate (MeJ)[a]

	control	MeJ
alcoholic degree (% v/v)	13.4 ± 0.6 a	12.7 ± 0.0 a
pH	3.69 ± 0.08 a	3.63 ± 0.08 a
total acidity (g/L)[b]	7.50 ± 0.04 a	7.45 ± 0.29 a
tartaric acid (g/L)	1.95 ± 0.13 a	1.98 ± 0.01 a
malic acid (g/L)	3.82 ± 0.15 a	3.95 ± 0.23 a
lactic acid (g/L)	0.05 ± 0.03 a	0.09 ± 0.00 a
volatile acidity (g/L)[c]	0.26 ± 0.09 a	0.18 ± 0.04 a
hue	0.50 ± 0.03 a	0.44 ± 0.01 a
color intensity (CI)	11.54 ± 0.46 a	15.66 ± 0.42 b
Folin-Ciocalteu index	43.3 ± 2.3 a	50.7 ± 0.6 b
total polyphenol index (TPI)	51.86 ± 3.20 a	59.71 ± 2.50 a
total anthocyanins (mg/L)	992 ± 77 a	1122 ± 82 a
ionised anthocyanins (mg/L)	216 ± 15 a	306 ± 17 b
total tannins (mg/mL)	2.50 ± 0.28 a	3.57 ± 0.64 a
polymerization index	1.33 ± 0.09 a	1.62 ± 0.10 b
total antioxidant activity (mmol TE/L)[d]	5.60 ± 0.34 a	5.89 ± 0.19 a

[a]Since the treatments were performed in triplicate, all parameters are listed with their standard deviation (n = 3). For each parameter, values with different letters are significantly different between the samples ($p \leq 0.05$). [b]As g/L tartaric acid. [c]As g/L acetic acid. [d]As mmol of Trolox equivalents per liter of wine.

Table 3. Individual Anthocyanin Content in Tempranillo Grape Berries (mg/kg) and Wines (mg/L) from Control Grapevines and from Grapevines Treated with Methyl Jasmonate (MeJ)[a]

	grape berries		wines	
	control	MeJ	control	MeJ
delphinidin-3-glc	256.94 ± 27.77 a	324.98 ± 20.85 a	57.80 ± 8.67 a	87.21 ± 5.44 b
cyanidin-3-glc	84.28±26.65 a	142.75 ± 15.07 a	7.03 ± 2.34 a	12.66 ± 1.20 a
petunidin-3-glc	164.80 ± 8.63 a	197.27 ± 1.24 b	63.22 ± 8.01 a	83.19 ± 0.09 b
peonidin-3-glc	115.37 ± 23.96 a	180.74 ± 1.32 b	25.11 ± 6.37 a	41.77 ± 0.73 b
malvidin-3-glc	342.44 ± 38.68 a	364.48 ± 39.13 a	210.18 ± 19.92 a	228.15 ± 14.81 a
delphinidin-3-acglc	4.49 ± 0.10 a	4.68 ± 0.58 a	5.37 ± 0.70 a	6.31 ± 0.00 a
cyanidin-3-acglc	0.83 ± 0.17 a	1.19 ± 0.06 a	0.23 ± 0.01 a	0.21 ± 0.00 a
petunidin-3-acglc	2.85 ± 0.26 a	2.94 ± 0.52 a	2.86 ± 0.24 a	3.19 ± 0.11 a
peonidin-3-acglc	0.20 ± 0.06 a	0.16 ± 0.06 a	0.79 ± 0.15 a	1.10 ± 0.04 a
malvidin-3-acglc	5.82 ± 1.56 a	5.12 ± 1.18 a	6.72 ± 1.38 a	6.39 ± 0.54 a
delphinidin-3-cmglc	24.37 ± 2.11 a	26.36 ± 1.92 a	6.90 ± 1.38 a	9.02 ± 1.05 a
cyanidin-3-cmglc	7.99 ± 1.27 a	11.70 ± 0.47 b	2.34 ± 0.38 a	4.16 ± 0.40 b
petunidin-3-cmglc	15.49 ± 2.82 a	15.67 ± 1.78 a	5.54 ± 1.20 a	6.65 ± 0.28 a
peonidin-3-cmglc	11.85 ± 1.51 a	15.88 ± 0.28 b	4.66 ± 0.84 a	7.41 ± 0.21 b
malvidin-3-*cis*-cmglc	0.97 ± 0.51 a	0.79 ± 0.13 a	0.46 ± 0.15 a	0.41 ± 0.01 a
malvidin-3-*trans*-cmglc	42.64 ± 14.34 a	37.95 ± 6.41 a	21.08 ± 6.82 a	21.47 ± 1.15 a
malvidin-3-cfglc	0.48 ± 0.16 a	0.31 ± 0.15 a	0.20 ± 0.07 a	0.23 ± 0.06 a
total anthocyanins	1082 ± 34 a	1333 ± 15 b	420 ± 40 a	520 ± 7 b
vitisin A	-	-	1.57 ± 0.20 a	1.59 ± 0.28 a
vitisin B	-	-	1.47 ± 0.09 a	1.68 ± 0.02 b

[a]Nomenclature abbreviations: glc, glucoside; acglc, acetylglucoside; cmglc, *trans-p-*coumaroylglucoside; cfglc, caffeoylglucoside. Since the treatments were performed in triplicate, all parameters are listed with their standard deviation (n = 3). For each parameter, values with different letters are significantly different between the samples ($p \leq 0.05$).

Table 4. Individual Flavonol Content in Tempranillo Grape Berries (mg/kg) and Wines (mg/L) from Control Grapevines and from Grapevines Treated with Methyl Jasmonate (MeJ)[a]

	grape berries		wines	
	control	MeJ	control	MeJ
myricetin-3-glcU	1.59 ± 0.33 a	2.20 ± 0.77 a	1.54 ± 0.16 a	1.91 ± 0.05 a
myricetin-3-gal	0.72 ± 0.43 a	0.87 ± 0.11 a	-	-
myricetin-3-glc	18.76 ± 2.68 a	22.63 ± 0.01 a	13.38 ± 2.28 a	17.90 ± 0.04 a
quercetin-3-gal	0.57 ± 0.29 a	1.01 ± 0.46 a	-	-
quercetin-3-glcU	4.60 ± 1.30 a	6.43 ± 2.76 a	2.45 ± 0.46 a	3.63 ± 0.81 a
quercetin-3-glc	6.20 ± 1.31 a	11.13 ± 3.03 a	3.00 ± 0.31 a	6.55 ± 1.17 b
quercetin-3-rut	0.39 ± 0.13 a	0.52 ± 0.33 a	-	-
laricitrin-3-glc	2.03 ± 0.63 a	2.30 ± 0.15 a	1.09 ± 0.11 a	1.23 ± 0.12 a
kaempferol-3-gal	0.18 ± 0.06 a	0.34 ± 0.17 a	-	-
kaempferol-3-glcU	0.16 ± 0.04 a	0.20 ± 0.01 a	-	-
kaempferol-3-glc	0.77 ± 0.40 a	1.61 ± 0.80 a	0.03 ± 0.03 a	0.16 ± 0.02 a
isorhamnetin-3-gal	0.11 ± 0.03 a	0.13 ± 0.02 a	-	-
isorhamnetin-3-glc	0.40 ± 0.10 a	0.80 ± 0.18 b	0.22 ± 0.04 a	0.39 ± 0.01 b
syringetin-3-glc	0.71 ± 0.23 a	0.89 ± 0.00 a	0.81 ± 0.13 a	0.92 ± 0.14 a
free-myricetin	-	-	4.77 ± 0.45 a	5.91 ± 0.00 b
free-quercetin	-	-	2.52 ± 0.21 a	2.86 ± 0.41 a
free-laricitrin	-	-	0.10 ± 0.01 a	0.10 ± 0.01 a
free-kaempferol	-	-	0.33 ± 0.04 a	0.48 ± 0.08 a
free-isorhamnetin	-	-	0.19 ± 0.01 a	$0.19 \pm .001$ a
free-syringetin	-	-	0.11 ± 0.03 a	0.20 ± 0.05 a
total flavonols	37.19 ± 7.34 a	51.06 ± 8.26 a	30.54 ± 2.59 a	42.42 ± 0.11 b

[a]Nomenclature abbreviations: glcU, glucuronide; gal, galactoside; glc, glucoside; rut, rutinoside. Since the treatments were performed in triplicate, all parameters are listed with their standard deviation (n = 3). For each parameter, values with different letters are significantly different between the samples ($p \leq 0.05$).

Table 5. Individual Flavanol Content in Tempranillo Grape Berries (mg/kg) and Wines (mg/L) from Control Grapevines and from Grapevines Treated with Methyl Jasmonate (MeJ)[a]

	grape berries		wines	
	control	MeJ	control	MeJ
procyanidin B1	13.95 ± 2.95 a	15.49 ± 0.25 a	13.16 ± 1.93 a	17.84 ± 0.78 a
epigallocatechin	1.39 ± 0.49 a	1.06 ± 0.15 a	12.16 ± 0.97 a	12.51 ± 1.66 a
catechin	65.51 ± 2.40 a	66.43 ± 14.40 a	43.90 ± 3.00 a	46.23 ± 4.60 a
procyanidin B2	17.58 ± 2.57 a	16.47 ± 1.58 a	6.60 ± 1.47 a	6.86 ± 0.40 a
epicatechin	34.75 ± 2.69 a	34.51 ± 3.88 a	18.60 ± 2.21 a	19.69 ± 0.12 a
epicatechin-3-gallate	9.61 ± 0.97 a	10.52 ± 0.69 a	0.38 ± 0.23 a	0.72 ± 0.33 a
total flavanols	142.78 ± 8.39 a	144.48 ± 20.97 a	94.81 ± 6.42 a	103.84 ± 2.76 a

[a]Since the treatments were performed in triplicate, all parameters are listed with their standard deviation (n = 3). For each parameter, values with the same letter are not significantly different between the samples ($p \leq 0.05$).

Table 6. non-Flavonoid Compounds in Tempranillo Grape Berries (mg/kg) and Wines (mg/L) from Control Grapevines and from Grapevines Treated with Methyl Jasmonate (MeJ)[a]

	grape berries		wines	
	control	MeJ	control	MeJ
hydroxybenzoic acids				
gallic acid	0.41 ± 0.17 a	0.38 ± 0.05 a	17.48 ± 2.05 a	19.88 ± 1.03 a
protocatechuic acid	-	-	3.60 ± 0.55 a	4.33 ± 0.01 a
hydroxycinnamic acids				
trans-caftaric acid	8.95 ± 1.35 a	9.69 ± 2.08 a	47.57 ± 2.83 a	48.10 ± 6.33 a
trans+*cis*-coutaric acids	9.04 ± 1.35 a	9.01 ± 1.36 a	46.82 ± 5.15 a	51.16 ± 6.81 a
trans-fertaric acid	0.68 ± 0.01 a	0.67 ± 0.00 a	1.94 ± 0.18 a	2.16 ± 0.09 a
cis-fertaric acid	0.21 ± 0.01 a	0.25 ± 0.00 a	-	-
caffeic acid	-	-	6.83 ± 1.21 a	9.21 ± 0.58 a
p-coumaric acid	-	-	2.07 ± 0.31 a	2.55 ± 0.19 a
ferulic acid	-	-	0.93 ± 0.22 a	1.27 ± 0.04 a
total	18.89 ± 2.67 a	19.62 ± 3.44 a	106.15 ± 7.64 a	114.45 ± 12.63 a
stilbenes				
trans-piceid	0.65 ± 0.17 a	3.65 ± 0.58 b	1.74 ± 0.31 a	5.39 ± 1.44 b
cis-piceid	0.39 ± 0.10 a	1.30 ± 0.06 b	0.34 ± 0.15 a	0.87 ± 0.59 a
trans-resveratrol	0.13 ± 0.05 a	0.13 ± 0.01 a	0.63 ± 0.14 a	1.31 ± 0.54 a
cis-resveratrol	0.10 ± 0.01 a	0.09 ± 0.00 a	0.18 ± 0.02 a	0.26 ± 0.08 a
total	1.26 ± 0.33 a	5.17 ± 0.61 b	2.98 ± 0.54 a	7.83 ± 2.65 b

[a]Since the treatments were performed in triplicate, all parameters are listed with their standard deviation (n = 3). For each parameter, values with different letters are significantly different between the samples ($p \leq 0.05$).

GRAPHIC FOR TABLE OF CONTENTS