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Improving phenolic and chromatic characteristics of Monastrell, Merlot and Syrah wines by using methyl jasmonate and benzothiadiazole

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Abstract

Aims: Phenolic compounds, including anthocyanins, flavonols and tannins, are considered very important from a health and technological point of view. While the concentration of these compounds in grapes depends on many factors, elicitors may be regarded as a new strategy for increasing their content in grapes and, consequently, in wines.

Methods and results: Berries of three grape varieties (Monastrell, Merlot and Syrah) were sprayed with two elicitors, BTH (benzothiadiazole) and MeJ (methyl jasmonate), during preharvest, and the phenolic characteristics (anthocyanins, flavonols and tannins) were studied in the grapes at harvest and in the corresponding wines at the end of alcoholic fermentation.

Conclusion: The results for both grapes and wines depended on the variety and the elicitor used.

Significance and impact of the study: These results can be useful for enhancing the phenolic composition in grapes and wines and for improving their quality, although the variety and the elicitor used are important factors to keep in mind.

Key words: Merlot, Syrah, Monastrell, wine, grape, BTH, MeJ, phenolic compounds.

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Introduction

Grapes are qualitatively and quantitatively rich in phenolic compounds, which are secondary metabolites widely distributed in the plant kingdom. These phenolic compounds include anthocyanins, flavonols and tannins, and are considered very important from a health point of view. Phenolics have received much attention in recent years because, in the human diet, they have been positively related to total antioxidant activity. Numerous epidemiological studies have shown that the long-term moderate consumption of wine is linked to a lower level of cardiovascular illness (Fernández-Marín *et al.*, 2014). Other researchers have suggested they show cancer chemopreventive activity and have beneficial effects against other illnesses such as Alzheimer's disease and urinary bladder dysfunction (Pezzuto, 2008).

The concentration of these compounds in grapes depends on many factors, including the variety, growing conditions, climate, harvest year and winemaking techniques (Bavaresco *et al.*, 2012). The use of elicitors as a strategy to improve the phenolic content of fruits, particularly grapes, stems from the results obtained using these compounds as agrochemicals to improve resistance against plant pathogens.

Although elicitation was first used as an alternative to conventional agrochemicals against pests (Buonaurio *et al.*, 2009), elicitors stimulate any type of plant defence. While they do not exert direct antimicrobial activity against phytopathogens, they are able to boost the plant innate immune systems, triggering a complex defence machinery known as systemic acquired resistance (SAR); Iriti and Faoro, 2007). These activators have also been shown to increase the level of polyphenols, and, in recent years, the phenolic content of grapes has been improved by means of pre and postharvest plant growth regulator treatments (Khan and Singh, 2007; Lara, 2013). Such enhancement has been attained using various compounds, including methyl jasmonate (MeJ) and benzothiadiazole (BTH). MeJ, a phytohormone derived from jasmonic acid, is a natural volatile compound commonly found in plants, where it plays an important role in suppressing several important diseases in fruits (Santos-Buelga and Scalbert, 2000; Tzortzakis and Economakis, 2007). It is also able to activate the enzymes responsible for the biosynthesis of polyphenols, such as PAL (phenylalanine ammonia lyase). For its part, BTH is a functional analogue of the plant endogenous hormone-like compound salicylic acid, which, in untreated plants, is required for the induction of defence genes, leading to broad spectrum, long lasting SAR (Iriti *et al.*, 2005).

There are many studies on the effect of preharvest application of MeJ and BTH to wine grapes. Such studies have pointed to increased levels of phenolic compounds in the treated grapes and corresponding wines for many varieties including Merlot (Iriti *et al.*, 2004), Monastrell (Ruiz-García *et al.*, 2012; Ruiz-García *et al.*, 2013a; Ruiz-García *et al.*, 2013b), Tempranillo (Portu *et al.*, 2015; Portu *et al.*, 2016), Groppello (Vitalini *et al.*, 2014) and Syrah (Fernández-Marín *et al.*, 2014). However, few researchers have studied the influence of preharvest treatments on several grape and wine varieties at the same time. Hence, the aim of this study was to increase the phenolic composition of wines made from three varieties by using two elicitors applied preharvest (BTH and MeJ) and to ascertain how the grape variety is a factor to be considered when grapes are treated with elicitors.

Materials and Methods

1. Plant material and open field treatments

The experiment was carried out in 2012 on three varieties of *Vitis vinifera* (Monastrell, Syrah and Merlot) grafted onto 1103 Paulsen and planted in 2002 in Bullas (Murcia, south-east Spain). The experimental plot consisted of vines trained to a vertically shoot positioned (VSP) spur pruned cordon, with six 2-bud spurs (12 nodes). The distance between rows was 3 m with 1.25 m between vines. The rows were oriented in an N-NW to S-SE direction. Plants were drip irrigated.

All treatments were applied to three replicates. A completely randomized experimental design was set up consisting of three replicates of 10 vines for each treatment. The field trial involved the application of two elicitors: BTH ([benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester], Sigma Aldrich, St. Louis, USA) at a concentration of 0.3 mM; and MeJ (methyl jasmonate; Sigma Aldrich, St. Louis, USA) at a concentration of 10 mM. The protocol used to apply the different treatments was described previously (Gómez-Plaza *et al.*, 2017). The concentrations were chosen according to the literature (Iriti *et al.*, 2004; Vezzulli *et al.*, 2007). Samples were harvested at optimum maturity. For analysis, five mature clusters per plant were randomly collected at harvest from treated and untreated grapevines of each treatment and replicate and immediately transported to the laboratory and frozen at -20 °C until analysis.

2. Vinifications

All the vinifications were made in triplicate in 50-L stainless steel tanks using 45 kg of grapes. Before alcoholic fermentation (AF) started, total acidity was corrected to 5.5 g/L and selected yeasts were added (10 g of dry yeast/100 kg of grapes, Laffort, DSM, Servian, France). The fermentative pomace contact period was 10 days. All the vinifications were conducted at 25±1 °C. Throughout the fermentation pomace contact period, the cap was punched down twice a day and the temperature and must density were recorded. At the end of this period, the wines were pressed at 1.5 bar in a 75-L tank membrane press. Free-run and press wines were combined and stored at room temperature. Wine oenological parameters were analyzed in triplicate at the end of AF.

3. Physicochemical determination in grapes at harvest

Grape analysis involved the traditional flesh measurements. Total soluble solids (°Brix) were measured using a digital refractometer (Atago RX-5000). Titratable acidity was measured using an automatic titrator (Metrohm, Herisau, Switzerland) with 0.1 N NaOH. The methodology used to carry out these analyses is described in OIV (2014).

4. Analysis of anthocyanins and flavonols in grapes and wines

Grapes were peeled with a scalpel, and the skins were stored at -20 °C until analysis. Samples (2 g) were immersed in methanol (40 mL) in hermetically closed tubes and placed on a stirring plate at 150 rpm and 25 °C. After 2 h, the methanolic extracts were filtered (0.45 µm) and analyzed by high-performance liquid chromatography (HPLC). Samples of wines were filtered through the 0.45-µm nylon filters. The samples were directly analyzed by HPLC according to Bautista-Ortín *et al.* (2005).

Waters Empower Pro software was used to process the data obtained. Compounds were identified by comparison of the UV spectra recorded with the diode array detector and those reported in the literature. In addition, HPLC–MS analysis was made to confirm the identity of each peak. An LC-MSD-Trap VL-01036 liquid chromatograph-ion trap mass detector (Agilent Technologies, Santa Clara, CA) equipped with electrospray ionization (ESI) was used. Elution was performed in the HPLC analysis conditions described by Bautista-Ortín *et al.* (2005). The heated capillary and voltage were maintained at 350 °C and 4 kV, respectively. Mass scans were measured from 100 to 800 *m/z*. Mass spectrometry data were acquired in the negative ionization mode and processed using Data Analysis 2.1 LC/MSD Trap software (Agilent). For quantification, DAD chromatograms were extracted at 520 nm (anthocyanins) using malvidin 3-*O*-glucoside chloride (Extrasynthèse, Genay, France) as external standard, and at 360 nm (flavonols) using quercetin (Sigma Aldrich) as external standard.

5. Analysis of tannins in grapes and wines

The seeds and skins of 10 berries were separated from the mesocarp and rinsed with distilled deionized water. Seeds and skins were extracted separately in hermetically closed falcon tubes with 10 mL 2:1 acetone/water at room temperature for 24 h on an orbital shaker at 200 rpm. The extraction was carried out in the dark. The extract was concentrated in a CentriVap concentrator (Labconco, United States) to remove acetone and re-dissolved in 2 mL methanol in a volumetric flask. Skin and seed proanthocyanidins were determined and analyzed according to a previously described method (Kennedy and Jones, 2001; Busse-Valverde *et al.*, 2010).

For wines, the samples were prepared by an optimization of the method described by Pastor del Río and Kennedy (2006). Wine (5 mL) was evaporated in a CentriVap concentrator (Labconco, United States), re-dissolved in 3 mL of water, and then passed through a C18-SPE column (1g Waters), previously activated with 10 mL of methanol followed by 10 mL of water. The cartridge was washed with 20 mL of water and the tannins were eluted with 10 mL of methanol, evaporated in a CentriVap and re-dissolved in 1 mL of methanol.

Proanthocyanidin cleavage products were estimated using their response factors relative to (+)-catechin (Sigma Aldrich, Spain), which was used as the quantitative standard. The results of these analyses enabled the total proanthocyanidin content, the apparent mean degree of polymerization (mDP), and the percentage of each constitutive unit to be obtained.

6. Sensory analysis

Wines (elaborated in 2012) were subjected to a triangular sensory test, for which ten members associated with the project were trained over several weeks. For this analysis, three samples were presented, two of which were identical. Samples were presented in random order in coded, clear 125 mL official glasses. Each taster selected the sample

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that he/she considered different (forced election method) and the sample that he/she preferred.

7. Statistical data treatments

Significant differences among grapes and wines and for each variable were assessed by analysis of variance (ANOVA) using Statgraphics 5.0 Plus. The Duncan test was used to separate the means ($p<0.05$) when the ANOVA test was significant.

Results and Discussion

1. Physicochemical analysis of grapes at harvest

The effects of the different treatments on the physicochemical characteristics of the control and treated grapes were determined (Table 1). In general, treated grapes were very similar and the effect of the treatments was very small. Differences in berry weight were only observed in Syrah grapes, which were heavier after both treatments. In the other two varieties, the treatments had no effect on grape size. Previous studies (Ruiz-García *et al.*, 2013b) found that the application of BTH, MeJ, BTH+MeJ and ABA (abscisic acid) to Monastrell grapes had no effect on berry weight.

The BTH-treated grapes from Merlot and the MeJ-treated grapes from Monastrell had higher sugar content than the corresponding control grapes, and higher total acidity values were observed in treated Monastrell grapes and MeJ-treated Syrah grapes when compared to control grapes. By contrast, Villango *et al.* (2015) measured lower acidity in Syrah grapes treated with a product obtained from yeast cell wall than in control grapes.

2. Anthocyanins in grapes and wines

Anthocyanins are natural colorants present in the skin of red grapes, and they play a key role in the organoleptic characteristics of wines (Lesschaeve and Noble, 2005). The biosynthesis of these compounds can be influenced by exogenous elicitors such as ABA, BTH, and BTH/MeJ mixture (Ruiz-García *et al.*, 2012; Ruiz-García *et al.*, 2013a; Ruiz-García *et al.*, 2013b).

The anthocyanin content of grapes and wines for the Syrah, Merlot and Monastrell varieties used in this research is shown in Table 2. The influence of the two treatments studied differed in grapes and wines as a function of the variety, although for all three varieties, anthocyanin concentrations were always higher in grapes than in wines.

For the Syrah variety, the highest concentrations of anthocyanins were found in BTH-treated grapes and in BTH and MeJ wines. In contrast, Portu *et al.* (2015) found that the foliar application of MeJ in Tempranillo induced anthocyanin synthesis in grapevines, increasing the concentration of several individual anthocyanins as well as the total content in both grapes and wines. More specifically, Table 2 also shows the results obtained for the individual anthocyanins in grapes and wines for both treatments applied to Syrah. Compared to the control samples, both treatments increased total 3-monoglucosides in grapes (by up to 27% for BTH and 22% for MeJ) and in wines (by up to 10% for BTH and 8% for MeJ) except cyanidin-3-glucoside in BTH-treated grapes and peonidin-3-glucoside in MeJ wines. In the case of acetylated compounds, both BTH and MeJ increased the petunidin-3-acetyl-glucoside content in grapes, while only MeJ increased the cyanidin-3-acetyl-glucoside content, and in wines, only BTH increased the concentration of cyanidin-3-acetyl-glucoside and peonidin-3-acetyl-glucoside. As regards the total content of acetylated compounds, an increase of 22% was observed in grapes when plants were sprayed with MeJ. Coumarates (cyanidin-3-coumaryl-glucoside and peonidin-3-coumaryl-glucoside) only increased in wines following BTH treatment but the total coumarate content remained unaltered.

Table 1 - Physicochemical parameters of grape berries at harvest.

	Merlot			Monastrell			Syrah		
	Control	BTH[1]	MeJ	Control	BTH	MeJ	Control	BTH	MeJ
Weight of 100 berries	116.7 a[2]	107.8 a	115.9 a	151.9 a	139.7 a	164.1 a	148.9 a	163.1 b	157.2 b
°Brix	24.9 a	25.5 b	24.3 a	19.3 a	19.6 ab	20.8 b	24.3 b	24.1 b	23.3 a
Total acidity (g/L)	3.7 a	3.5 a	4.1 a	3.9 a	4.5 b	4.5 b	4.4 a	4.5 a	5.5 b
Skin/pulp (g)	0.14 a	0.15 a	0.15 a	0.12 a	0.13 a	0.13 a	0.11 ab	0.13 b	0.09 a

[1]BTH: benzothiadiazole; MeJ: methyl jasmonate
[2]Different letters in the same row indicate significant differences according to Duncan test ($p<0.05$)

Table 2 - Anthocyanins in Syrah, Merlot and Monastrell grapes (mg/kg) and wines (mg/L).

	SYRAH					
	Grapes			Wines		
	Control	BTH	MeJ	Control	BTH	MeJ
Df-3-gl[1]	42.3 a[3]	67.0 b	76.2 c	26.8 a	44.3 c	35.1 b
Cy-3-gl	17.5 a	16.7 a	23.7 b	1.5 a	2.4 b	3.0 c
Pet-3-gl	117.5 a	144.5 b	156.1 c	50.4 a	55.8 b	55.4 b
Peo-3-gl	55.0 a	83.0 b	86.5 b	32.7 a	40.0 c	30.9 a
Mv-3-gl	604.5 a	754.9 c	683.7 b	448.2 a	475.9 b	478.2 b
Df-3-ac-gl	20.2 a	29.0 b	29.9 b	7.6 a	11.7 c	8.5 b
Cy-3-ac-gl	3.3 a	3.7 a	4.3 b	10.9 a	15.5 b	12.7 a
Pet-3-ac-gl	29.1 a	41.0 b	41.1 b	1.4 b	1.0 a	1.2 ab
Peo-3-ac-gl	60.3 a	61.2 a	60.1 a	15.5 a	21.1 b	18.2 ab
Mv-3-ac-gl	469.4 b	473.9 b	393.9 a	181.6 b	161.5 a	168.0 a
Mv-3-cum-gl (cis)	18.9 b	18.4 b	15.0 a	5.4 b	4.9 b	3.7 a
Df-3-cum-gl/peo-3-caf	40.5 a	43.2 a	41.2 a	7.4 b	6.5 a	7.1 ab
Mv-3-caf-gl	32.9 b	21.0 a	17.9 a	-	-	-
Cy-3-cum-gl	13.3 a	13.2 a	12.4 a	0.9 a	1.3 b	0.9 a
Pet-3-cum-gl	61.1 a	61.1 a	57.0 a	13.4 a	12.6 a	12.1 a
Peo-3-cum-gl	143.0 b	141.5 b	131.6 a	26.8 a	30.9 b	23.2 a
Mv-3-cum-gl (trans)	641.5 c	571.2 b	473.4 a	77.3 ab	83.8 b	75.4 a
Vitisins	-			4.9 a	6.0 a	5.6 a
Total	2370.4 a	2545.1 b	2304.0 a	913.8 a	975.4 c	939.5 b
	MERLOT					
	Grapes			Wines		
	Control	BTH	MeJ	Control	BTH	MeJ
Df-3-gl	86.0 a[3]	127.0 b	154.8 c	50.9 a	52.1 a	59.1 b
Cy-3-gl	23.0 a	47.2 b	43.7 b	5.7 a	5.2 a	7.6 b
Pet-3-gl	114.7 a	144.0 b	158.6 b	53.6 a	55.1 a	61.2 b
Peo-3-gl	88.2 b	73.8 a	127.1 c	54.4 c	40.7 a	45.6 b
Mv-3-gl	667.5 a	680.6 a	737.8 b	420.1 b	416.4 b	398.3 a
Df-3-ac-gl	31.2 a	39.2 b	52.3 c	8.5 a	9.6 b	11.0 c
Cy-3-ac-gl	6.2 a	7.7 a	13.7 b	7.7 a	9.4 b	11.1 c
Pet-3-ac-gl	40.1 a	48.5 b	62.5 c	0.8 a	1.0 b	0.9 b
Peo-3-ac-gl	44.3 ab	40.1 a	48.2 b	11.8 a	12.4 ab	12.6 b
Mv-3-ac-gl	343.2 ab	355.0 b	328.2 a	114.0 a	128.9 b	111.1 a
Mv-3-cum-gl (cis)	11.6 b	11.0 b	7.2 a	2.9 b	3.0 b	2.1 a
Df-3-cum-gl/peo-3-caf	27.6 a	31.9 b	37.5 c	4.7 a	5.6 b	4.8 a
Mv-3-caf-gl	26.6 a	33.1 b	26.0 a	-	-	-
Cy-3-cum-gl	8.3 a	9.1 a	13.6 b	5.4 a	6.9 b	6.5 b
Pet-3-cum-gl	31.9 a	35.0 b	36.9 b	1.2 a	1.7 b	1.2 a
Peo-3-cum-gl	43.6 b	39.7 a	43.3 b	11.5 b	11.4 b	10.0 a
Mv-3-cum-gl (trans)	229.6 b	244.1 c	193.7 a	51.2 b	67.1 c	47.4 a
Vitisins	-	-	-	5.4 a	5.6 a	6.4 b
Total	1823.8 a	1967.3 b	2085.3 c	809.9 b	832.2 c	797.0 a
	MONASTRELL					
	Grapes			Wines		
	Control	BTH	MeJ	Control	BTH	MeJ
Df-3-gl	81.9 b	58.7 a	103.5 c	14.6 a	20.7 b	44.1 c
Cy-3-gl	18.9 a	37.4 b	84.0 c	1.2 a	2.5 b	7.7 c
Pet-3-gl	135.7 b	103.8 a	142.7 c	31.3 a	38.9 b	66.8 c
Peo-3-gl	65.8 a	78.0 b	96.1 c	10.8 a	13.5 b	29.4 c
Mv-3-gl	503.9 b	441.5 a	636.9 c	242.5 a	258.8 b	326.7 c
Df-3-ac-gl	6.2 b	5.4 a	9.8 c	0.5 a	0.7 b	0.6 ab
Cy-3-ac-gl	2.5 a	4.3 b	5.3 c	0.8 a	0.9 b	1.2 c
Pet-3-ac-gl	9.8 b	8.8 a	12.1 c	1.4 a	1.5 b	2.8 c
Peo-3-ac-gl	4.5 a	6.6 b	6.5 b	11.5 a	12.8 b	13.9 c
Mv-3-ac-gl	37.8 a	42.7 b	44.8 c	4.6 a	4.7 a	6.8 b
Mv-3-cum-gl (cis)	8.6 a	9.7 c	9.1 b	2.3 b	1.5 a	4.5 c
Df-3-cum-gl/peo-3-caf	25.9 a	25.7 a	28.8 b	2.0 b	2.8 c	1.6 a
Mv-3-caf-gl	9.3 a	14.6 c	12.6 b	-	-	-
Cy-3-cum-gl	16.3 a	22.8 b	22.8 b	3.6 c	3.1 b	2.6 a
Pet-3-cum-gl	41.3 ab	40.1 a	43.6 b	6.4 c	5.0 b	4.3 a
Peo-3-cum-gl	25.9 a	30.8 b	31.6 b	4.4 a	7.0 b	7.9 c
Mv-3-cum-gl (trans)	185.8 b	167.7 a	191.8 b	32.3 a	32.4 a	40.3 b
Vitisins	-	-	-	1.9 b		
Total	1180.3 b	1098.7 a	1482.6 c	372.3 a	409.0 b	563.5 c

[1]Abbreviations: Df-3-gl: delphinidin-3-glucoside; Cy-3-gl: cyanidin-3-glucoside; Pet-3-gl: petunidin-3-glucoside; Peo-3-gl: peonidin-3-glucoside; Mv-3-gl: malvidin-3-glucoside; ac: acetyl glucosides; cum: coumaryl glucosides; caf: caffeate glucoside

[2]BTH: benzothiadiazole; MeJ: methyl jasmonate

[3]Different letters in the same row indicate significant differences according to Duncan test ($p<0.05$)

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Table 3 - Flavonols in Syrah, Merlot and Monastrell grapes (mg/kg) and wines (mg/L).

	SYRAH					MERLOT					MONASTRELL				
	Grapes			Wines		Grapes			Wines		Grapes			Wines	
	Control	BTH[2]	MeJ	Control	BTH	MeJ	Control	BTH	MeJ	Control	BTH	MeJ	Control	BTH	MeJ
Mir-3-glu[1]	2.0 b[3]	1.8 a	2.2 c	18.5 a	18.7 a	24.5 b	2.1 c	1.4 b	1.2 a	13.6 b	14.0 c	12.7 a	2.0 b	1.8 a	2.2 c	18.5 a	18.7 a	24.5 b
Mir-3-gal	-	-	-	1.2 b	1.0 a	1.2 b	-	-	-	3.3 a	4.8 b	4.5 b	-	-	-	1.2 b	1.0 a	1.2 b
Q-3-gal	-	-	-	0.8 a	0.8 a	0.9 b	-	-	-	5.4 b	6.2 c	4.7 a	-	-	-	0.8 a	0.8 a	0.9 b
Q-3-glc	4.3 b	4.5 b	3.8 a	11.1 b	8.6 a	14.5 c	4.8 c	2.8 a	3.8 b	0.9 a	4.9 b	6.4 c	4.3 b	4.5 b	3.8 a	11.1 b	8.6 a	14.5 c
Q-3-gln	2.7 b	1.6 a	1.8 a	1.0b	0.8 a	1.3 c	1.7 c	1.5 a	1.6 b	3.5 a	1.1 b	1.5 c	2.7 b	1.6 a	1.8 a	1.0b	0.8 a	1.3 c
Lar-3-glu	1.1 b	1.1 b	0.9 a	2.5 a	2.2 a	2.3 a	1.2 c	0.7 a	0.9 b	2.4 b	3.1 c	2.1 a	1.1 b	1.1 b	0.9 a	2.5 a	2.2 a	2.3 a
Kam-3-gal	0.3 b	0.2 a	0.2 a	0.3 a	0.5 c	0.4 b	0.3 b	0.2 a	0.3 b	1.2 b	1.3 b	1.0 a	0.3 b	0.2 a	0.2 a	0.3 a	0.5 c	0.4 b
Kam-3-glu	0.8 ab	0.9 b	0.7 a	8.5 a	9.5 b	8.7 a	0.5 b	0.3 a	0.6 b	7.0 c	6.6 b	3.8 a	0.8 ab	0.9 b	0.7 a	8.5 a	9.5 b	8.7 a
Iso-3-glu	0.1 a	0.2 b	0.2 b	1.4 a	1.4 a	1.7 b	1.1 c	1.0 b	0.6 a	5.4 b	5.7 b	2.9 a	0.1 a	0.2 b	0.2 b	1.4 a	1.4 a	1.7 b
Sir-3-glu	0.3 ab	0.4 b	0.2 a	1.1 a	1.3 b	1.3 b	0.2 b	0.1 a	0.2 b	2.4 b	2.6 c	1.5 a	0.3 ab	0.4 b	0.2 a	1.1 a	1.3 b	1.3 b
Total	11.4 c	10.6 b	9.9 a	46.4 a	45.0 a	57.4 b	11.8 c	8.0 a	9.1 b	45.0 b	50.2 c	41.2 a	11.4 c	10.6 b	9.9 a	46.4 a	45.0 a	57.4 b

1Abbreviations: Mir: myricetin; Q: quercetin; Lar: laricitrin; Kam: kaempferol; Iso: isorhamnetin; Sir: syringetin; glu: O-glucoside; gal: O-galactoside; gln: O-glucuronide
2BTH: benzothiadiazole; MeJ: methyl jasmonate ; 3Different letters in the same row indicate significant differences according to Duncan test ($p<0.05$)

The results of the anthocyanin analysis in Merlot grapes and wines are shown in the second part of Table 2. In this variety, the highest concentrations of anthocyanins were found in MeJ-treated grapes (up to 14% increase) followed by BTH-treated grapes (up to 8%), and only a slight increase was observed in BTH wines (up to 3%). Previous studies demonstrated an increase in the anthocyanin content of Merlot grapes after BTH application, accompanied by increased resistance to *Botrytis* attack (Iriti *et al.*, 2005).

As can be seen, both treatments (MeJ and BTH) increased most individual anthocyanins in grapes (glucosides, acetates and coumarates) except malvidin-3-acetate and coumarate. In MeJ-treated Tempranillo grapes, Portu *et al.* (2016) reported an increase in the content of delphinidin-, cyanidin-, petunidin- and peonidin-3-glucoside, besides peonidin-3-acetyl-glucoside and cyanidin-3-coumaryl-glucoside. In the wines of our experiment, glucoside concentrations increased when grapes were treated with MeJ, acetates increased with both treatments, and coumarates increased when grapes were treated with BTH.

Finally, for the Monastrell variety, the highest concentrations of anthocyanins were found in MeJ-treated grapes (up to 25% increase) and in MeJ and BTH wines (up to 51% and 10% increase, respectively). The influence of variety on the effect of both treatments was evident. Ruiz-García *et al.* (2013a) previously reported that the impact of MeJ was clone-dependent. In another study carried out over two years (2009 and 2010), the same authors reported an increase in skin anthocyanin content in preharvest BTH- and MeJ-treated Monastrell grapes (Ruiz-García *et al.*, 2013a). The results obtained pointed to higher total monomeric anthocyanin values in the 2009 wines than in the corresponding 2010 wines. No differences due to treatments were observed in 2009, whereas the anthocyanin concentration was higher in the wines made from MeJ-treated grapes in 2010 (Ruiz-García *et al.*, 2013a). Finally, Ruiz-García *et al.* (2013b) also observed a statistically significant increase in the anthocyanin concentration of Monastrell grapes and wines when BTH+MeJ was applied.

The results for individual anthocyanins in Monastrell grapes and wines are shown in the third part of Table 2. As can be seen, almost all individual anthocyanins increased in grapes as a result of both treatments, although the increase in total anthocyanins was only significant in MeJ-treated grapes. Many authors have described the effect of MeJ treatment on anthocyanin accumulation in

plants: e.g. strawberry fruits (Pérez *et al.*, 1997), *Vitis vinifera* (Zhang *et al.*, 2002), and tulip leaves (Saniewski *et al.*, 2003). Our results showed that MeJ had a stronger effect in grapes than BTH, since tri-OH forms (delphinidin, petunidin and malvidin) did not increase when grapes were treated with BTH. However, in wines, both treatments produced higher levels of glucosides and acetylated anthocyanins compared to the control samples, but not higher levels of coumarate anthocyanins (except delphinidin and peonidin in BTH wines and malvidin and peonidin in MeJ wines).

3. Flavonols in grapes and wines

The application of BTH or MeJ has previously been shown to be an interesting option for increasing the levels of grape phenolic compounds (Portu *et al.*, 2015). Flavonols are very close to anthocyanins in the biosynthetic pathway - indeed, they share most of the pathway - so that any increase in enzyme activity upstream of the flavonoid biosynthetic pathway may also affect the concentration of these compounds (Ruiz-García *et al.*, 2012). The results obtained for grape and wine flavonols for the three varieties studied are summarized in Table 3. Neither myricetin-3-glucoside nor quercetin-3-glucoside was detected in grapes but both were detected in wines. The results obtained for Syrah are shown in Table 3 (first part). As can be seen, statistically significant differences were found for total flavonols in BTH-treated grapes (increases of up to 36%) but not in the corresponding wines. As regards individual flavonols, the differences in the Syrah variety were generally more significant in grapes than in wines. In BTH-treated grapes, statistical differences from control levels were found for all the compounds analyzed, except kaempferol-3-galactoside, while in MeJ-treated grapes statistically significant differences from the control sample were found in quercetin-3-glucuronide, kaempferol-3-galactoside, kaempferol-3-glucoside and syringetin-3-glucoside. De la Peña-Moreno *et al.* (2010) reported an increase in the levels of quercetin and kaempferol in red raspberry fruits exposed to MeJ postharvest. By contrast, in wines, only slight differences between treatments were observed when compared to control samples: statistically significant differences were found in quercetin-3-glucoside in both treatments and in kaempferol-3-galactoside in BTH wines.

The results for flavonols in the Merlot variety are shown in Table 3 (second part). As can be seen, there were significant differences in grape total flavonol content between the control and the treatments, control grapes obtaining higher values for this parameter. However, statistically significant differences from the control existed in the wines elaborated with BTH-treated grapes. In agreement with our results, Ruiz-García *et al.* (2012) found that the concentration of flavonols in wine made from Monastrell showed only small differences due to grape treatments (MeJ and BTH), the differences only being significant for the wines made from BTH-treated grapes in one year (2009). Here, none of the treatments increased the level of total or individual flavonols in grapes at harvest, but statistical differences from control levels were found in total flavonols in BTH wines and in some of the individual compounds in both treatments. In BTH wines, all compounds except kaempferols (3-galactoside and 3-glucoside) and isorhamnetin increased in concentration compared with control wines, while in MeJ wines increases were only observed in the case of myricetin-3-glucoside, quercetin-3-glucoside and quercetin-3-glucuronide.

The results for flavonols in the Monastrell variety are shown in Table 3 (third part). As can be seen, there were no significant differences in total flavonol content between the control and the treated grapes, but statistically significant differences from control values were found for the wines made from MeJ-treated grapes. As regards individual flavonols, few differences could be observed in grapes at harvest, with only myricetin-3-glucoside showing a higher concentration in MeJ-treated grapes than in control grapes, and isorhamnetin-3-glucoside showing a higher concentration for both treatments. In wines, statistical differences were found for all individual flavonols (except myricetin-3-galactoside and kaempferol-3-glucoside) in MeJ wines, and for some compounds (kaempferol-3-glucoside and galactoside and syringetin-3-glucoside) in BTH wines. Similarly, Ruiz-García *et al.* (2012) observed that control wines and MeJ wines had similar flavonol content. In a two-year experiment in which MeJ and BTH were applied preharvest to Monastrell grapes, Ruiz-García *et al.* (2013a) showed an increase in the flavonol content of treated grapes at harvest. On the other hand, these authors also reported differences between years for Monastrell grapes, suggesting that years with lower temperatures and higher humidity may provide suitable conditions for pathogen development and consequently help elicitor-treated plant react more efficiently.

The year this experiment was run was very dry and warm: only 130 mm of rain were measured from May to July and 53 mm from July to September, with maximum temperatures of 37, 37, and 32°C in July,

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Table 4 - Tannins in Syrah, Merlot and Monastrell grapes (mg/kg) and wines (mg/L).

SYRAH	Skin grape			Seed grape			Wine		
	Control	BTH[2]	MeJ	Control	BTH	MeJ	Control	BTH	MeJ
mDP[1]	8.4 a[3]	16.3 b	17.5 c	4.7 a	5.6 b	5.6 b	2.8 a	3.3 b	3.5 b
%Gal	2.3 a	4.7 b	2.4 a	16.4 a	16.2 a	16.5 a	6.5 a	7.4 b	7.2 b
%Cat T	2.5 a	2.8 b	3.9 c	6.5 a	6.4 a	6.2 a	16.3 b	14.4 a	14.6 a
%Epi T	8.5 c	2.4 b	1.5 a	8.2 c	6.5 a	7. 8 b	13.7 c	12.2 b	7.8 a
%E3OG T	1.2 c	0.2 a	0.6 b	5.1 b	4.5 a	4.4 a	3.7 a	4.4 a	4.4 a
%EgC E	40.3 b	35.2 a	36.2 a	-	-	-	18.6 a	22.3 c	21.4 b
%Cat E	1.3 a	1.7 b	1.6 b	5.4 a	6.5 b	6.2 b	7.1 b	6.5 a	6.9 b
%Epi E	44.4 a	51.6 c	49.6 b	63.8 a	64.1 a	61.2 a	36.3 a	38.9 b	39.8 b
%E3OG E	1.9 a	3.3 c	2.4 b	11.6 a	11.7 a	11.7 a	2.9 a	2.9 a	3.0 b
Total	368.7 a	366.6 a	443.0 b	1395.4 a	1559.7 ab	1608.3 b	263.7 a	297.7 c	284.3 b
MERLOT	Skin grape			Seed grape			Wine		
	Control	BTH[2]	MeJ	Control	BTH	MeJ	Control	BTH	MeJ
mDP	11.1 a	12.4 b	15.4 c	4.4 a	6.5 b	5.5 ab	3.7 a	3.9 b	3.8 ab
%Gal	2.8 c	1.7 b	1.3 a	8.3 a	14.4 b	10.2 ab	5.5 a	5.3 a	5.4 a
%Cat T	2.5 a	3.3 b	3.7 b	5.6 a	6.6 b	5.7 a	15.3 a	15.7 a	15.4 a
%Epi T	0.8 b	0.5 a	0.4 a	13.6 c	9.7 b	7.9 a	8.6 b	8.3 ab	7.5 a
%E3OG T	0.1 a	0.3 b	0.3 b	2.8 b	1.0 a	4.6 c	2.5 a	2.3 a	3.0 b
%EgC E	13.0 b	15.0 c	12.4 a	-	-	-	23.4 a	26.1 b	22.0 a
%Cat E	2.8 b	2.5 a	2.5 a	10.7 c	7.2 a	8.5 b	5.5 a	5.7 a	6.4 b
%Epi E	30.0 a	28.8 a	40.0 b	62.8 a	67.4 b	63.8 ab	41.2 a	40.1 a	43.1 b
%E3OG E	2.5 b	1.8 a	1.6 a	4.2 a	10.2 b	9.8 b	3.0 b	3.0 b	2.4 a
Total	1669.0 a	1779.1 a	2977.3 b	1142.6 a	2080.4 b	2129.9 b	451.2 a	458.0 a	501.5 b
MONASTRELL	Skin grape			Seed grape			Wine		
	Control	BTH[2]	MeJ	Control	BTH	MeJ	Control	BTH	MeJ
mDP	13.7 a	15.7 b	17.5 c	5.5 a	6.4 b	5.4 a	3.3 a	3.5 b	4.3 c
%Gal	25.4 b	20.6 a	38.4 c	5.9 b	5.3 a	5.8 b	2.5 ab	2.2 a	2.6 b
%Cat T	4.6 a	4.5 a	4.3 a	6.8 ab	6.3 a	7.3 b	22.9 b	22.8 b	18.5 a
%Epi T	0.3 a	0.5 b	0.3 a	1.7 b	1.6 b	1.2 a	5.8 a	7.3 b	5.5 a
%E3OG T	0.2 a	0.3 b	0.4 c	2.4 b	2.4 b	1.9 a	0.5 a	0.6 b	0.5 a
%EgC E	8.6 b	7.7 a	9.5 c	-	-	-	15.1 b	13.5 a	18.0 c
%Cat E	2.5 b	1.9 a	1.6 a	7.5 a	7.4 a	8.7 b	1.7 a	1.9 a	7.5 b
%Epi E	45.8 a	49.2 b	46.1 a	67.6 a	67.1 a	67.0 a	51.2 c	48.4 b	47.1 a
%E3OG E	1.0 b	0.9 a	0.9 a	2.4 b	2.4 b	1.9 a	2.1 a	2.3 b	2.0 a
Total	1810.5 b	1843.1 b	1484.5 a	2051.7 b	2519.4 c	1752.7 a	207.9 a	239.5 b	298.8 c

[1]Abbreviations: mDP: mean degree of polymerization; %Gal: percentage of galloylation; %Cat T: percentage of terminal (+)-catechin; %Epi T: percentage of terminal (-)-epicatechin; %E3OG T: percentage of terminal (-)-epicatechin 3-O-gallate; %Cat E: percentage of extension (+)-catechin; %Epi E: percentage of extension (-)-epicatechin; %EgC E: percentage of extension epigallocatechin; %E3OG E: percentage of extension (-)-epicatechin 3-O-gallate

[2]BTH: benzothiadiazole; MeJ: methyl jasmonate

[3]Different letters in the same row indicate significant differences according to Duncan test ($p<0.05$)

August and September, respectively (data not shown; from Murcia Agricultural Information System).

4. Tannins in grapes and wines

Tannins are an important component of wine quality contributing to both perceived mouthfeel and long-term colour stability. Seeds contain a higher concentration of tannins than skin and a higher proportion of galloylated units, whereas skin tannins have a higher mDP than seed tannins.

The tannin levels of Syrah grapes (skin and seeds) and wines are shown in Table 4. Statistically significant differences from the control values were detected for total tannins in skin and seeds from MeJ-treated grapes and in wine from both treatments. Gómez-Plaza *et al.* (2017) also found higher concentrations of tannins in Syrah grapes treated with MeJ at harvest. In our study, both treatments (BTH and MeJ) also had an effect on mDP in the skin, seeds and wines. The percentage of galloylation was higher in BTH grape skins and in both BTH and MeJ wines. The percentage of tannin galloylation affects both the bitterness and astringency of wines (Lesschaeve and Noble, 2005). An increase in maceration time may explain the higher percentages of galloylation in higher quality wines (greater extraction of seed tannins, which are more galloylated) (Busse-Valverde *et al.*, 2010). However, other winemaking techniques, such as the use of macerating enzymes or prefermentative cold maceration, could also increase the percentage of galloylation of wine tannins (He *et al.*, 2010), although the maceration time was similar in all our wines.

Regarding the composition of tannins in general and the terminal units in particular, in the Syrah variety (Table 4-first part), differences from control levels were more pronounced in skin and wine tannins than in seed tannins. As regards the terminal units, only (+)-catechin showed statistically significant differences from control levels for both treatments in skin tannins, but higher differences were found in the extension units: (+)-catechin, (-)-epicatechin and epicatechin-3-*O*-gallate content increased with respect to the control samples for both treatments. In seeds, only (+)-catechin content increased in both treatments and in wines, (-)-epicatechin content increased in both treatments and epicatechin-3-*O*-gallate content increased in the MeJ treatment.

As regards the Merlot variety, statistical analysis identified significant differences from the control for total tannins in skin, seeds and wines in the MeJ treatment and only in seeds in the BTH treatment (Table 4-second part). The increase of mDP in skin, seeds and wines was only observed with the BTH treatment, while the MeJ treatment only affected the mDP of the skin tannins. As regards terminal units, only the percentage of (+)-catechin and epicatechin-3-*O*-gallate increased (for both treatments) in skin, (+)-catechin in seeds following BTH treatment, and epicatechin-3-*O*-gallate in seeds of MeJ-treated grapes and MeJ wines. Considering the extension units, Table 4 points to an increase in MeJ skin and wine epicatechin, BTH and MeJ seed epicatechin-3-O-gallate, and BTH skin and wine epigallocatechin. This last compound is a prodelphinidin and only appears in grape skin and, therefore, in wines made with these skins. Both (+)-catechin and (-)-epicatechin reached a higher percentage in wines following MeJ treatment.

Finally, in the Monastrell variety (Table 4-third part), the differences were evident in skin, seeds and wines. Total tannins only increased over control levels in the seeds of BTH-treated grapes and in the wines of both treatments. Also, an increase in mDP was observed in skin and wine tannins for both treatments but only in seeds following BTH treatment. The percentage of galloylation was enhanced only in the skin of MeJ-treated grapes. An increase in the terminal units (-)-epicatechin and epicatechin-3-*O*-gallate was observed in BTH skins and wines and an increase in the extension unit epigallocatechin gallate was observed in MeJ skins and wines. The increases in the different tannin compounds resulted in an increase in the total tannin content of the wines made in both treatments.

As has been seen, responses to the treatments depended on the variety. Other authors have mentioned a similar dependence on variety: for example, Vitalini *et al.* (2011) in a study of the effect of BTH and chitosan on the melatonin content of grapes, and Gómez-Plaza *et al.* (2017) in a study of the effects of elicitors applied during the ripening period in Syrah, Merlot and Monastrell grapes.

5. Sensory analysis

To determine whether the results observed in the differently treated wines could be detected at sensory level, the wines were tested using a triangular test (Table 5). Again, the effect that the treatments had on the sensory analysis depended on the variety. In Syrah wines, no clear difference or preference for treated wines was observed. However, in the case of Merlot, the wines elaborated with BTH-treated grapes were clearly differentiated and preferred. Finally, in Monastrell wines, both treatments could be differentiated and most panellists preferred the

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Table 5 - Results of the triangle test performed by ten panellists for wines at the end of alcoholic fermentation.

Sample	No. of correct answers (%)	Preferred sample
Syrah wines		
Control-BTH	50%	Control
Control-MeJ	40%	MeJ-Control (50%)
Merlot wines		
Control-BTH	75%	BTH
Control-MeJ	50%	Control
Monastrell wines		
Control-BTH	75%	Control
Control-MeJ	62.5%	MeJ

MeJ wine to the control, while the opposite was observed for the BTH treatment.

Conclusion

The results obtained in this study show that the treatments applied were variety-dependent. Thus, in Syrah, the BTH treatment led to higher values of the studied parameters in both grapes and wines, while in Merlot, the MeJ treatment led to higher values in grapes and the BTH treatment to higher values in wines. Finally, in Monastrell grapes and wines, the best results were obtained with the MeJ treatment.

From a technological point of view, the results were more striking in wines than in grapes, although the results were not always favourable in the sensory analysis. Again, the variety played an important role since the preferred Merlot wine was that made from BTH-treated grapes and the preferred Monastrell wine was that made from the MeJ-treated grapes, while there was no clear preference in the case of Syrah wines.

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