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Changes on Grape Aroma Composition as a Consequence of Foliar Application of Methyl Jasmonate and Nano-Sized Particles Doped with Methyl Jasmonate

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Abstract: In recent years, foliar application of elicitors to the vineyard has been increasingly used, in particular, elicitation with methyl jasmonate (MeJ). However, due to the high cost of this compound, it is necessary to find a form of application in which the amount to be used is considerably reduced. Therefore, the aim of this work was study for the first time the influence of foliar application of nanoparticles doped with MeJ (ACP-MeJ) and foliar application of methyl jasmonate (MeJ), using a dose of 1 mM versus 10 mM, respectively, on volatile composition of Tempranillo grapes during two consecutive vintages. Grape volatile composition was determined by SPME-GC-MS. The obtained results reveal that MeJ application increased the concentration of terpenoids, and total C6 compounds in 2019 and 2020, and C13 norisoprenoids in 2019. In addition, ACP-MeJ enhanced the amount of terpenoids, and benzenoids in 2020. These are encouraging results considering that the ACP-MeJ dose was 10 times lower than that of MeJ. Therefore, the foliar application of MeJ supported on nanoparticles could be a tool in order to improve grape volatile composition, favoring a more viable and sustainable viticulture.

Keywords: nanoparticles; methyl jasmonate; volatile compounds; grape varietal aroma; elicitors

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1. Introduction

Aroma is one of the most important parameters to determine must and wine quality, also influences the grape flavor and contributes to the sensory character of the wine [1–4]. The grape volatile compounds belong to several groups: terpenoids, C_{13} norisoprenoides, benzenoid compounds, esters, C6 compounds, alcohols, thiols and methoxypyrazines [5–7]. The amount of these compounds depend on several factors such as grape variety, season, terroir, grape maturity, viticultural practices, etc. [1,8–10]. Foliar fertilization is a technique that is increasingly used as allows a quick and efficient assimilation of the products applied to the plant, reducing soil contamination and costs [10–12].

Foliar application of elicitors, molecules capable of activating the defensive systems of plants, can increase the synthesis of secondary metabolites [12–14]. Methyl jasmonate is a volatile organic compound derived from jasmonic acid [15,16]. This elicitor has been mainly implicated as a mediator of plant responses triggered by wounding and insect feeding and is involved in the pathogen resistance [15,17,18]. Foliar application of methyl jasmonate has been shown to increase the synthesis of secondary metabolites such as amino acids [19], phenolic compounds [14,18,20], and volatile compounds [5,18–21]. Despite this, methyl jasmonate is a compound with a very high cost and with low chemical stability.

In the last decade, nanotechnology has opened new horizons in several disciplines, including and agriculture [22]. Nanotechnology is providing very interesting results in

agriculture by improving the efficiency of agrochemicals [23,24]. Specifically in viticulture, there are few studies [25] in which nanocarriers have been applied to vineyards to improve the efficiency of fertilizers (i.e., urea) or elicitors (i.e., MeJ) [26–30]. In this line, biomimetic calcium phosphate nanoparticles (ACP-NPs) have been proposed as promising MeJ nanocarrier providing slow release kinetic and protection against thermal degradation [31]. ACP-NPs are non-toxic and biocompatible nanomaterials widely used in biomedicine for drug delivery, dental remineralization or bone tissue engineering [32]. But, the effect of the foliar application of this nanoelicitor on grape aromatic composition has not been studied so far, only in the wine volatile composition, i.e., fermentative aromas [33].

Hence, this work aims at evaluating the influence of foliar application of nanoparticles doped with MeJ (ACP-MeJ) and foliar application of methyl jasmonate (MeJ) in conventional form on volatile composition of *Vitis vinifera* L. cv. Tempranillo grapes during two vintages.

2. Materials and Methods

2.1. Vineyard Site, Grapevine Treatments and Samples

This study was conducted, during the 2019 and 2020 vintages, on *Vitis vinifera* L. cv. Tempranillovines belonging to an experimental vineyard located at Finca La Grajera, Logroño, La Rioja (Spain). These vines were planted in 1997 using R-110 rootstock and treated according to local viticultural practices. They were trained in a VSP (vertical shoot positioned) trellis system, with a spacing between vines of 2.80 m between rows, and 1.25 m within the same row. For further information, climatic data were obtained from the Agroclimatic Information Service (SIAR), which were collected by an automatic weather station located near the area. During 2019, from the beginning of April to 1 September, the accumulated rainfall was 247.8 L/m^2, and the average temperatures were: 27 °C the maximum, 13.8 °C the mean, and 3.7 °C the minimum. For the year 2020, in the same period, the accumulated rainfall was 217.8 L/m^2, and the average temperatures were: 26.3 °C the maximum, 13.8 °C the mean, and 3.7 °C the minimum. Foliar applications of free methyl jasmonate (MeJ) and amorphous calcium phosphate nanoparticles functionalized with MeJ (ACP-MeJ) were studied. To carry out the field experiments, free MeJ aqueous solution (10 mM) and ACP-MeJ aqueous suspension (1 mM MeJ) were prepared following previous [19,27,34]. Tween 80 were used in both cases as wetting agent (1 mL/L). ACP-MeJ nanoparticles were synthesized and fully characterized as described in detail elsewhere [31]. All treatments were applied first at veraison and second one week later. The concentration of treatment applied to the leaves of each plant was 200 mL/plant in each of the two applications. For the control only the plants were sprayed with the aqueous solution of Tween 80. Each of the treatments was carried out in triplicate, and each replicate consisted of 10 vines. All treatments were arranged in a complete randomized block design.

The berries were harvested at their optimum point of technological maturity (weight of 100 berries constant, and 13% (v/v) of probable alcohol). Once harvested, they were destemmed and crushed until the must was obtained. The general parameters of all the musts were then measured, and aliquots of each must sample were frozen (−20 °C) for subsequent analysis of the aromatic composition.

2.2. General Parameters Determination

Enological parameters (°Brix, probable alcohol, pH, total acidity . . .) were determined by official methods established by the OIV [35]. The remaining general parameters such as glucose + fructose fractions, glucose (and fructose indirectly, as subtraction of glucose + fructose − glucose), malic acid, total phenols and nitrogen, were determined by enzymatic methods, with the Miura One equipment (TDI, Barcelona, Spain). The results obtained for these parameters are shown as the mean ± standard deviation (n = 3).

2.3. Analysis of Grape Volatile Compounds by HS-SPME-GC-MS

Determination of volatile compounds in the musts was carried out by head space solid-phase microextraction (HS-SPME) and their subsequent analysis by gas chromatog-

raphy (GC) coupled to mass spectrometry (MS), according to the method described by Garde-Cerdán et al., 2018 [34]. The SPME fiber used was divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 µm) (Supelco, Bellenfonte, PA, USA). In 20 mL vials (Supelco), 9 mL of sample, 2.5 g NaCl and 10 µL of internal standard (2-octanol) were added. After adding a stir bar, the vial was closed and placed in the GC-MS (Agilent, Palo Alto, CA, USA). Sample conditioning was done at 60 °C, for 15 min and with stirring. After this step, the fiber was automatically inserted into the headspace in order to the extraction of the volatile compounds could take place, for 105 min, with agitation.

After the extraction process was completed, the fiber was immediately introduced into the GC injection port at 250 °C and held for 15 min for desorption of the compounds of interest. The capillary column used for analyte separation is SPB™-20 (30 m × 0.25 mm I.D. × 0.25 µm film thickness) (Supelco). Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The chromatographic conditions used were: initial temperature, 40 °C for 5 min, a temperature gradient of 2 °C/min, up to a final temperature of 220 °C, to be maintained for 20 min (total time = 115 min). The ionization of the volatile compounds was performed at 70 eV. The detector worked at full scan (35—300 m/z). Identification was carried out using the NIST library and comparing with mass spectra and retention time of chromatographic standards, when available, as well as with data found in the literature. Semi-quantification was performed by relating the areas of each compound to the area and known concentration of the internal standard.

Since the treatments were performed in triplicate, the results of grape volatile compounds are expressed as the mean concentration and standard deviation of the three replicates (n = 3).

2.4. Statistical Analyses

Statistical analysis of the data was performed with the SPSS statistical package version 21.0 for Windows (SPSS, Chicago, IL, USA). Analysis of variance (ANOVA) ($p < 0.05$) was performed for general parameters and volatile compound data. To evaluate possible differences between treatments, the Duncan test was performed at the 95% probability level. A multivariate factor analysis was also performed (with treatment and season as factors) considering oenological parameters and grape aromatic compounds. Finally, a discriminant analysis was performed to classify the samples according to their volatile composition.

3. Results and Discussion

3.1. Effect of the Foliar MeJ and ACP-MeJ Treatments on the Must General Parameters

Table 1 shows the enological parameters in the grapes from control and vines treated with methyl jasmonate (MeJ) and with nanoparticles doped with MeJ (ACP-MeJ), in 2019 and 2020 seasons. In 2019, MeJ treatment significantly decreased °Brix, probable grade, glucose + fructose (Glu + Fru), glucose (Glu), and fructose (Fru) content with respect to control grapes, while total acidity, total phenols, amino nitrogen, and yeast assimilable nitrogen (YAN) increased when vines were foliarly treated with MeJ. However, ACP-MeJ treatment showed no significant differences with respect to the control in any of the parameters studied except for total phenols, which concentration increased (Table 1). In 2020 season, MeJ and ACP-MeJ foliar application did not affect must enological parameters. This result are similar to those reported by Garde-Cerdán et al., 2018 [34] which found only small or no differences in these parameters after MeJ application. Although overall precipitation and average temperatures were similar in 2019 and 2020, August rainfall was 11.5 L/m^2 in 2019 and 32.9 L/m^2 in 2020. Since this month is where the berry ripening process is completed, this may be the reason why the weight of 100 berries is higher in 2020 than in 2019 (Table 1).

Table 1. General parameters in grapes from control, methyl jasmonate (MeJ) and nanoparticles doped with MeJ (ACP-MeJ) foliar treatments, in 2019 and 2020 seasons.

	2019			2020		
	Control	**MeJ**	**ACP-MeJ**	**Control**	**MeJ**	**ACP-MeJ**
Weight of 100 berries (g)	113.68 ± 11.07 a	141.81 ± 27.18 a	116.94 ± 4.62 a	199.57 ± 7.27 a	207.67 ± 40.39 a	194.90 ± 20.65 a
°Brix	24.70 ± 0.72 b	22.23 ± 1.17 a	23.37 ± 0.49 ab	22.30 ± 0.92 a	22.17 ± 2.31 a	22.37 ± 0.38 a
Probable alcohol (% *v*/*v*)	14.63 ± 0.49 b	12.92 ± 0.80 a	13.71 ± 0.35 ab	12.97 ± 0.63 a	12.89 ± 1.58 a	13.01 ± 0.26 a
pH	3.83 ± 0.05 a	3.78 ± 0.10 a	3.82 ± 0.09 a	3.76 ± 0.01 a	3.70 ± 0.07 a	3.73 ± 0.06 a
Total acidity (g/L) *	4.61 ± 0.11 a	5.20 ± 0.36 b	5.13 ± 0.26 ab	4.12 ± 0.33 a	4.54 ± 1.08 a	4.03 ± 0.21 a
Glu + Fru (g/L)	249.86 ± 9.97 b	215.50 ± 12.29 a	231.40 ± 10.82 ab	216.42 ± 10.70 a	218.62 ± 26.56 a	223.84 ± 2.98 a
Glu (g/L)	120.18 ± 5.13 b	102.88 ± 6.89 a	110.89 ± 4.94 ab	107.31 ± 4.54 a	106.08 ± 12.84 a	108.61 ± 2.98 a
Fru (g/L)	129.68 ± 4.84 b	112.62 ± 5.43 a	120.51 ± 6.26 ab	109.11 ± 6.53 a	112.54 ± 13.76 a	114.72 ± 0.98 a
Malic acid (g/L)	2.24 ± 0.24 a	2.54 ± 0.32 a	2.51 ± 0.56 a	1.21 ± 0.08 a	1.54 ± 0.22 a	1.39 ± 0.18 a
Total phenols (mg/L)	1185.33 ± 72.31 a	1306.57 ± 61.35 b	1351.40 ± 27.32 b	541.60 ± 64.02 a	603.07 ± 73.82 a	582.70 ± 66.02 a
Ammonium nitrogen (mg N/L)	78.00 ± 8.22 a	106.34 ± 15.68 a	101.40 ± 20.40 a	121.16 ± 3.52 a	101.66 ± 19.58 a	114.66 ± 6.24 a
Amino nitrogen (mg N/L)	118.51 ± 14.33 a	202.11 ± 50.59 b	175.71 ± 24.66 ab	152.53 ± 14.33 a	139.63 ± 35.64 a	152.24 ± 5.50 a
YAN (mg N/L)	196.51 ± 21.18 a	308.45 ± 64.76 b	277.11 ± 44.31 ab	273.69 ± 17.69 a	241.29 ± 55.05 a	266.90 ± 11.62 a

* As g/L of tartaric acid. YAN: yeast assimilable nitrogen. All parameters are listed with their standard deviation (n = 3). For each season and parameter, different letters indicate significant differences between the samples ($p \leq 0.05$).

3.2. Influence of the Foliar MeJ and ACP-MeJ Treatments on Must Volatile Compounds

Figures 1–3 and Table 2 show the results of must volatile primary aroma content in control and in samples from treated grapevines with methyl jasmonate (MeJ) and with nanoparticles doped with MeJ (ACP-MeJ), in 2019 and 2020 seasons. A total of 37 compounds were identified and semi-quantified, including terpenoids, C_{13} norisoprenoids, benzenoid compounds, alcohols, carbonyl compounds, C6 compounds, and other compounds.

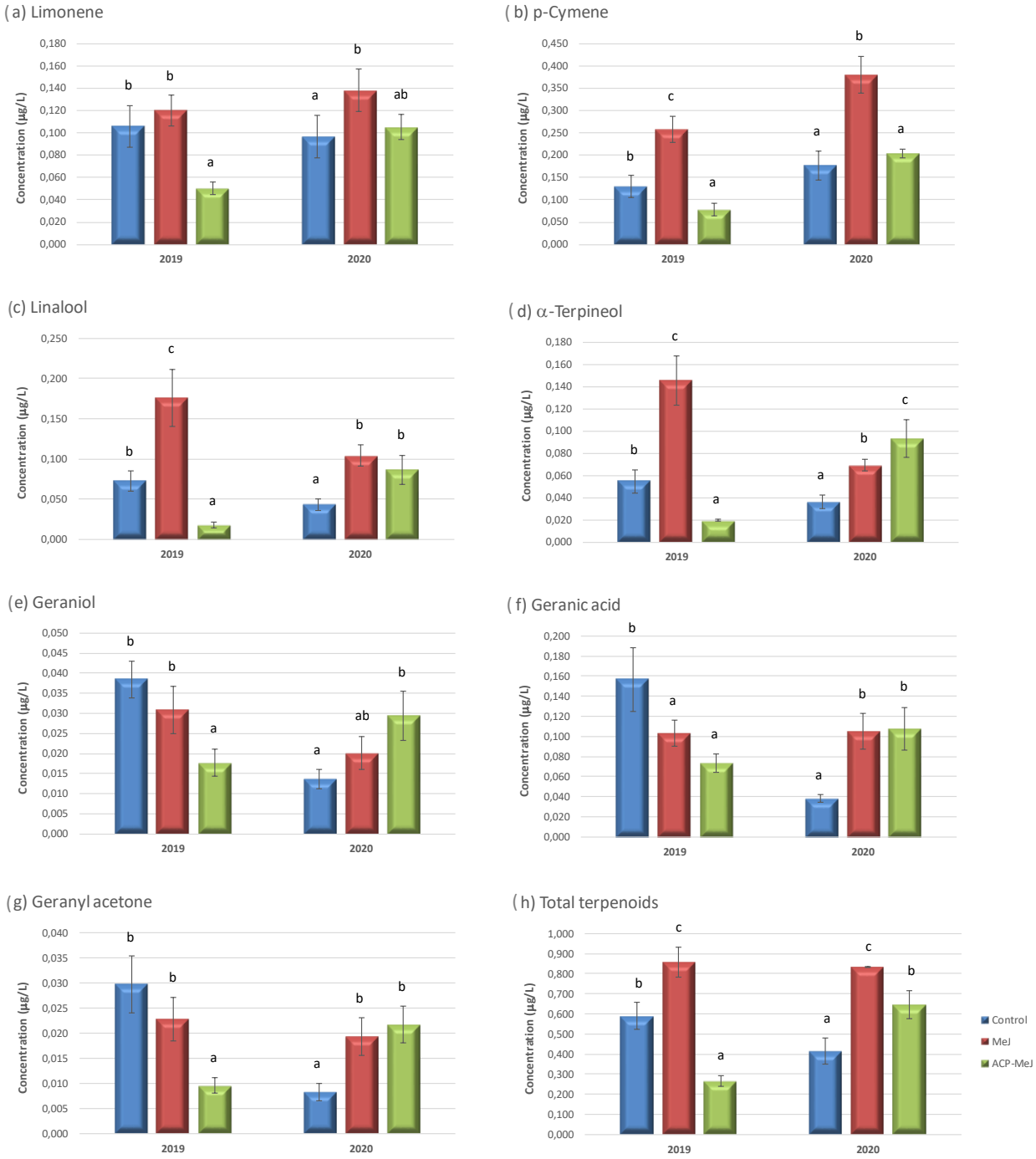

Figure 1. Terpenoids concentration (µg/L) in grapes from control, methyl jasmonate (MeJ) and nanoparticles doped with MeJ (ACP-MeJ) foliar treatments, in 2019 and 2020 seasons. All parameters listed with their standard deviation (n = 3). For each season and compound, different letters indicate significant differences between samples ($p \leq 0.05$).

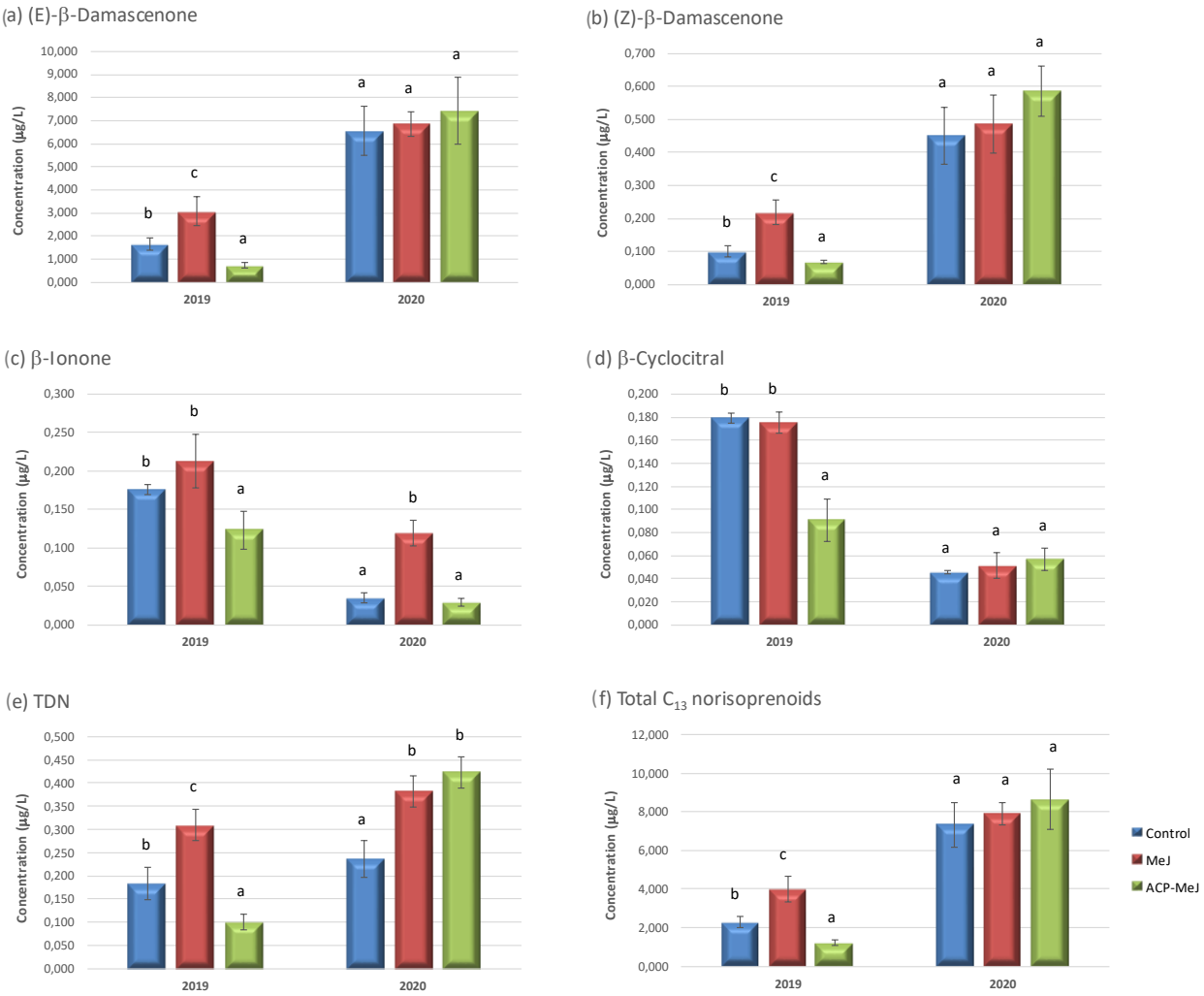

Figure 2. C_{13} norisoprenoids concentration (µg/L) in grapes from control, methyl jasmonate (MeJ) and nanoparticles doped with MeJ (ACP-MeJ) foliar treatments, in 2019 and 2020 seasons. All parameters listed with their standard deviation ($n = 3$). For each season and compound, different letters indicate significant differences between samples ($p \leq 0.05$). TDN: 1,1,6-trimethyl-1,2-dihydronaphthalene.

Figure 1 shows the concentration of terpenoids found in the control samples and in the grapes from the treatments with MeJ and ACP-MeJ, in 2019 and 2020 seasons. In 2019, limonene (Figure 1a), geraniol (Figure 1e), and geranyl acetone (Figure 1g) decreased their concentration in ACP-MeJ grapes relative to control and MeJ grapes. This effect had already been observed in *Vitis vinifera* L. cv. Tempranillo variety after the application of MeJ [34]. The MeJ-based treatment showed no significant differences with the control for these compounds.

For the same year, p-cymene (Figure 1b), linalool (Figure 1c), and α-terpineol (Figure 1d), which are very important terpenoids for grape and wine aroma [36], increased their content in MeJ grapes, and decreased it in ACP-MeJ samples with respect to control one. In the case of geranic acid (Figure 1f), both treatments significantly decreased the amount of this compound. Finally, in the same year, total terpenoids concentration (Figure 1h) increased in grapes from the foliar application of MeJ, and decreased in grapes treated with ACP-MeJ with respect to control grapes.

In 2020, limonene and p-cymene (Figure 1a,b) increased their concentration in MeJ grapes relative to control grapes. This effect had already been observed in *Vitis vinifera* 'Garnacha' variety after the application of MeJ [12]. The MeJ-doped nanoparticles treatment showed no significant differences in those compounds with the control samples. In the

Garde-Cerdán et al., 2018 [34] study, it is shown that the synthesis of p-cymene increases upon application of MeJ. Moreover, for the same year, linalool (Figure 1c), geranic acid (Figure 1f) and geranyl acetone (Figure 1g), significantly increased their concentration in MeJ-treated and ACP-MeJ-treated samples with respect to the control. In the case of α-terpineol (Figure 1d), both foliar treatments increased the content of this compound in the grapes, with ACP-MeJ increasing to a greater extent. Regarding geraniol (Figure 1e), only the ACP-MeJ treatment significantly increased the amount of this compound with respect to the control grapes, despite being a treatment with a concentration 10 times lower. In this season, the total concentration of terpenoids (Figure 1h) increased significantly with both treatments with respect to the control grapes, being more effective the application with MeJ. The increase in the amount of terpenoids after foliar application with MeJ has been previously demonstrated by other groups [5,12,37]. However, some studies have also found that the content of total terpenoids decreases when MeJ is applied [34,38]. In general, the treatments increased the amount of several terpenoids found in the grapes (Figure 1). This may be due to the foliar treatments were applied during veraison, moment when free terpenoids start to be produced [12,39]. These compounds are high volatile compounds, and have very low perception threshold, and therefore represent one of the most important group of aromatic compounds [12,40], and among these, linalool, α-terpineol, and geraniol, which are some of the most odoriferous monoterpenes [5].

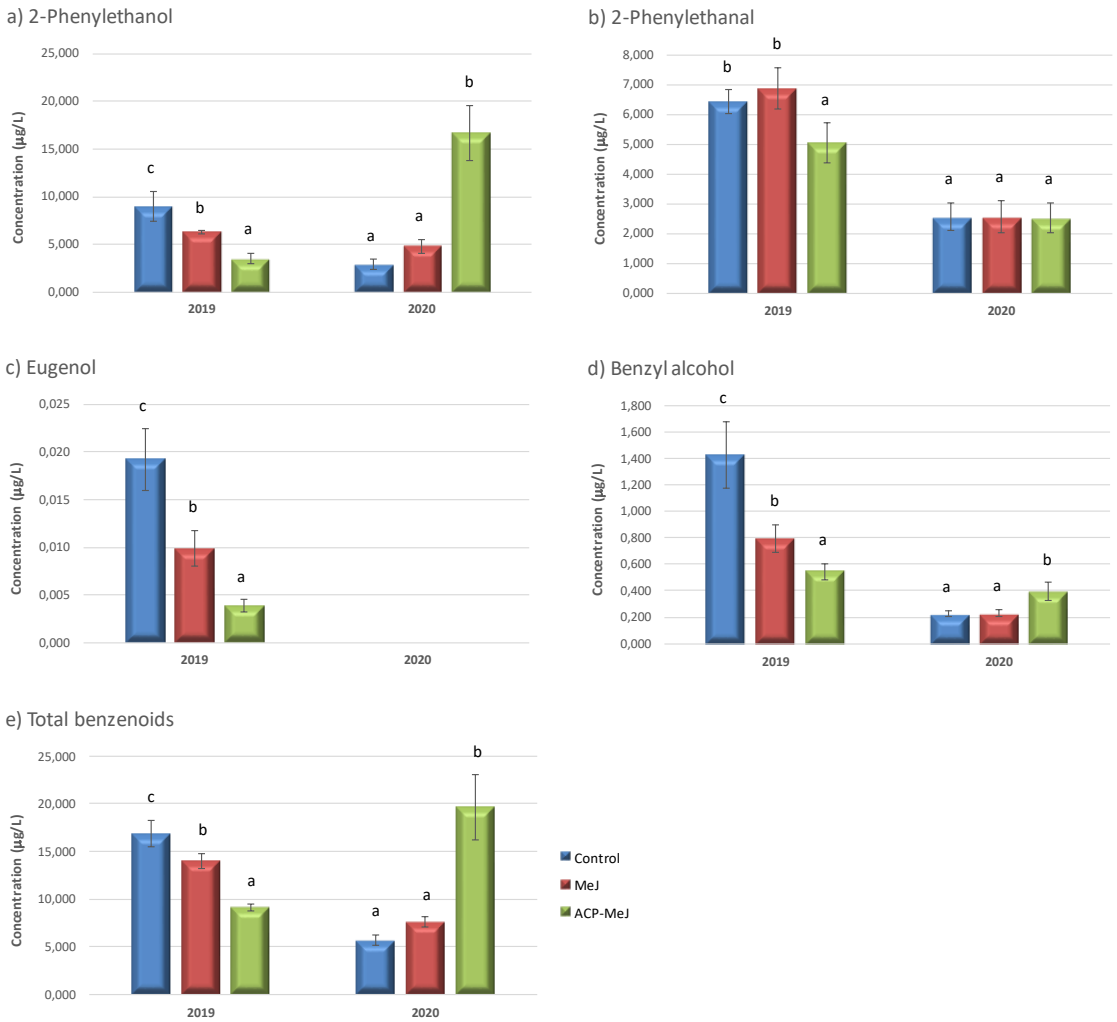

Figure 3. Benzenoid compounds concentration (µg/L) in grapes from control, methyl jasmonate (MeJ) and nanoparticles doped with MeJ (ACP-MeJ) foliar treatments, in 2019 and 2020 seasons. All parameters listed with their standard deviation (n = 3). For each season and compound, different letters indicate significant differences between samples ($p \leq 0.05$).

Table 2. Alcohols, carbonyl compounds, C6 compounds and other compounds concentration (μg/L) in grapes from control, methyl jasmonate (MeJ) and nanoparticles doped with MeJ (ACP-MeJ) foliar treatments, in 2019 and 2020 seasons.

	2019			2020		
	Control	**MeJ**	**ACP-MeJ**	**Control**	**MeJ**	**ACP-MeJ**
Alcohols						
n-Heptanol	0.062 ± 0.010 c	0.046 ± 0.008 b	0.028 ± 0.005 a	0.047 ± 0.002 a	0.044 ± 0.009 a	0.045 ± 0.009 a
n-Octanol	0.191 ± 0.014 b	0.174 ± 0.017 b	0.107 ± 0.013 a	0.326 ± 0.018 b	0.234 ± 0.042 a	0.238 ± 0.048 a
n-Nonanol	0.064 ± 0.006 b	0.059 ± 0.010 b	0.031 ± 0.007 a	0.197 ± 0.036 b	0.245 ± 0.048 b	0.093 ± 0.015 a
1-Octen-3-ol	0.595 ± 0.043 b	0.296 ± 0.063 a	0.243 ± 0.031 a	0.174 ± 0.036 b	0.074 ± 0.006 a	0.147 ± 0.030 b
2-Ethyl-1-hexanol	3.088 ± 0.060 b	1.798 ± 0.309 a	1.625 ± 0.137 a	1.870 ± 0.131 b	0.863 ± 0.132 a	2.140 ± 0.446 b
Total	*4.001 ± 0.108 b*	*2.373 ± 0.387 a*	*2.035 ± 0.167 a*	*2.613 ± 0.048 b*	*1.460 ± 0.156 a*	*2.663 ± 0.502 b*
Carbonyl compounds						
Heptanal	0.055 ± 0.009 b	0.034 ± 0.007 a	0.033 ± 0.004 a	0.014 ± 0.002 b	0.007 ± 0.001 a	0.010 ± 0.001 a
(E)-2-Octenal	0.059 ± 0.005 a	0.051 ± 0.009 a	0.051 ± 0.006 a	0.043 ± 0.009 b	0.024 ± 0.004 a	0.042 ± 0.007 b
Nonanal	0.204 ± 0.039 b	0.115 ± 0.028 a	0.083 ± 0.011 a	0.381 ± 0.074 b	0.143 ± 0.025 a	0.236 ± 0.040 a
(E)-2-Nonenal	0.065 ± 0.007 a	0.068 ± 0.007 a	0.065 ± 0.007 a	0.047 ± 0.008 b	0.031 ± 0.001 a	0.024 ± 0.005 a
Decanal	0.076 ± 0.013 b	0.070 ± 0.011 b	0.046 ± 0.009 a	0.112 ± 0.023 b	0.068 ± 0.014 a	0.040 ± 0.005 a
(E,E)-2,4-Hexadienal	1.177 ± 0.245 b	1.567 ± 0.261 b	0.691 ± 0.110 a	0.711 ± 0.133 b	0.208 ± 0.015 a	0.836 ± 0.109 b
(E,E)-2,4-Nonadienal	0.097 ± 0.011 b	0.112 ± 0.026 b	0.059 ± 0.001 a	0.040 ± 0.005 b	0.026 ± 0.005 a	0.046 ± 0.007 b
γ-Decalactone	0.125 ± 0.024 b	0.157 ± 0.030 b	0.054 ± 0.008 a	0.146 ± 0.029 a	0.141 ± 0.021 a	0.274 ± 0.044 b
6-Methyl-3,5-heptadien-2-one	0.086 ± 0.017 b	0.079 ± 0.015 b	0.046 ± 0.009 a	0.022 ± 0.005 a	0.029 ± 0.004 a	0.027 ± 0.005 a
Total	*1.942 ± 0.278 b*	*2.254 ± 0.286 b*	*1.128 ± 0.102 a*	*1.515 ± 0.258 b*	*0.676 ± 0.049 a*	*1.535 ± 0.106 b*
C6 compounds						
n-Hexanol	5.904 ± 1.031 b	7.018 ± 1.447 b	3.479 ± 0.575 a	22.311 ± 3.544 a	42.324 ± 4.178 b	19.316 ± 4.032 a
n-Hexanal	22.040 ± 2.145 b	28.064 ± 5.929 b	8.021 ± 1.150 a	11.784 ± 1.942 b	16.831 ± 2.431 c	7.163 ± 1.427 a
(Z)-3-Hexen-1-ol +(E)-2-Hexen-1-ol	1.027 ± 0.187 b	0.340 ± 0.065 a	0.361 ± 0.081 a	0.669 ± 0.115 a	1.080 ± 0.206 b	0.553 ± 0.107 a
(E)-2-Hexenal	5.474 ± 1.044 b	10.305 ± 2.251 c	1.346 ± 0.166 a	9.629 ± 0.776 a	19.002 ± 3.906 b	8.177 ± 0.496 a
Total	*34.445 ± 3.815 b*	*45.727 ± 8.718 c*	*13.206 ± 1.925 a*	*44.393 ± 4.949 a*	*79.237 ± 5.398 b*	*35.209 ± 5.113 a*
Other compounds						
Hexyl acetate	n.d.	n.d.	n.d.	0.206 ± 0.043 a	0.721 ± 0.159 b	0.554 ± 0.115 b
Methyl jasmonate	0.064 ± 0.006 a	0.077 ± 0.009 a	0.121 ± 0.016 b	1.738 ± 0.381 b	0.222 ± 0.038 a	0.114 ± 0.022 a

All parameters are listed with their standard deviation (n = 3). For each season and compound, different letters indicate significant differences between the samples ($p \leq 0.05$). n.d.: not detected.

Figure 2 shows the concentration of C_{13} norisoprenoids found in the control and in the grapes from the applications to vines of MeJ and ACP-MeJ, in 2019 and 2020 seasons. In 2019, (E)-β-damascenone (Figure 2a), (Z)-β-damascenone (Figure 2b), and 1,1,6-trimetil-1,2-dihidronaftaleno (TDN) (Figure 2e), increased their concentration in MeJ grapes and decreased their concentration in ACP-MeJ samples with respect to control. The (E)-β-damascenone (Figure 2a) was the most abundant C_{13} norisoprenoid in the samples, predominantly over the rest of the compounds of this group. This fact is expected because this compound is one of the most abundant norisoprenoid in the grapes [12,41]. TDN is one of the most polarising, and maybe the less studied C_{13} norisoprenoid [39], its typical aroma descriptor is pretolor kerosene. In the case of β-ionone (Figure 2c), which provides violet notes [42], and β-cyclocitral (Figure 2d), both significantly decreased their amount in ACP-MeJ grapes relative to control and MeJ samples. Regarding the total C_{13} norisoprenoids (Figure 2f) in 2019, MeJ treatment increased its content with respect to control grapes. This could be probably due to the fact that the MeJ increases the activity of the enzymes involved in the synthesis of these compounds [43], which derive from biodegradation of carotenoids [40,44], whereas ACP-MeJ treatment decreased it. Therefore, despite applying the same product, the dose was 10 times lower, and maybe it was too low to affect enzyme activity.

In 2020, (E)-β-damascenone (Figure 2a), (Z)-β-damascenone (Figure 2b), and β-cyclocitral (Figure 2d), did not suffer variations in their content in MeJ and ACP-MeJ grapes with respect to control samples. In this season, β-ionone (Figure 2c) significantly increased its amount in MeJ grapes with respect to ACP-MeJ and control grapes. The increase of β-ionone with MeJ may be justified because β-ionone is a derivative of β-carotene [39], and MeJ accelerates its degradation [45]. TDN (Figure 2e) increased its concentration in grapes from both treatments (MeJ and ACP-MeJ) with respect to control grapes. As for total C_{13} norisoprenoids (Figure 2f), in 2020 neither treatment had a significant effect on its amount with respect to control samples. Interestingly, C_{13} norisoprenoids, derived from the breakdown of carotenoids via chemical, photochemical and oxidase-coupled degradation or enzymatic cleavage [5], generally unchanged with the MeJ treatments in 2020. These results contrast with those obtained by Gutiérrez-Gamboa et al., 2019 [38], where the application with MeJ decreased the amount of C_{13} norisoprenoids.

Terpeneoids and C_{13} norisoprenoids are very important in the floral aroma of grapes. Respect to the C_{13} norisoprenoids, β-damascenone and β-ionone are the most important, since they strongly contribute to the desirable flavor and odor in wines, due to their low perception thresholds [40,46].

Figure 3 shows the concentration of benzenoids found in the control and in the grapes from the foliar treatments with MeJ and ACP-MeJ, in 2019 and 2020 seasons. In 2019, 2-phenylethanol (Figure 3a), eugenol (Figure 3c) and benzyl alcohol (Figure 3d) decreased their concentration in grapes from both treatments (MeJ and ACP-MeJ) with respect to control grapes, with significantly lower content in ACP-MeJ samples. In the study of Marín-San Román et al., 2020 [12], 2-phenylethanol also decreased when MeJ is applied to vines. 2-Phenylethanal (Figure 3b) significantly decreased its amount in grapes from ACP-MeJ treatment with respect to control and MeJ samples. Finally, in 2019, the content of total benzenoids (Figure 3e) decreased with both treatments respect to control samples, with significantly lower content in ACP-MeJ grapes. This trend was also observed in the work of Gutiérrez-Gamboa et al., 2019 [38].

Regarding to the 2020 season, the concentration of 2-phenylethanol (Figure 3a) and benzyl alcohol (Figure 3d) increased in ACP-MeJ grapes with respect to those from the other two samples. 2-Phenylethanol and benzyl alcohol, which in grapes derive from aromatic amino acids, were the principal benzenoid compounds [5]. The content of 2-phenylethanal in grapes (Figure 3b) showed no significant differences in MeJ treatments with respect to the control. Eugenol was not detected in grapes in this second season (Figure 3c). The concentration of total benzenoids (Figure 3e) significantly increased with the ACP-MeJ treatment with respect to the control and MeJ ones.

Terpenoids, C_{13} norisoprenoids, and some benzenoid compounds are the most important grape aroma compounds present in the pulp and skin of the berries in both free and glycoside forms. These compounds are transferred to the wine during the winemaking and depend on the process used [47].

Table 2 shows the concentration of alcohols, carbonyl compounds, C6 compounds, and other compounds in grapes from the control, MeJ, and ACP-MeJ foliar treatments, in 2019 and 2020 seasons.

As regards to the alcohols in the 2019 season, the concentration of n-heptanol decreased with both MeJ and ACP-MeJ treatments with respect to the control grapes, being significantly lower the concentration in ACP-MeJ grapes. Both, n-octanol and n-nonanol decreased their concentrations in ACP-MeJ grapes with respect to control and ACP-MeJ samples; while the content of 1-octen-3-ol and 2-ethyl-1-hexanol decreased in grapes from both treatments (MeJ and ACP-MeJ) to the same extent. In 2019, the concentration of total alcohols decreased with both MeJ treatments respect to the control one (Table 2). In 2020, n-heptanol concentration did not change with either treatment (MeJ and ACP-MeJ). The content of n-octanol decreased in samples from both treatments (MeJ and ACP-MeJ) with respect to the control grapes. The n-nonanol content decreased only in the ACP-MeJ samples with respect to the control and MeJ grapes. In contrast, 1-octen-3-ol and 2-ethyl-1-hexanol only decreased in MeJ grapes relative to control and ACP-MeJ samples. The total alcohols content, in 2020, decreased in MeJ grapes, while ACP-MeJ did not show significant differences with the control (Table 2).

Among the carbonyl compounds, in 2019, heptanal and nonanal decreased their concentrations in the grapes from both treatments with respect to the control. The concentration of (E)-2-octenal and (E)-2-nonenal did not show significant differences between treatments. The content of decanol, (E,E)-2,4-hexadienal, (E,E)-2,4-nonadienal, γ-decalactone, and 6-methyl-3,5-heptadien-2-one, decreased only with the ACP-MeJ treatment with respect to control and MeJ grapes. This same trend was followed by the content of total carbonyl compounds in grapes (Table 2). In 2020, heptanal, nonanal, (E)-2-nonenal, and decanal decreased their concentration when grapevines were treated with MeJ and ACP-MeJ with respect to control grapes. Moreover, the concentration of (E)-2-octenal, (E,E)-2,4-hexadienal, and (E,E)-2,4-nonadienal decreased only when grapevines were treated with the MeJ with respect to the control and ACP-MeJ samples. On the contrary, γ-decalactone decreased its content in ACP-MeJ treated grapes with respect to control and MeJ ones. The concentration of 6-methyl-3,5-heptadien-2-one in grapes was not affected by the foliar applications. Total carbonyl compounds, in 2020, decreased in MeJ grapes compared to control and ACP-MeJ samples (Table 2).

Regarding the C6 compounds, which, in high concentrations, can provide negative notes, in 2019, the concentration of n-hexanol and n-hexanal decreased in ACP-MeJ grapes compared to control and MeJ samples. The content of (Z)-3-hexen-1-ol + (E)-2-hexen-1-ol decreased in both MeJ and ACP-MeJ grapes relative to the control ones. The concentration of (E)-2-hexenal significantly increased in MeJ grapes respect to the control, whereas its content decreased in ACP-MeJ samples (Table 2). Total C6 compounds followed the same trend as the latter compound, as well as was observed by Gutiérrez-Gamboa et al., 2019 [38]. In 2020, n-hexanol, (Z)-3-hexen-1-ol + (E)-2-hexen-1-ol, and (E)-2-hexenal increased their concentration in MeJ grapes respect to the other samples; since these compounds were the majority, total C6 compounds followed the same trend. Garde-Cerdán et al., 2018 [34] demonstrated that the application of MeJ increased the content of C6 compounds in the *Vitis vinifera* L. cv. Tempranillo variety. However, n-hexanal content increased in MeJ grapes, but decreased in ACP-MeJ samples compared to control. In this way, the increase in C6 aldehydes observed in the studied grapes, as a consequence of MeJ treatment, could be due to modification of the pathways involved in the formation of fatty acids [21]. These compounds are responsible for green aromas [40,48].

For the rest of the aroma compounds determined in the *Vitis vinifera* L. cv. Tempranillo grapes, in 2019, only methyl jasmonate was quantified, which increased its concentration

in grapes from ACP-MeJ foliar treatment with respect to control and MeJ samples (Table 2). In 2020, hexyl acetate, which increased its concentration with both treatments (MeJ and ACP-MeJ), and methyl jasmonate, which decreased its concentration with both treatments (MeJ and ACP-MeJ), were quantified in grapes.

The differences in the amount of volatile compounds found between vintages may be due to climatic differences between them. For example, the difference in average precipitation in August (11.5 L/m^2 in 2019 and 32.9 L/m^2 in 2020). Moreover, as can be seen in Table 1, in the case of control berries and ACP-Mej treated berries, the probable alcohol content is higher in 2019 than in 2020, which may affect the content of volatile compounds in the grapes.

3.3. Factorial (Treatment, Season and Their Interaction) and Discriminant Analysis of the Aroma Compounds in Vitis vinifera L. cv. Tempranillo Grapes from 2019 and 2020 Seasons

Tables 3 and 4 show the factorial analysis of the general parameters (Table 3) and volatile compounds (Table 4) of the grapes with the two factors studied: treatment (control, MeJ, ACP-MeJ) and season (2019 and 2020).

In Table 3, it can be seen that MeJ foliar treatments did not affect must enological parameters, except in the amount of total phenols, where the application of MeJ and ACP-MeJ increased its concentration with respect to those from the control grapes. However, some annual differences were observed. In fact, the weight of 100 berries, and the ammonium nitrogen were significantly lower in the grapes harvested in the 2019 than in 2020, while values of total acidity, fructose (Fru), malic acid, and total phenols were significantly lower in 2020 than in 2019. For any enological parameter, there was no significant interaction between the two factors (treatment and season) but for the ammonium nitrogen, amino nitrogen, and YAN.

Regarding to the treatment factor, Table 4 shows that, for terpenoids, MeJ foliar application increased the grape concentration of limonene, p-cymene, linalool, α-terpineol, and total terpenoids with respect to control and ACP-MeJ grapes. For the remaining terpenoids, MeJ had no significant effect respect to control samples. On the other hand, ACP-MeJ applications showed no effect on the studied terpenoids. For C_{13} norisoprenoids, MeJ foliar application increased the concentration of β-ionone, and TDN with respect to the other two samples (control and ACP-MeJ). β-Cyclocitral content was similar in control and MeJ samples but higher than in ACP-MeJ one. The application of ACP-MeJ only increased the concentration of TDN with respect to the control grapes, being MeJ the most effective treatment (Table 4). MeJ treatment did not increase the concentration of benzenoid compounds. However, this treatment decreased the concentration of eugenol and benzyl alcohol with respect to the control samples. On the other hand, ACP-MeJ treatment increased the 2-phenylethanol concentration, and, since this is the most abundant benzenoid, ACP-MeJ foliar application also increased the concentration of total benzenoids respect to the control and MeJ treatments. On the other hand, foliar application of ACP-MeJ decreased the concentration of 2-phenylethanal, eugenol, and benzyl alcohol with respect to control and MeJ grapes (Table 4). For alcohols, MeJ treatment decreased the amount of n-octanol, 1-octen-3-ol, 2-ethyl-1-hexanol, and total alcohols, while the other compounds remained unaffected. In the case of the ACP-MeJ treatment, its application to vines decreased the grape concentration of all alcohols respect to the control one. For carbonyl compounds, MeJ foliar application did not increase the concentration of any of them respect to the control. However, MeJ application decreased the concentration of heptanal, (E)-2-octenal, nonanal, decanal, and total carbonyl compounds with respect to the control. ACP-MeJ foliar application decreased the concentration of heptanal, nonanal, (E)-2-nonenal, decanal, 6-methyl-3,5-heptadien-2-one, and total carbonyl compounds with respect to the control and increased the content of (E)-2-octenal respect to MeJ application (Table 4). Regarding C6 compounds, MeJ treatment increased the concentration of n-hexanol, n-hexanal, (E)-2-hexenal, and total C6 compounds with respect to the control and ACP-MeJ grapes. Foliar application of ACP-MeJ did not increase the concentration of C6

compounds, but decreased the amount of the n-hexanal, (Z)-3-hexen-1-ol + (E)-2-hexen-1-ol, (E)-2-hexenal, and total C6 compounds with respect to control grapes. For other aroma compounds, like hexyl acetate and methyl jasmonate, both MeJ treatments increased the concentration of hexyl acetate and decreased the concentration of methyl jasmonate with respect to the control samples. As regards the season factor, some compounds were found in greater quantities in 2019 and others in 2020 (Table 4). In 2019, the terpenoids: geraniol, geranic acid, and geranyl acetone; the C_{13} norisoprenoids: β-ionone, and β-cyclocitral; the benzenoid compounds: 2-phenylethanal, eugenol, benzyl alcohol and the total benzenoid compounds; the alcohols: 1-octen-3-ol, and 2-ethyl-1-hexanol, and the total alcohols; the carbonyl compounds: heptanal, (E)-2-octenal, (E)-2-nonenal, (E,E)-2,4-hexadienal, (E,E)-2,4-nonadienal, and 6-methyl-3,5-heptadien-2-one, and the total carbonyl compounds; and the C6 compound: n-hexanal, were found in greater quantities than in 2020. On the contrary, in the second season, the terpenoids: limonene, and p-cymene and the total terpenoids; the C_{13} norisoprenoids: (E) and (Z)-β-damascenone, and TDN, and the total C_{13} norisoprenoids; the benzenoid compound: 2-phenylethanol; the alcohols: n-octanol, and n-nonanol; the carbonyl compounds: nonanal, and γ-decalactone; the C6 compounds: n-hexanol, (Z)-3-hexel-1-ol + (E)-2-hexen-1-ol, and (E)-2-hexenal, and the total C6 compounds; and the hexyl acetate and methyl jasmonate were found in higher amounts than those from 2019. In this case, the treatment-season interaction was significant for all compounds except β-ionone, n-octanol, (E)-2-octenal, and (E)-2-hexenal (Table 4).

In order to classify the different samples, discriminant analysis was performed on data expressing the concentration of volatile compounds in control, MeJ, and ACP-MeJ samples. The results are shown in Figure 4. In 2019 season (Figure 4a), Function 1 explained a very high percentage of variance 90.5% and Function 2 explained only 9.5%, so the total of variance explained was 100%. The variables that contributed the most to the discriminant model were α-terpineol, 2-ethyl-1-hexanol, geraniol, and 1-hexanol (Function 1) and 2-ethyl-1-hexanol, geraniol, and TDN (Function 2). The discriminant model showed a good separation among the different samples. In the case of the data from 2020 (Figure 4b), Function 1 explained 89.8% and Function 2 explained 10.2%, (total variance explained = 100%). The variables that contributed the most to the discriminant model were: (Z)-3-hexen-1-ol, β-ionone, 2-phenylethanol, and 6-methyl-3,5-heptadiene-2-one for Function 1 and 2-phenylethanol, 2-ethyl-1-hexanol, and (E)-2-octenal for Function 2. Again, the discriminant showed a very good separation among the different samples. Figure 4c shows the discriminant analysis for both seasons, with treatment as factor. It can be observed that there is a good separation between the treatments. Function 1 explained 56.4% of the variance and Function 2 explained 43.6% (total variance = 100%). The variables with the highest contribution were p-cymene, and (E)-2-nonenal for Function 1, and heptanal, (E)-2-nonenal, and decanal for Function 2. Considering the sample as factor (Figure 4d), Function 1 explained almost all the variance 98.7%, and Function 2 only 0.7%, so the total of variance explained was 99.4%. Again, the discriminant shows a good separation between all samples. The variables that contributed the most to the discriminant model were (E)-β-damascenone, p-cymene, 1-octanol, and linalool for Function 1, and p-cymene, 2-phenylethanol, 1-octanol, and 1-hexanol for Function 2.

Table 3. Multifactor analysis of variance of general parameters of the musts with the two factors studied: treatment (control, MeJ, ACP-MeJ) and season (2019 and 2020).

	Weight of 100 Berries (g)	°Brix	Probable Alcohol (% *v*/*v*)	pH	Total Acidity (g/L)	Glu + Fru (g/L)	Glu (g/L)	Fru (g/L)	Malic Acid (g/L)	Total Phenols (mg/L)	Ammonium Nitrogen (mg N/L)	Amino Nitrogen (mg N/L)	YAN (mg N/L)
Treatment (T)													
Control	156.63 a	23.50 a	13.80 a	3.79 a	4.37 a	233.14 a	113.74 a	119.39 a	1.73 a	863.47 a	99.58 a	135.52 a	235.10 a
MeJ	174.74 a	22.20 a	12.91 a	3.74 a	4.87 a	217.06 a	104.48 a	112.58 a	2.04 a	954.82 b	104.00 a	170.87 a	274.87 a
ACP-MeJ	155.92 a	22.87 a	13.36 a	3.77 a	4.58 a	227.37 a	109.75 a	117.62 a	1.95 a	967.05 b	108.03 a	163.97 a	272.00 a
Season (S)													
2019	124.14 a	23.43 a	13.75 a	3.81 a	4.98 b	232.25 a	111.32 a	120.94 b	2.43 b	1281.10 b	95.25 a	165.44 a	260.68 a
2020	200.71 b	22.28 a	12.96 a	3.73 a	4.23 a	219.46 a	107.33 a	112.12 a	1.38 a	575.79 a	112.49 b	148.13 a	260.63 a
Interaction													
$T \times S$	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	*	*	*

Glu: glucose, Fru: fructose, YAN: yeast assimilable nitrogen. For each parameter and factor, different letters indicate significant differences between samples ($p \leq 0.05$). Interaction: *, $p \leq 0{,}05$; N.S., not significant ($p > 0.05$).

Table 4. Multifactor analysis of variance of grape aroma compounds (expressed as μg/L) with the two factors studied: treatment (control, MeJ, ACP-MeJ) and season (2019 and 2020).

	Treatment (T)			Season (S)		
	Control	**MeJ**	**ACP-MeJ**	**2019**	**2020**	**Interaction (T × S)**
Terpenoids						
Limonene	0.101 b	0.129 c	0.078 a	0.092 a	0.113 b	*
p-Cymene	0.154 a	0.319 b	0.141 a	0.156 a	0.254 b	*
Linalool	0.058 a	0.140 b	0.052 a	0.089 a	0.078 a	***
α-Terpineol	0.045 a	0.107 b	0.056 a	0.073 a	0.066 a	***
Geraniol	0.026 a	0.025 a	0.024 a	0.029 b	0.021 a	***
Geranic acid	0.098 a	0.104 a	0.090 a	0.111 b	0.084 a	***
Geranyl acetone	0.019 ab	0.021 b	0.016 a	0.021 b	0.016 a	***
Total	*0.501 a*	*0.846 b*	*0.457 a*	*0.570 a*	*0.632 b*	***
C_{13} norisoprenoids						
(E)-β-Damascenone	4.093 a	4.964 a	4.080 a	1.815 a	6.944 b	*
(Z)-β-Damascenone	0.275 a	0.353 a	0.327 a	0.129 a	0.508 b	*
β-Ionone	0.105 b	0.186 c	0.076 a	0.170 b	0.061 a	N.S.
β-Cyclocitral	0.112 b	0.113 b	0.074 a	0.148 b	0.051 a	***
TDN	0.210 a	0.346 c	0.262 b	0.197 a	0.347 b	***
Total	*4.795 a*	*5.942 a*	*4.936 a*	*2.500 a*	*7.949 b*	*
Benzenoid compounds						
2-Phenylethanol	5.917 a	5.528 a	10.099 b	6.254 a	8.109 b	***
2-Phenylethanal	4.492 b	4.722 b	3.794 a	6.128 b	2.544 a	*
Eugenol	0.010 c	0.005 b	0.002 a	0.011 b	n.d. a	***
Benzyl alcohol	0.826 b	0.511 a	0.468 a	0.921 b	0.282 a	***
Total	*11.245 a*	*10.766 a*	*14.362 b*	*13.314 b*	*10.935 a*	***
Alcohols						
n-Heptanol	0.055 b	0.045 ab	0.037 a	0.045 a	0.045 a	**
n-Octanol	0.258 b	0.204 a	0.173 a	0.157 a	0.266 b	N.S.
n-Nonanol	0.130 b	0.152 b	0.062 a	0.051 a	0.178 b	**
1-Octen-3-ol	0.384 b	0.185 a	0.195 a	0.378 b	0.131 a	***
2-Ethyl-1-hexanol	2.479 c	1.330 a	1.883 b	2.170 b	1.624 a	***
Total	*3.307 c*	*1.916 a*	*2.349 b*	*2.803 b*	*2.245 a*	***
Carbonyl compounds						
Heptanal	0.034 b	0.020 a	0.021 a	0.041 b	0.010 a	*
(E)-2-Octenal	0.051 b	0.038 a	0.047 b	0.054 b	0.037 a	N.S.
Nonanal	0.292 b	0.129 a	0.159 a	0.134 a	0.253 b	*
(E)-2-Nonenal	0.056 b	0.049 ab	0.045 a	0.066 b	0.034 a	*
Decanal	0.094 c	0.069 b	0.043 a	0.064 a	0.073 a	*
(E,E)-2,4-Hexadienal	0.944 a	0.888 a	0.763 a	1.145 b	0.585 a	***
(E,E)-2,4-Nonadienal	0.069 a	0.069 a	0.053 a	0.090 b	0.037 a	***
γ-Decalactone	0.135 a	0.149 a	0.164 a	0.112 a	0.0187 b	***
6-Methyl-3,5-heptadien-2-one	0.054 b	0.054 b	0.036 a	0.070 b	0.026 a	*
Total	*1.729 b*	*1.465 a*	*1.331 a*	*1.775 b*	*1.242 a*	***
C6 compounds						
n-Hexanol	14.107 a	24.671 b	11.398 a	5.467 a	27.984 b	***
n-Hexanal	16.912 b	22.448 c	7.592 a	19.375 b	11.926 a	*
(Z)-3-Hexen-1-ol + (E)-2-Hexen-1-ol	0.848 b	0.710 b	0.457 a	0.576 a	0.767 b	***
(E)-2-Hexenal	7.552 b	14.653 c	4.761 a	5.708 a	12.269 b	N.S.
Total	*39.419 b*	*62.482 c*	*24.207 a*	*31.126 a*	*52.946 b*	**
Other compounds						
Hexyl acetate	0.103 a	0.361 b	0.277 b	n.d. a	0.494 b	***
Methyl jasmonate	0.901 b	0.149 a	0.117 a	0.087 a	0.691 b	***

TDN: 1,1,6-trimethyl-1,2-dihydronaphthalene. For each parameter and factor, different letters indicate significant differences between samples ($p \leq 0.05$). Interaction: *, $p \leq 0.05$, **, $p \leq 0.01$, ***, $p \leq 0.001$, and N.S., not significant ($p > 0.05$).

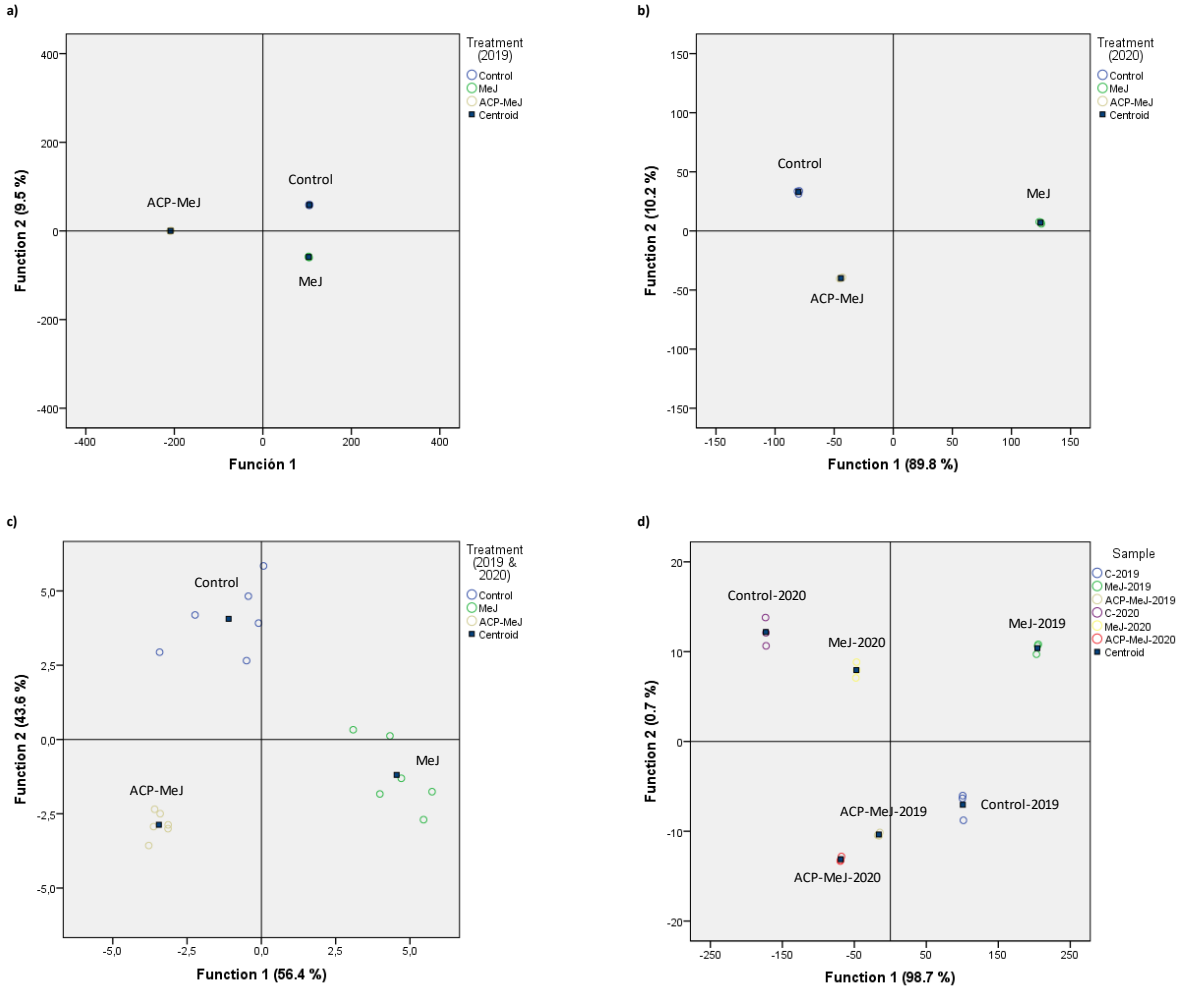

Figure 4. Discriminant analysis of volatile compounds content (μg/L) in grapes from control, methyl jasmonate (MeJ) and nanoparticles doped with this elicitor (ACP-MeJ) treatments, in (**a**) 2019, (**b**) 2020, and (**c**) 2019 & 2020 seasons, carried out with the treatment as factor; and (**d**) carried out with the sample as factor.

4. Conclusions

The use of elicitors through foliar applications to *Vitis vinifera* L. cv. Tempranillo grapevines affected grape volatile composition. Methyl jasmonate (MeJ) treatment increased the concentration in the grapes of total terpenoids, and total C6 compounds in 2019 and 2020, and the total C_{13} norisoprenoids in 2019; while decreased the concentration of total benzenoid compounds in 2019, total carbonyl compounds in 2020, and total alcohols in both seasons. In addition, ACP-MeJ increased the amount in the grapes of total terpenoids, and total benzenoid compounds in 2020; whereas decreased the content of total terpenoids, total C_{13} norisoprenoids, total benzenoid compounds, total alcohols, total carbonyl compounds, and C6 compounds in 2019. These results are not completely conclusive since this is the first time that foliar application of ACP-MeJ has been performed in *Vitis vinifera* L. cv. Tempranillo grapevines to evaluate the effect on grape aroma. Nevertheless, the results suggest that MeJ is still a better option than ACP-MeJ in order to enhance the grape volatile composition, but considering that the applied dose in the ACP-MeJ treatment was 10 times lower than that applied in the MeJ conventional treatment, it can be said that nanotechnology has given very positive results in order to improve the grape aromatic quality.

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Application of Elicitors, as Conventional and Nano Forms, in Viticulture: Effects on Phenolic, Aromatic and Nitrogen Composition of Tempranillo Wines

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Abstract: The phenolic, aromatic and nitrogen composition of a wine determines its organoleptic profile and quality. Elicitors have been used as a tool to stimulate the plant's defense systems, favoring the synthesis of secondary metabolites. In this pioneering study, the elicitor methyl jasmonate in conventional form (MeJ) and in nanoparticle form (ACP-MeJ), with a concentration ten times lower, was applied in a Tempranillo vineyard over two seasons. The phenolic, nitrogen and volatile composition and the sensory properties of the MeJ-based wines were determined. The results showed that the effects of foliar applications of MeJ modify the wine composition. Thus, although the total concentration of most of the groups of phenolic compounds was not altered, several compounds, such as petunidin-3-glucoside, quercetin-3-glucoside, epigallocatechin and most of the stilbenes, increased, in both years, in the treated wines. Amino acids were influenced differently in each of the years studied, and volatile compounds generally did not improve in the treated wines. However, the ACP-MeJ wines were the best rated by the tasters, highlighting their equilibrium on the taste and their genuineness and odor quality. Therefore, foliar applications of ACP-MeJ can be considered a useful tool to improve wine quality.

Keywords: methyl jasmonate; nanoparticles; wine taste properties; foliar application; phenolic compounds; amino acids; aroma

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1. Introduction

Foliar applications of phytohormones, compounds that regulate plant development, have been effective in reducing the adverse effects of different abiotic stresses on several plant species [1]. Jasmonic acid and its methyl ester, methyl jasmonate (MeJ), play an essential role in the regulation of reactions associated with biotic and abiotic stresses in plants [2] and acts as a signal molecule and inductor of plant secondary metabolites [3–5]. These compounds are endogenous messenger molecules that are ubiquitous in a wide range of higher plant species, where their levels are high in the reproductive tissues and flowers, but very low in the mature leaves and roots [6]. MeJ activates the defense mechanisms of plants in response to pathogens, insect wounds and various environmental stresses [7] as well as modulates many crucial processes in plant growth and development, such as vegetative growth, cell cycle regulation, anthocyanin biosynthesis, fruit ripening, nitrogen and phosphorus uptake and glucose transport, among other processes [6,8]. Different studies have shown that MeJ applications in the vineyard induce an improvement or modification of the nitrogen, volatile and phenolic composition of grapes of different varieties and under different climatic conditions. Thus, Garde-Cerdán et al. [9] reported

an increase in some amino acids in Tempranillo musts after applying MeJ in the vineyard. Other authors such as Flores et al. [10] showed that the MeJ application as a postharvest treatment enhances anthocyanin accumulation in grapes, and Larrondo et al. [11] reported that the MeJ application is able to stimulate accumulation of stilbene in leaves and berries of grapevines. Portu et al. [3] observed increases of secondary metabolites in Tempranillo grapes after MeJ application in grapevines. Meanwhile, Garde-Cerdán et al. [12] described the influence of MeJ applications to vineyards on grape volatile composition. In their study on Grenache grapes, Marín-San Román et al. [13] observed that MeJ applications in the vineyard increased the content of volatile compounds, mainly favoring terpenoids and C13 norisoprenoids. In addition, the mixed phenylalanine + MeJ treatment favored the increase in terpenoids and benzenoids content in the grapes. However, few authors have studied the effect of foliar applications of MeJ on the nitrogen, phenolic and volatile composition of the wines. Therefore, to the best of our knowledge, only authors such as Ruiz-García et al. [14] and Ruiz-García and Gómez-Plaza [15] in Monastrell, Portu et al. [4] in Tempranillo, and Gil-Muñoz et al. [16] in Monastrell, Merlot and Syrah, reported increases of secondary metabolites in grapes and wines after using a foliar application of MeJ in the vineyard.

Nowadays, nanotechnology is becoming as a promising tool with great potential to release agrochemicals to the crops in a more efficient and safer way [17]. Compared to bulk materials, nanomaterials (size < 100 nm) are generally highly reactive, due to their high surface to volume ratio and their small dimensions [18]. The use of nanoparticles in agriculture could reduce the quantities of chemical products applied in the field, since it has been demonstrated that they minimize product losses, increase product absorption by the plant and inhibit rapid changes in the chemical properties of nutrients [19]. Thus, Parra-Torrejón et al. [20] developed amorphous calcium phosphate nanoparticles (ACP) (mimicking the precursor phase of bone mineral) doped with MeJ, which allows the particles to be retained on the leaf surface for a longer period of time, increasing the efficiency of MeJ action after foliar application, being delivered slowly and gradually over time [21]. This also makes it possible to reduce some of the disadvantages of foliar applications of MeJ, such as its high volatility, low water solubility and its high economic cost [22]. Additionally, Xiong et al. [23] and Epple [24] have shown that ACP used in agriculture as fertilizers is safe as long as bioavailability, movement in soils and human toxicity issues are taken into account. Although, in viticulture, the use of nanoparticles is increasing, especially as an environmentally sustainable fertilizer (for instance, urea-doped nanoparticles such as reported Gaiotti et al. [18] and Pérez-Álvarez et al. [19]) or a winegrowing practice that improves nitrogen plant uptake, increasing the nitrogen quality of the grapes [21], their implications in the composition of wines as a final product in the wine sector are not receiving as much attention. Therefore, the aim of this work was to study, for the first time, the effects of foliar treatments of MeJ, in conventional and nano-size form (with a dose of MeJ ten times lower than the conventional form), on phenolic, aromatic and nitrogen composition of Tempranillo wines over two vintages.

2. Materials and Methods

2.1. Vineyard Site, Grapevine Treatments, Vinification and Samples

The trial was conducted during 2019 and 2020 seasons on an experimental vineyard of Tempranillo (*Vitis vinifera* L.) cultivar grafted onto R-110 rootstock, located in Finca La Grajera, Logroño, La Rioja (Spain). The vines were trained to a VSP (vertical shoot positioned) trellis system and were planted in 1997 with 2.80 m intra-row × 1.25 m inter-row space. The annual rainfall and mean temperature in 2019 and 2020, were, respectively, 519 and 498 mm and 13.8 °C for both seasons. In the grape-growing period from 1 April to end-September, the rainfall and the mean temperature were 248 and 218 mm, and 18.3 and 18.6 °C, in the 2019 and 2020 seasons, respectively.

The experiment design included 10 vines per replicate of each treatment, and they were arranged in a complete randomized block design, in three randomized blocks, assigned to the following treatments: (i) control, (ii) foliar application of methyl jasmonate (MeJ), and

(iii) foliar application of nanoparticles doped with this elicitor (ACP-MeJ). Control plants were sprayed only with a water solution of Tween 80, used as wetting agent (1 mL/L). To carry out the MeJ-based treatments, aqueous solutions were prepared with a MeJ concentration of 10 mM (according to previous works, Garde-Cerdán et al. [9,12]) and 1 mM of ACP-MeJ, according to Pérez-Álvarez et al. [21], using Tween 80. The foliar applications of each of the three treatments were performed twice, at veraison and one week later, applying 200 mL/plant over leaves for each application.

Grapes from all grapevines and treatments were manually harvested at their optimum technological maturity, i.e., when the weight of 100 berries remained constant and the probable alcohol reached 13 (% *v*/*v*). At the winery, the grape clusters were destemmed and crushed separately for each treatment and repetition. The resulted pomace was introduced in one 30 L tank for each one to carry out the maceration-fermentation. Therefore, 9 elaborations were carried out (3 treatments × 3 repetitions/treatment). They were protected by the addition of 50 mg SO_2/kg of grapes and inoculated (at a dosage of 20 g/hL) with a commercial *Saccharomyces cerevisiae* strain (Safoeno SC22, Fermentis, Marcq-en-Barœul, France) responsible for carrying out alcoholic fermentation (at 20 +/− 2 °C). Once the alcoholic fermentation was finished (i.e., when sugar concentration was lower than 2.5 g/L), the wines were racked and placed in 12 L tanks. Then, a commercial *Oenococcus oeni* strain (Viniflora CiNe, CHR Hansen, Hørsholm, Denmark) at 1 g/hL was inoculated into the wines, in order to perform the malolactic fermentation (at 17 +/− 1 °C). For each wine and for each group of compounds studied (amino acids, phenolic compounds and volatile compounds), aliquots samples were frozen and stored at −20 °C until their analysis.

2.2. Determination of Enological Parameters of Wines

The basic enological parameters, alcoholic degree, pH, total acidity and volatile acidity were analyzed using the official methods established by OIV [25]. Malic acid, lactic acid, amino and ammonium nitrogen content, which sum represent the yeast assimilable nitrogen (YAN), and total phenols were determined using a Miura One enzymatic equipment (Tecnología Difusión Ibérica, TDI, Barcelona, Spain). Total anthocyanins content was measured by bleaching using sulfur dioxide [26]. Color intensity (CI) was determined by spectrophotometric absorbance and expressed as the sum of the absorbance at 420, 520 and 620 nm. Total polyphenols index (TPI) was determined by spectrophotometric absorbance at 280 nm after previous dilution of samples.

As the field treatments and the vinifications were performed in triplicate, the results of these parameters are shown as the average of three analyses (n = 3).

2.3. Analysis of Wine Phenolic Compounds by HPLC-DAD

2.3.1. Sample Preparation for the Analysis of Non-Anthocyanin Phenolic Compounds

An amount of 3 mL of each wine sample was diluted with 3 mL of 0.1 N HCl and later was passed through the PCX SPE cartridges (500 mg, 6 mL; Bond Elut Plexa, Agilent, Palo Alto, CA, USA), previously conditioned (5 mL of methanol and 5 mL of water). Then, the cartridges were washed with 5 mL of 0.1 N HCl and 5 mL of water [3]. In order to analyze the non-anthocyanin phenolic compounds (flavonols, flavanols, hydroxybenzoic and hydroxycinnamic acids and stilbenes), the anthocyanin-free fraction was used. The non-anthocyanin phenolic compounds fraction was eluted with 3 × 5 mL of ethanol and dried at 35 °C in a centrifugal evaporator (miVac, Genevac Ltd., Lpswich, Suffolk, UK) and re-solved in 1.5 mL of 20% (*v*/*v*) methanol aqueous solution.

2.3.2. Analysis of Phenolic Compounds by HPLC-DAD

Phenolic compounds were analyzed utilizing an Agilent 1260 Infinity II chromatograph (Palo Alto, Santa Clara, CA, USA) equipped with a diode array detector (DAD). According to Portu et al. [3], wine samples were filtered and injected with a flow rate of 0.630 mL/min on a Lichrospher® 100 RP-18 reversed-phase column (250 × 4.0 mm; 5 µm packing: Agilent, Santa Clara, CA, USA) with a pre-column Lichrospher® 100 RP-18

(4 × 4 mm; 5 µm packing; Agilent, Santa Clara, CA, USA), both thermostated at 40 °C. In order to analyze the anthocyanins, 10 µL of wine sample was injected, using two different eluents: (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 gradient used for the anthocyanin separation was: 0 min, 6% B; 15 min, 30% B; 30 min, 50% B; 35 min, 60% B, 38 min, 60% B, 46 min, 6% B. In order to analyze the non-anthocyanin phenolic compounds fraction, 20 µL of sample was injected and three eluents were used: (A) and (B) as for anthocyanins and a third eluent, (C) methanol/water/formic acid (90:1.5:8.5, *v*/*v*/*v*). The gradient used for the non-anthocyanin separation was: 0 min, 4% B and 0% C; 7 min, 4% B and 0% C; 38 min, 17% B and 13% C; 52 min, 30% B and 20% C; 52.5 min, 40% B and 30% C; 57 min, 50% B and 50% C; 58 min, 50% B and 50% C; 65 min, 4% B and 0% C.

The retention times of available pure compounds and the UV-Vis data obtained from authentic standards and/or published in previous studies [27] were used for identifying the phenolic compounds. In order to quantify the compounds, DAD chromatograms were extracted at 520 nm (anthocyanins), 360 nm (flavonols), 320 nm (hydroxycinnamic acids and stilbenes) and 280 nm (gallic acid and flavanols) and the calibration graphs of the respective standards ($R^2 > 0.99$) were used. If no standard was available, quantification was performed according to the calibration of the most similar compound. Therefore, for the quantification of the anthocyanins in the samples, malvidin-3-*O*-glucoside was used, for flavonols, quercetin-3-*O*-glucoside was used, for free hydroxycinnamic acids and the corresponding tartaric esters, *trans*-caftaric acid was used, for procyanidins B1 and B2 the catechin calibration was used, for epigallocatechin the epicatechin was used, and for *trans*-piceid and *trans*-resveratrol calibration their respective *cis* isomers were used. Phenolic compounds' concentrations in wines were expressed as milligrams per liter of wine (mg/L).

Since field treatments and vinifications were performed in triplicate, the results for phenolic compounds are the average of the analyses of three samples ($n = 3$).

2.4. Determination of Wine Aromatic Compounds by GC-MS

The determination of the wine volatile compounds was carried out based on Garde-Cerdán et al.'s [28] method. Briefly, 8 mL of each wine sample was centrifuged (3220× *g*, at 4 °C for 15 min) and placed in a 10 mL tube containing a magnetic stir bar and 10 µL of the internal standard 2-octanol (Sigma-Aldrich, Madrid, Spain). The wine volatile compounds extraction was performed by stirring the sample with 400 µL of dichloromethane (Merck, Darmstadt, Germany) for 15 min. After cooling for 10 min at 0 °C, the organic phase was separated by centrifugation (5031× *g*, 10 min, 4 °C) and the extract was recovered into a vial.

The analytes determination was carried out using a Gas Chromatograph (GC) with a Mass Detector (MS) (Agilent, Santa Clara, CA, USA) and a VF-Wax 52 CB (60 m × 0.25 mm i.d. × 0.25 µm) capillary column (Agilent, Santa Clara, CA, USA) was used. The volume of injection of each sample was 2 µL and the injector temperature was programmed from 40 °C to 250 °C, at 180 °C/min. The oven temperature was held for 2 min at 50 °C. After that, the oven was programmed to increase at 3 °C/min from 50 °C to 250 °C. The detector was operated at electronic impact mode (70 eV), with an acquisition range (*m*/*z*) from 29 to 260. The NIST library and the comparison of results with the mass spectrum of available standards (Sigma-Aldrich) was used to identify the volatile compounds. A semi-quantification was carried out, relating the areas of each volatile compound with the area and the known concentration of 2-octanol, the internal standard. The concentrations of wine aromatic compounds were expressed as milligrams per liter of wine (mg/L).

As the field treatments and vinifications were performed in triplicate, the results of wine volatile compounds are shown as the average of three analyses ($n = 3$).

2.5. Analysis of Wine Nitrogen Compounds by HPLC-DAD

The analysis of amino acids in wines was performed according to the methodology reported by Garde-Cerdán et al. [29]. Briefly, amino acids were derivatizated in a basic methanolic medium reaction performed in a screw-cap test tube over 30 min in an ultrasound bath (Sonorel Digital 10 P, Bandelin, Berlin, Germany): 1.75 mL of borate buffer 1 M (pH 9), 750 µL of methanol (Merck, Darmstadt, Germany), 1 mL of sample (previously filtered), 20 µL of internal standard (L-2-aminoadipic acid, 1 g/L) (Sigma-Aldrich, Madrid, Spain) and 30 µL of derivatization reagent diethyl ethoxymethylenemalonate (DEEMM) (Sigma-Aldrich, Spain) were mixed. In order to complete degradation of excess DEEMM and reagent by-products, the wine sample was heated at 70–80 °C in a constant temperature heater (Dri-Block DB 3D, Techne, Newcastle upon Tyne, England) for 2 h.

The analyses were performed using a Shimadzu Nexera X2 Ultra High-Performance Liquid Chromatograph (UHPLC) (Shimadzu, Kyoto, Japan) equipped with an automatic liquid sampler and a diode array detector (DAD). An ACE HPLC column (C18-HL) (Aberdeen, Scotland) with particle size 5 µm (250 mm × 4.6 mm) was used in order to perform the chromatographic separation. According to Garde-Cerdán et al. [29], two eluents, previously filtered through a 0.45 µm Durapore ® membrane pore filter (Merck), were used as mobile phases (gradient elution): Phase (A), 25 mM acetate buffer, pH 5.8, with 0.4 g of sodium azide; phase (B), 80:20 (% v/v) mixture of acetonitrile and methanol (Merck). DAD monitoring at 280, 269 and 300 nm was used for detection. The injected volume of derivatized samples was 50 µL. The target compounds aspartic acid, glutamic acid, asparagine, serine, glutamine, histidine, glycine, threonine + citrulline, arginine, α-alanine, γ-aminobutyric acid (Gaba), proline, tyrosine, valine, methionine, cysteine, isoleucine + tryptophan, leucine, phenylalanine, ornithine and lysine were separated, identified and quantified. The identification was performed according to the retention times and the UV–Vis spectral characteristics of their corresponding standards (Sigma-Aldrich) when derivatizated. Quantification was carried out by using the calibration graphs ($R^2 > 0.98$) of the respective standards in 0.1 N HCl, which underwent the same process of derivatization as the samples. The concentrations of amino acids in wine samples were expressed as milligrams per liter (mg/L).

Since the field treatments and vinifications were performed in triplicate, the results of free amino acids correspond to the average of 3 analyses (n = 3).

2.6. Sensory Analysis of the Wines

Approximately 12 months after the completion of malolactic fermentation, the 9 wines of each year were sensorially evaluated by a 12-member panel who were experienced with Appellation D'Origine Contrôlée (A.O.C., Rioja) Rioja wine tasting methodology. For this, the wines were evaluated in a comparative way, using a totally randomized-order blind tasting system. An amount of 50 mL of wine, approximately, was served to each taster in standard tasting glasses, each one with a random three-digit combination code. The wines were kept at a cool temperature until just before they were served to each of the tasters. Each panel member was provided with a specific tasting file comprising the general odor and taste attribute, following the 100-point method approved by the OIV [30]. It has a scale for each evaluated attribute ranging between 40 (insufficient) to 100 (excellent). The tasting file also included a descriptive evaluation of olfactory attributes (raisined, reds, blacks and white fruit, floral, spicy, alcoholic, herbaceous-vegetal, balsamic, underbrush-forest floor, lactic, oxidation and reduction) as well as the gustatory characteristics (sweetness, acidity, bitterness, alcohol, astringency and equilibrium), on an intensity scale of 1 to 6 (1 the lowest and 6 the highest). These descriptors were selected according to the standard attributes from A.O.C. Rioja Tempranillo wines.

Since the treatments and the wines were performed in triplicate, the results of the sensory analysis of the wines correspond to the average of 3 analyses (n = 3).

2.7. Statistical Analysis

The statistical elaboration of the data was performed using SPSS Version 21.0 statistical package for Windows (SPSS, Chicago, IL, USA). General parameters and phenolic, aromatic and nitrogen compounds data were processed using a two-way variance analysis (ANOVA) ($p \leq 0.05$). The differences between means were compared using the Duncan test ($p \leq 0.05$).

3. Results and Discussion

3.1. Effect of MeJ and ACP-MeJ Foliar Applications on Wine Enological Parameters

General parameters of wines elaborated with grape samples after the applied control, methyl jasmonate (MeJ) and nanoparticles doped with methyl jasmonate (ACP-MeJ) treatments in the vineyard in 2019 and 2020 are shown in Table 1. In 2019, wines from the MeJ and ACP-MeJ groups had lower alcohol content than those from the control treatment. This result could be an advantage of the foliar application of these elicitors as a strategy to reduce the alcohol content of wines, which is strongly demanded by the consumer, and which is increasing due to the climate change. Furthermore, ACP-MeJ reduced the total acidity of the wines with respect to the control wines and MeJ increased the volatile acidity (Table 1). However, all the volatile acidity values were well below 0.6 g/L, which is usually perceived as a spoilage character for wine [31]. The MeJ and ACP-MeJ treatments increased the yeast assimilable nitrogen (YAN) content in wines regarding the control wines. The YAN content is relevant since nitrogen has a key role in the formation of aromatic compounds in wine as well as biogenic amines. Thus, higher amounts of residual nitrogen in wines, together with other factors, increase the risks of microbiological instability and the production of ethyl carbamate and biogenic amines in wines [32]. Regarding the total anthocyanins, wines from the MeJ group had increased content in comparison to the ACP-MeJ wines. The color index (CI) values in wines from ACP-MeJ treatment were reduced with respect to the control wines, with intermediate values for the wines from the MeJ treatment. However, the pH, lactic acid and TPI values did not change in the treated wines with respect to the control wines (Table 1).

Table 1. Basic enological parameters in wines from control, methyl jasmonate (MeJ) and nanoparticles doped with this elicitor (ACP-MeJ) treatments, in 2019 and 2020 seasons.

	2019			2020		
	Control	**MeJ**	**ACP-MeJ**	**Control**	**MeJ**	**ACP-MeJ**
Alcoholic degree (%, *v*/*v*)	13.97 ± 0.31 b	12.57 ± 0.25 a	12.93 ± 0.64 a	12.47 ± 0.70 a	12.18 ± 1.59 a	12.42 ± 0.12 a
pH	3.96 ± 0.07 a	3.90 ± 0.10 a	3.97 ± 0.08 a	3.66 ± 0.08 a	3.70 ± 0.04 a	3.70 ± 0.09 a
Total acidity (g/L) *	4.27 ± 0.10 b	4.08 ± 0.06 ab	3.96 ± 0.15 a	4.43 ± 0.59 a	4.38 ± 0.23 a	4.26 ± 0.17 a
Volatile acidity (g/L) **	0.23 ± 0.02 a	0.28 ± 0.03 b	0.24 ± 0.02 a	0.22 ± 0.02 b	0.18 ± 0.01 a	0.21 ± 0.02 b
Lactic acid (g/L)	1.32 ± 0.10 a	1.36 ± 0.07 a	1.36 ± 0.13 a	0.86 ± 0.07 a	1.14 ± 0.15 b	0.99 ± 0.13 ab
YAN (mg N/L)	18.06 ± 2.08 a	41.65 ± 3.90 c	27.50 ± 1.16 b	30.36 ± 0.54 a	28.40 ± 12.49 a	27.35 ± 8.26 a
Total phenols (mg/L)	2440.83 ± 123.16 a	2160.37 ± 221.12 a	2300.20 ± 236.75 a	1116.63 ± 106.69 a	1263.07 ± 224.95 a	1231.77 ± 75.81 a
Total anthocyanins (mg/L)	1117.33 ± 69.97 ab	1225.67 ± 98.64 b	1019.67 ± 97.01 a	130.99 ± 20.13 a	158.53 ± 18.35 a	155.49 ± 11.41 a
Color index	18.27 ± 1.03 b	17.53 ± 1.81 ab	15.06 ± 0.80 a	6.05 ± 0.55 a	7.70 ± 2.13 a	7.12 ± 0.53 a
TPI	70.83 ± 3.47 a	66.43 ± 7.95 a	64.55 ± 5.79 a	36.82 ± 4.05 a	41.04 ± 8.69 a	40.39 ± 2.33 a

* As g/L tartaric acid; ** as g/L acetic acid. YAN: yeast assimilable nitrogen; TPI: total pholyphenols index. All parameters are listed with their standard deviation (n = 3). For each season and compound, different letters indicate significant differences between the samples ($p \leq 0.05$).

In 2020, the effects of the treatments applied in the vineyard on the enological parameters were less than those described for the 2019 samples. Thus, volatile acidity was reduced in the MeJ wines compared to the wines from the other two treatments (control and ACP-MeJ), but lactic acid was higher than in the control wines (Table 1). These seasonal differences are probably because of the differences between the precipitations in both seasons. Thus, the accumulated rainfall in 2019 (519.7 mm) was higher than in 2020

(497.60 mm), as well as the rainfall through the grapevine cycle (April–September), which was higher in 2019 (247.8 mm) vs. 2020 (217.8 mm), whereas the average temperature in both seasons was the same (13.8 °C).

The slight differences observed in the enological parameters of the wines are in agreement with the results obtained by Pérez-Álvarez et al. [21] in cv. Monastrell musts after ACP-MeJ applications.

3.2. Influence of the Foliar MeJ and ACP-MeJ Treatments on Wine Phenolic Compounds

Tables 2 and 3 show the phenolic composition of wines (mg/L) elaborated from grapes of Tempranillo vines foliarly treated with control, methyl jasmonate (MeJ) and nanoparticles doped with MeJ (ACP-MeJ) treatments, in the 2019 and 2020 seasons.

Table 2. Anthocyanins content (mg/L) in wines from control, methyl jasmonate (MeJ) and nanoparticles doped with this elicitor (ACP-MeJ) treatments, in 2019 and 2020 seasons.

	2019			2020		
	Control	**MeJ**	**ACP-MeJ**	**Control**	**MeJ**	**ACP-MeJ**
Delphinidin-3-*O*-glc	14.67 ± 2.72 a	17.06 ± 1.23 a	15.15 ± 1.64 a	6.48 ± 0.67 a	11.03 ± 1.09 b	7.42 ± 0.66 a
Cyanidin-3-*O*-glc	2.21 ± 0.06 a	2.44 ± 0.41 a	2.03 ± 0.28 a	1.57 ± 0.07 a	1.78 ± 0.19 a	1.67 ± 0.04 a
Petunidin-3-*O*-glc	20.48 ± 3.40 a	22.94 ± 3.45 a	21.37 ± 1.49 a	13.81 ± 2.37 a	18.22 ± 1.49 b	14.18 ± 7.61 a
Peonidin-3-*O*-glc	6.38 ± 0.60 a	9.43 ± 0.84 b	6.59 ± 0.52 a	2.83 ± 0.56 a	4.11 ± 0.55 b	3.03 ± 0.21 a
Malvidin-3-*O*-glc	89.68 ± 8.97 a	101.81 ± 5.10 a	94.83 ± 4.25 a	82.84 ± 8.04 a	80.27 ± 17.19 a	84.50 ± 4.07 a
Total non-acylated	133.42 ± 15.69 a	153.68 ± 9.56 a	139.96 ± 8.17 a	107.53 ± 11.53 a	115.40 ± 18.82 a	110.81 ± 5.49 a
Delphinidin-3-*O*-acglc	2.51 ± 0.24 a	2.68 ± 0.13 a	2.51 ± 0.17 a	2.39 ± 0.19 a	2.48 ± 0.38 a	2.42 ± 0.03 a
Cyanidin-3-*O*-acglc	1.35 ± 0.00 a	1.37 ± 0.00 b	1.35 ± 0.01 a	1.36 ± 0.01 b	1.37 ± 0.01 b	1.34 ± 0.00 a
Petunidin-3-*O*-acglc	2.61 ± 0.20 a	2.67 ± 0.15 a	2.59 ± 0.14 a	2.59 ± 0.23 a	2.64 ± 0.44 a	2.64 ± 0.02 a
Peonidin-3-*O*-acglc	2.12 ± 0.07 a	2.60 ± 0.26 b	2.17 ± 0.03 a	1.74 ± 0.10 a	1.81 ± 0.17 a	1.78 ± 0.03 a
Malvidin-3-*O*-acglc	5.93 ± 0.46 a	6.24 ± 0.09 a	6.25 ± 0.33 a	6.73 ± 0.44 a	6.25 ± 0.94 a	6.72 ± 0.23 a
Delphinidin-3-*O*-cmglc	3.76 ± 0.35 a	4.28 ± 0.37 a	4.04 ± 0.41 a	3.81 ± 0.57 a	3.59 ± 0.68 a	4.05 ± 0.11 a
Cyanidin-3-*O*-cmglc	1.79 ± 0.09 a	2.09 ± 0.17 b	1.87 ± 0.10 ab	1.79 ± 0.11 a	1.89 ± 0.29 a	1.84 ± 0.01 a
Petunidin-3-*O*-cmglc	2.90 ± 0.19 a	3.30 ± 0.16 a	3.12 ± 0.39 a	2.86 ± 0.35 a	3.19 ± 0.45 a	2.93 ± 0.05 a
Peonidin-3-*O*-cmglc	2.37 ± 0.11 a	2.91 ± 0.23 b	2.52 ± 0.13 a	2.28 ± 0.20 a	2.44 ± 0.48 a	2.35 ± 0.06 a
Malvidin-3-*O*-*cis*-cmglc	1.71 ± 0.03 a	1.74 ± 0.01 a	1.84 ± 0.07 b	1.82 ± 0.02 a	1.70 ± 0.09 a	1.83 ± 0.06 a
Malvidin-3-*O*-*trans*-cmglc	9.33 ± 0.46 a	10.37 ± 0.38 a	10.41 ± 1.08 a	9.84 ± 1.52 a	11.45 ± 2.60 a	10.30 ± 0.53 a
Malvidin-3-O-cfglc	1.99 ± 0.09 a	2.23 ± 0.17 b	2.04 ± 0.03 ab	1.59 ± 0.06 a	1.59 ± 0.26 a	1.65 ± 0.03 a
Total acylated	38.37 ± 2.22 a	42.48 ± 0.97 a	40.71 ± 2.68 a	38.80 ± 3.65 a	40.41 ± 6.21 a	39.85 ± 0.99 a
Total anthocyanins	171.80 ± 17.75 a	193.92 ± 14.13 a	176.46 ± 17.25 a	146.33 ± 15.18 a	155.81 ± 24.83 a	150.66 ± 5.81 a
Vitisin A	2.00 ± 0.16 b	1.73 ± 0.04 a	1.74 ± 0.01 a	1.51 ± 0.02 a	1.53 ± 0.04 a	1.52 ± 0.04 a
Vitisin B	1.97 ± 0.12 a	2.18 ± 0.18 a	2.06 ± 0.04 a	1.78 ± 0.05 a	1.85 ± 0.23 a	1.94 ± 0.02 a

Nomenclature abbreviations: glc, glucoside; acglc, acetylglucoside; cmglc, *trans*-p-coumaroylglucoside; cfglc, caffeoylglucoside. All parameters are listed with their standard deviation ($n = 3$). For each season and compound, different letters indicate significant differences between the samples ($p \leq 0.05$).

In 2019, regarding the non-acylated anthocyanins, only wines from the MeJ treatment increased the peonidin-3-*O*-glc content in comparison to the control and ACP-MeJ wines. The content of some of the acylated anthocyanins in the wines was affected by the treatments. Thus, the concentration of cyanidin-3-*O*-aglc, peonidin-3-*O*-aglc, cyanidin-3-*O*-cmglc, peonidin-3-*O*-cmglc and malvidin-3-*O*-cfglc increased in MeJ wines with respect to the control ones. Wines from the ACP-MeJ treatment increased the malvidin-3-*O*-*cis*-cmlg content with respect to the control wines (Table 2). In this first year of the study, neither the total non-acylated anthocyanins nor the acylated anthocyanins and total anthocyanins content of the wines were affected by the application of the elicitors in the vineyard. However, the vitisin A content decreased in wines from the two MeJ treatments in comparison to the content in the control wines (Table 2).

Table 3. Flavonols, flavanols, phenolic acids and stilbenes content (mg/L) in wines from control, methyl jasmonate (MeJ) and nanoparticles doped with this elicitor (ACP-MeJ) treatments, in 2019 and 2020 seasons.

	2019			2020		
	Control	**MeJ**	**ACP-MeJ**	**Control**	**MeJ**	**ACP-MeJ**
Flavonols						
Myricetin-3-glcU	12.16 ± 1.20 a	10.40 ± 1.63 a	11.47 ± 0.46 a	6.64 ± 0.39 a	6.71 ± 0.62 a	8.80 ± 1.04 b
Myricetin-3-gal	15.56 ± 0.34 a	13.33 ± 1.19 a	14.14 ± 1.53 a	8.14 ± 1.05 a	9.49 ± 1.06 ab	12.17 ± 2.01 b
Myricetin-3-glc	110.56 ± 6.68 a	105.43 ± 17.27 a	102.34 ± 3.46 a	31.94 ± 6.38 a	47.86 ± 5.78 b	51.43 ± 3.08 b
Quercetin-3-glcU	85.40 ± 11.76 b	60.07 ± 6.79 a	83.28 ± 5.93 b	11.35 ± 1.11 a	13.12 ± 1.76 a	16.93 ± 2.02 b
Quercetin-3-glc	94.97 ± 11.20 b	74.64 ± 6.63 a	78.78 ± 7.67 ab	57.77 ± 6.23 a	76.74 ± 9.28 b	78.10 ± 7.89 b
Laricitrin-3-glc	17.50 ± 1.22 a	15.95 ± 1.78 a	16.45 ± 0.34 a	10.79 ± 0.37 a	11.79 ± 1.22 a	14.98 ± 1.21 b
Kaempferol-3-gal	1.58 ± 0.23 a	1.30 ± 0.23 a	1.46 ± 0.10 a	0.16 ± 0.01 a	0.19 ± 0.03 ab	0.22 ± 0.04 b
Kaempferol-3-glcU + 3-glc	7.24 ± 1.14 b	4.95 ± 0.61 a	4.50 ± 0.87 a	0.70 ± 0.10 a	0.78 ± 0.07 ab	0.97 ± 0.13 b
Isorhamnetin-3-glc	1.73 ± 0.24 a	1.66 ± 0.28 a	1.46 ± 0.14 a	0.23 ± 0.04 a	0.38 ± 0.04 b	0.37 ± 0.01 b
Syringetin-3-glc	11.25 ± 1.06 a	10.67 ± 1.73 a	10.45 ± 0.26 a	8.92 ± 0.59 a	10.40 ± 1.24 ab	12.01 ± 1.69 b
Free-myricetin	12.56 ± 0.46 b	15.85 ± 2.44 c	7.74 ± 0.65 a	18.61 ± 3.15 a	30.71 ± 5.01 ab	35.77 ± 8.77 b
Free-quercetin	18.85 ± 1.69 b	18.73 ± 3.00 b	9.69 ± 1.17 a	14.36 ± 1.39 a	17.09 ± 2.46 a	24.01 ± 4.52 b
Free-kaempferol	10.09 ± 0.69 b	11.42 ± 1.48 b	5.48 ± 0.52 a	3.95 ± 0.32 a	3.93 ± 0.09 a	4.37 ± 0.73 a
Free-laricitrin	2.34 ± 0.06 a	2.36 ± 0.22 a	2.09 ± 0.29 a	4.70 ± 0.29 a	5.37 ± 1.12 a	5.45 ± 0.85 a
Free-isorhamnetin + syringetin	0.54 ± 0.05 b	0.64 ± 0.07 b	0.33 ± 0.03 a	0.38 ± 0.03 a	0.40 ± 0.05 a	0.38 ± 0.07 a
Total flavonols	402.34 ± 29.87 a	343.84 ± 40.47 a	339.59 ± 43.65 a	178.57 ± 6.30 a	225.67 ± 55.20 a	260.12 ± 41.43 a
Flavanols						
Catechin	16.62 ± 1.12 a	18.37 ± 2.85 a	17.74 ± 2.56 a	8.18 ± 1.57 a	8.17 ± 1.05 a	7.49 ± 1.52 a
Epicatechin	19.02 ± 1.22 a	18.49 ± 3.53 a	16.60 ± 1.46 a	10.07 ± 1.46 a	14.32 ± 2.04 b	12.28 ± 1.33 ab
Epicatechin-3-gallate	17.24 ± 1.84 a	16.71 ± 3.22 a	16.38 ± 1.86 a	n.d.	n.d.	n.d.
Epigallocatechin	1.50 ± 0.23 a	2.32 ± 0.37 b	1.83 ± 0.32 ab	6.14 ± 0.93 a	7.45 ± 0.73 a	8.22 ± 1.31 a
Procyanidin B1	7.47 ± 0.96 a	15.93 ± 1.11 b	7.95 ± 1.24 a	2.64 ± 0.42 a	4.46 ± 0.57 b	4.01 ± 0.60 b
Procyanidin B2	16.34 ± 1.50 b	8.06 ± 1.53 a	9.31 ± 0.77 a	n.d.	n.d.	n.d.
Total flavanols	81.99 ± 2.40 a	87.77 ± 16.59 a	75.51 ± 9.57 a	26.13 ± 4.77 a	35.72 ± 3.47 b	32.01 ± 4.52 ab
Hydroxybenzoic acid						
Gallic acid	29.84 ± 4.11 b	20.17 ± 2.87 a	26.62 ± 0.72 b	14.46 ± 1.04 a	18.89 ± 1.26 b	16.24 ± 2.58 ab
Hydroxycinnamic acids (HCAs)						
trans-Caftaric acid	4.42 ± 0.53 b	2.27 ± 0.51 a	2.99 ± 0.68 a	9.19 ± 1.00 a	12.23 ± 1.04 b	8.80 ± 1.47 a
trans + *cis*-Coutaric acids	2.65 ± 0.29 c	1.70 ± 0.32 b	0.92 ± 0.14 a	7.07 ± 0.71 a	8.98 ± 0.83 b	7.58 ± 0.65 ab
trans-Fertaric acid	1.12 ± 0.10 a	0.93 ± 0.14 a	0.97 ± 0.23 a	1.48 ± 0.04 a	1.90 ± 0.28 b	1.87 ± 0.18 b
Caffeic acid	30.43 ± 0.71 b	22.49 ± 2.48 a	29.30 ± 1.93 b	12.11 ± 2.28 a	14.50 ± 3.05 a	14.52 ± 3.09 a
p-Coumaric acid	10.52 ± 0.98 ab	7.95 ± 0.10 a	10.79 ± 2.10 b	7.30 ± 1.46 a	8.35 ± 1.55 a	8.82 ± 1.73 a
Ferulic acid	2.31 ± 0.29 a	1.83 ± 0.31 a	2.23 ± 0.11 a	2.08 ± 0.37 a	2.63 ± 0.30 a	2.61 ± 0.41 a
Total HCAs	52.19 ± 3.53 a	43.97 ± 10.35 a	49.30 ± 8.58 a	39.24 ± 2.48 a	48.36 ± 3.65 b	44.06 ± 5.48 ab
Stilbenes						
trans-Piceid	3.55 ± 0.22 a	3.43 ± 0.56 a	3.27 ± 0.12 a	0.87 ± 0.08 a	1.56 ± 0.20 b	1.62 ± 0.10 b
cis-Piceid	0.24 ± 0.04 a	0.47 ± 0.06 b	0.38 ± 0.07 b	0.95 ± 0.13 ab	0.87 ± 0.09 a	1.19 ± 0.16 b
trans-Resveratrol	0.58 ± 0.02 a	0.74 ± 0.12 b	0.51 ± 0.06 a	1.87 ± 0.07 a	2.96 ± 0.22 b	2.97 ± 0.58 b
cis-Resveratrol	0.63 ± 0.10 a	0.67 ± 0.06 a	0.61 ± 0.04 a	0.50 ± 0.04 a	0.73 ± 0.15 b	0.75 ± 0.11 b
Total stilbenes	5.15 ± 0.43 a	5.23 ± 1.11 a	4.86 ± 0.18 a	4.28 ± 0.37 a	5.93 ± 0.91 a	6.07 ± 1.53 a

Nomenclature abbreviations: glcU, glucuronide; gal, galactoside; glc, glucoside. All parameters are listed with their standard deviation (n = 3). For each season and compound, different letters indicate significant differences between the samples ($p \leq 0.05$). n.d.: not detected.

In 2020, the content of non-acylated anthocyanins in the wines was more affected by the treatments than in 2019, although the total non-acylated anthocyanins content did not

show a difference in the treated wines compared to the control wines. Delphinidin-3-*O*-glc, petunidin-3-*O*-glc and peonidin-3-*O*-glc content in wines from MeJ treatment were higher than the content of control and ACP-MeJ wines (Table 2). Regarding the acylated anthocyanins, only cyaniding-3-*O*-acglc content was affected, decreasing in the ACP-MeJ wines with respect to the wines from both the MeJ and control treatments. In 2020, the foliar treatments did not affect the total acylated anthocyanins or total anthocyanins or either of the two vitisins determined in the wines (Table 2). Anthocyanins are the compounds responsible for the color of grapes and red wines. Their synthesis takes place in the skins and the profile or proportion in which each one is found in the grape is specific to each variety [33], which makes it possible to distinguish varieties [34] or even characterize certain wines [35]. The results of the total anthocyanin contents obtained in our study did not match with the increase in the phenolic composition of both grapes and wines, especially the content of total and non-acylated anthocyanins found by other authors after the foliar application of MeJ on plants of Barbera [36], Monastrell [14], Syrah [37], Tempranillo [3,4] and Graciano [5]. Thus, it has been shown that malolactic fermentation of wines, which leads to an increase in pH and changes in chemical composition, influences the ability of anthocyanins to react with other compounds such as pyruvic acid, acetaldehyde, or various copigments such as phenolic acids [38]. In this sense, after applications with MeJ in grapes of Syrah, Fernández-Marín et al. [37] reported significant decreases in the concentration of anthocyanins in the wines once malolactic fermentation was completed with respect to freshly pressed wines.

In 2019, many of the individual flavonols in the wines were affected by foliar treatments as shown in Table 3. Thus, quercetin-3-glcU, quercetin-3-glc and kaempferol-3-galcU + 3-glc content decreased in wines from the MeJ treatment with respect to the control wines, meanwhile, free-myricetin content was the highest in MeJ wines. The ACP-MeJ treatment reduced the content of free-myricetin, free-quercetin, free-kaempferol and free-isoharmenetin + syringetin with respect to the wines of both control and MeJ treatments (Table 3). However, in the wines from 2020, it was observed that the ACP-MeJ treatment increased the content of all flavonols except free-kaempferol, free-laricitrin and free-isoharmenetin + syringetin, in comparison to the control wines. MeJ treatment increased the myricetin-3-glc, quercetin-3-glc and isorhamnetin-3-glc content in the wines compared to these from the control treatment (Table 3). Although the treatments favored the synthesis of some of the flavonols studied in the wines, compared to the control, the total flavonol compounds were not affected in either of the two years of study (Table 3). Similar to the anthocyanins, flavonols are located in the skin of grapes, and are of great importance in the color stability of red wines due to their copigmentation reactions with the anthocyanins [39]. Furthermore, flavonols contribute to the taste sensations of wine, since quercetin derivatives are related to the wine bitterness, while other compounds such as syringetin-3-glc contribute to the wine astringency [40]. Although anthocyanins and flavonols largely share their synthesis pathway, the response to MeJ treatments observed in the wines was diverse.

Thus, after the application of MeJ on a Graciano variety vineyard, Portu et al. [5] also observed in the second year of the study significant increases in flavonols content, both in grapes and wines, while in the first year, they did not observe differences between control and treated samples. Portu et al. [3] reported a significant increase of 40% of flavonols content in Tempranillo wines from MeJ grapes treated in comparison to the control ones, mainly due to the increase in the concentration of quercetin-3-glc, kaempferol-3-glc, isorhamnetin-3-glc and free myricetin content. However, in another study with Tempranillo wines, Portu et al. [4] did not observe differences in flavonols concentration between those from the MeJ treated grapes and the control.

Regarding the flavanols content in wines, in 2019, MeJ treatment increased the epigallocatechin and procyanidin B1 concentration respect to the control wines, but both treatments reduced the procyanidin B2 content compared to the control wines. In the 2020 wines, neither epicatechin-3-gallate nor procyanidin B2 were detected. On the other hand, the MeJ treatment

increased the epicatechin content, and both treatments increased the procyanidin B1 content in comparison to the content in the control wines.

Thus, only in the 2020 wines, total flavonol content increased in the MeJ treatment wines compared to the control (Table 3). These results did not match with those reported by other authors such as Portu et al. [3–5] after studying wines from grapes that have been treated with MeJ, which did not show differences in flavanols content compared to untreated wines. Flavanols are located in the skin of grapes and also, mainly, in the pips [41]. They are of special relevance in the taste properties of wines [42], being closely related to the astringency of wines as well as their color stability [40].

The only hydroxybenzoic acid determined in the wine samples, gallic acid, decreased its content in the MeJ wines in 2019 but increased in those of 2020, in comparison to the control wines (Table 3). This acid is found in high concentration in grapes and also in wines, especially those aged in oak barrels, since it is released by the hydrolysis of hydrolysable tannins in the wood [43]. In general, the applications with the MeJ-elicitor have not modified the content of this hydroxybenzoic acid in the treated wines, compared to the control as also reported Portu et al. [3,5].

Among the hydroxycinnamic acids analyzed in the wines, in 2019, neither of the two treatments favored their increase in comparison to the control wines. In the 2020 wines, MeJ treatment increased the concentration of *trans*-caftaric acids, *trans* + *cis*-coutaric acids and *trans*-fertaric acid, whereas ACP-MeJ also increased the *trans*-fertaric acid content compared to the control wines (Table 3). Total hydroxycinnamic acids in the wines were only increased with respect to the control treatment in 2020 MeJ wines. Hydroxycinnamic compounds, released by hydrolysis during fermentation, can have a great organoleptic impact on the wines, since they react with anthocyanins to form copigments, and thus contribute to the color stability of young wines [39]. Moreover, in aged wines and because of the activity of contaminating yeasts such as *Brettanomyces/Dekkera*, these compounds are precursors of the ethylphenols, volatile compounds with unpleasant notes in wines [44]. As previously mentioned in the case of the hydroxybenzoic acid content, foliar applications with MeJ carried out by other authors had no effect on the content of hydroxycinnamic acids in wines.

Finally, regarding the stilbenes, in the 2019 wines, both treatments increased the *cis*-piceid content and the MeJ treatment also increased the *trans*-resveratrol content in comparison to the control wines (Table 3). For its part, the 2020 wines showed an increase in all stilbenes (except *cis*-piceid in the case of the MeJ treatment), compared to the control wines. However, this increase was not reflected in the total stilbene content of the wines, which statistically did not differ from the control in either the 2019 or 2020 wines (Table 3). Stilbenes are phytoalexins synthesized by the plant in response to fungal attacks and other situations of biotic and abiotic stress [45]. Their concentration in grapes and wines depends on multiple factors such as the intrinsic properties of grape variety, climate, cultivar management, season and enological procedures [46]. In 2020, *trans*-resveratrol was the major stilbene in wines, accounting for up to 49% of the total stilbenes content (Table 3). This agrees with the results of other authors, who reported that *trans*-resveratrol was the major stilbene in wines [45]. However, in wines from 2019, *trans*-piceid was the majority stilbene comprising around 66% of the total stilbenes, followed by *trans*-resveratrol (48%) content (Table 3).

Portu et al. [3] observed that the application of the MeJ elicitor produced increases in *trans*-piceid content in their Tempranillo wines, doubling the content of total stilbenes compared to the control wines. Portu et al. [5] observed significant increases in Graciano wines after applications in the vineyard with MeJ and important trends of increase (between 30 and 13% higher than the control) in the case of Tempranillo wines, compared to the control wines. Authors such as Vezzulli et al. [36], Ruiz García et al. [14] and Fernández-Martín et al. [37] also reported increases of the total stilbenes content in wines after applying MeJ to the Barbera, Monastrell and Syrah grapevines, respectively. However, Portu et al. [4] observed that the differences in stilbenes concentration found in the Tempranillo grapes treated with MeJ were not reflected in the wines. After applying MeJ (at dose of 5 mM and

10 mM) and nanoparticles of MeJ (1 mM) to Monastrell grapevines, Parra-Torrejón et al. [20] observed that all of the treatments increased the *trans*-resveratrol concentration in wines, while the *cis*-resveratrol content only increased compared to the control wines when MeJ was applied at 5 mM and as nano-MeJ. Additionally, all the MeJ-based treatments, including the nano-MeJ (with five and ten times lower MeJ concentration than conventional MeJ treatments), increased the *cis*- and *trans*-piceid concentration in their Monastrell wines compared to the control ones.

3.3. Effect of the Foliar MeJ and ACP-MeJ Applications on Wine Aromatic Compounds

Figure 1 shows the concentration (mg/L) of esters in the wines made with grapes after the application in the vineyard of control, methyl jasmonate (MeJ) and nanoparticles doped with MeJ (ACP-MeJ) treatments in the 2019 and 2020 seasons. In 2019, acetate esters (isoamyl acetate and 2-phenyletyl acetate) as well as the total acetate esters content were reduced in the treatments compared to the control, especially in ACP-MeJ wines (Figure 1a–c). However, even in the ACP-MeJ treatment wines, which had the lowest concentration of both acetate esters, its content was above the perception thresholds of 0.03 mg/L in the case of isoamyl acetate, with banana aroma, and 0.25 mg/L in the case of 2-phenylethyl acetate, with rose aroma [47]. In 2020, wines from the MeJ treatment had lower content of both acetate esters and the total acetate esters than the control wines, but the wines from ACP-MeJ treatment had intermediate values of 2-phenylethyl acetate (Figure 1a–c).

Regarding the ethyl esters, in 2019 wines, the concentration of ethyl hexanoate, ethyl octanoate, ethyl decanoate, C6 + C8 + C10 ethyl ester, diethyl succinate as well as the total ethyl esters in ACP-MeJ wines was the lowest. Furthermore, the ethyl lactate content was similar in both MeJ and ACP-MeJ wines, but lower than in the control wines (Figure 1d–j). In 2020, there was a tendency to show a lower content of most of the ethyl esters in MeJ wines. However, this reduction was significant, compared to control wines, only for the content of ethyl decanoate, diethyl succinate and total ethyl esters (Figure 1d–j). Among all the ethyl esters found in the wines of all treatments and in both seasons, only ethyl hexanoate (perception threshold = 0.014 mg/L) and ethyl octanoate (0.05 mg/L) contribute with pleasant fruity and floral notes [48]. However, other esters such as ethyl decanoate, ethyl lactate and diethyl succinate would be below its perception threshold (0.2 mg/L, 154 mg/L and 6 mg/L, respectively) [49] in all samples, not contributing directly to the aroma of the wines. The content of total esters in 2019 and 2020 wines reflected what was commented for acetate esters and ethyl esters individually, since, in 2019, the elicitor-treated wines reduced the total esters content compared to the control wines and, in 2020, the MeJ wine was the one with the lowest total esters content compared to the wines from the other two treatments (control and ACP-MeJ) (Figure 1k).

In 2019, the concentration of higher alcohols in the elicitor-treated wines decreased compared to the control wines. In the case of the isoamyl alcohol, 2-phenylethanol, (E)-3-hexenol and total alcohols content, the MeJ wines had intermediate values and ACP-MeJ wines had the lowest concentration (Figure 2a–g). In 2020, the treatments affected the content of higher alcohols in the wines to a lesser extent. Thus, the MeJ treatment reduced the isoamyl alcohol and (E)-3-hexenol content regarding the control wines and n-hexanol and (E)-3-hexenol in comparison to the ACP-MeJ wines (Figure 2a–g). Only in the case of the ACP-MeJ wines for the 2019 season was the concentration of isoamyl alcohol lower than the perception threshold (30 mg/L). This compound contributes with aromatic notes of cheese and alcohol [31]. The 2-phenylethanol concentration was higher in the 2019 wines compared to those from 2020, where there was no difference between treatments although all of the wines were above the threshold. The 2-phenylethanol perception is related with floral notes, rose and honey when its concentration is above the threshold established in wines for this compound (14 mg/L) [49]. The higher alcohols group was the most abundant of the three studied, followed by esters and acids, as found by Garde-Cerdán et al. [28] in their Tempranillo and Tempranillo Blanco wines. The low concentration of compounds of

this group observed in all of our wines (less than 300 mg/L), suggests that they contribute to the desirable complexity of the wines [50]. However, if the concentration of these higher alcohols exceeded 400 mg/L, their contribution would negatively influence the aroma of the wines [28].

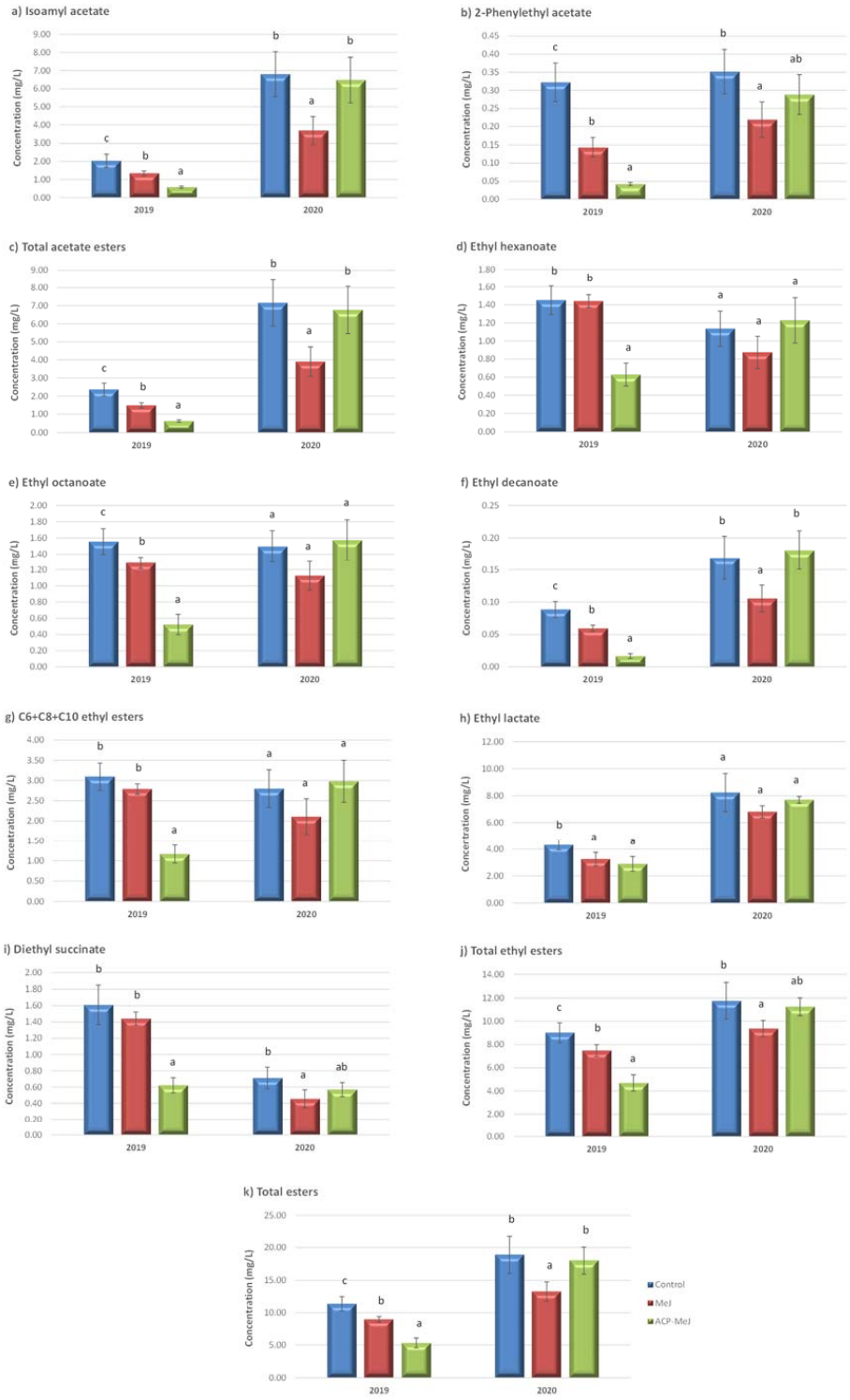

Figure 1. Esters concentration (mg/L) in control wines and from methyl jasmonate (MeJ) and apatite doped with methyl jasmonate (ACP-MeJ) treatments, in the two seasons (2019 and 2020). All parameters are listed with their standard deviation (n = 3). For each season and compound, different letters indicate significant differences between the samples ($p \leq 0.05$).

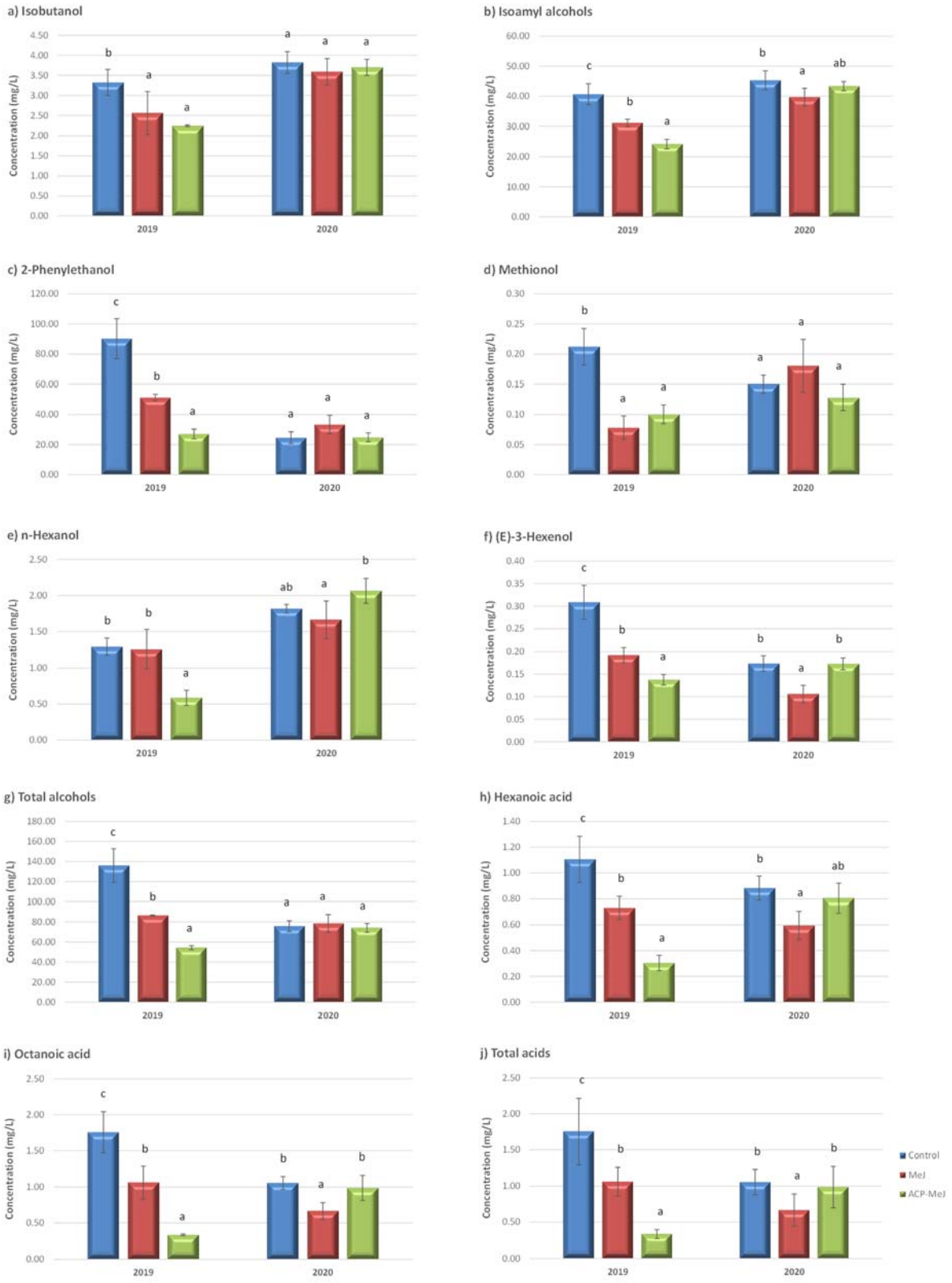

Figure 2. Alcohols and acids concentration (mg/L) in control wines and from methyl jasmonate (MeJ) and apatite doped with methyl jasmonate (ACP-MeJ) treatments, in the two seasons (2019 and 2020). All parameters are listed with their standard deviation (n = 3). For each season and compound, different letters indicate significant differences between the samples ($p \leq 0.05$).

In samples from the 2019 season, elicitor treatments reduced the content of both hexanoic and octanoic acids, as well as total acids content regarding the control wines, with intermediate values for the MeJ treatment samples (Figure 2h–j). In the case of the 2020 samples, MeJ treatment decreased the hexanoic acid content in wines compared to control ones, and also reduced the octanoic acid and total acids content in comparison to the wines of both control and ACP-MeJ treatments (Figure 2h–j). For both acids (hexanoic and octanoic), the concentrations of the 2019 ACP-MeJ wines were found to be below their perception thresholds (0.42 and 0.50 mg/L, respectively) [48]. This may be a positive aspect since these compounds can contribute a fresh flavor to the wines or, conversely, an unpleasant rancid flavor if they are in excess [51].

3.4. Influence of the Foliar MeJ and ACP-MeJ Treatments on Wine Nitrogen Compounds

Table 4 shows the amino acids content of wines from the control, methyl jasmonate (MeJ) and MeJ-doped nanoparticle (ACP-MeJ) treatments from the 2019 and 2020 seasons. In 2019, MeJ treatment increased the content of aspartic acid, asparagine, threonine + citrulline, γ-aminobutyric acid and ornithine in wines compared to control wines. In addition, the wines from this MeJ treatment had the highest concentrations of leucine, phenylalanine and lysine, followed by those from the ACP-MeJ treatment, compared to the control, which had the lowest concentrations. The ACP-MeJ treatment increased the glycine content in comparison to the control wines (Table 4).

Table 4. Amino acids content (mg/L) in wines from control, methyl jasmonate (MeJ) and nanoparticles doped with this elicitor (ACP-MeJ) treatments, in 2019 and 2020 seasons.

	2019			2020		
	Control	**MeJ**	**ACP-MeJ**	**Control**	**MeJ**	**ACP-MeJ**
Aspartic acid	0.07 ± 0.02 a	1.40 ± 0.46 b	0.54 ± 0.27 a	7.27 ± 0.65 b	6.67 ± 0.56 b	4.16 ± 0.68 a
Glutamic acid	3.44 ± 0.88 a	5.73 ± 2.25 a	5.34 ± 0.96 a	16.38 ± 1.32 b	17.88 ± 4.51 b	9.62 ± 1.03 a
Asparagine	3.36 ± 0.82 a	5.78 ± 1.23 b	4.90 ± 1.00 ab	8.22 ± 1.31 a	7.62 ± 1.12 a	6.43 ± 1.13 a
Serine	3.17 ± 1.01 a	3.13 ± 1.49 a	3.39 ± 0.33 a	7.71 ± 1.11 a	7.44 ± 1.00 a	6.31 ± 0.43 a
Glutamine	2.79 ± 0.20 a	1.99 ± 0.95 a	1.95 ± 0.20 a	6.89 ± 1.01 a	5.27 ± 1.33 a	6.17 ± 0.79 a
Histidine	5.25 ± 1.10 a	5.51 ± 1.44 a	4.91 ± 0.24 a	13.10 ± 2.38 b	7.83 ± 1.43 a	11.93 ± 2.52 ab
Glycine	6.48 ± 0.41 a	8.85 ± 1.89 ab	9.11 ± 0.91 b	15.29 ± 2.04 a	14.80 ± 3.26 a	12.47 ± 1.72 a
Threonine + Citrulline	1.82 ± 0.22 a	3.46 ± 1.13 b	2.98 ± 0.21 ab	10.62 ± 1.23 a	8.12 ± 1.82 a	9.28 ± 1.70 a
Arginine	6.02 ± 0.28 a	6.51 ± 0.36 a	6.17 ± 0.93 a	7.09 ± 1.85 b	4.34 ± 0.69 a	6.82 ± 0.62 b
Alanine	3.52 ± 0.99 a	8.05 ± 3.37 a	7.64 ± 1.78 a	26.21 ± 5.20 b	21.56 ± 2.53 ab	17.13 ± 1.70 a
γ-Aminobutyric acid	9.06 ± 1.37 a	16.69 ± 5.17 b	15.29 ± 2.24 ab	14.24 ± 1.83 b	15.17 ± 2.61 b	6.13 ± 0.78 a
Proline	647.05 ± 45.92 a	726.77 ± 110.61 a	742.09 ± 52.52 a	2172.04 ± 120.58 ab	1816.80 ± 218.65 a	2243.38 ± 189.88 b
Tyrosine	0.63 ± 0.05 a	1.96 ± 1.72 a	0.94 ± 0.15 a	6.68 ± 0.67 c	4.94 ± 0.80 b	2.98 ± 0.42 a
Valine	0.67 ± 0.06 a	2.32 ± 1.72 a	1.22 ± 0.30 a	7.46 ± 0.96 b	6.34 ± 0.87 b	4.13 ± 0.57 a
Methionine	0.56 ± 0.10 a	0.86 ± 0.47 a	0.59 ± 0.20 a	1.69 ± 0.28 b	1.39 ± 0.28 b	0.53 ± 0.12 a
Cysteine	0.44 ± 0.06 b	0.27 ± 0.06 a	0.27 ± 0.08 a	0.36 ± 0.04 a	0.38 ± 0.06 a	0.39 ± 0.06 a
Isoleucine + Tryptophan	0.93 ± 0.07 a	1.87 ± 0.96 a	1.53 ± 0.10 a	7.60 ± 0.76 b	7.25 ± 0.90 b	5.02 ± 0.93 a
Leucine	1.40 ± 0.32 a	4.84 ± 1.00 c	3.44 ± 0.03 b	12.94 ± 2.44 b	7.72 ± 0.90 a	4.94 ± 0.96 a
Phenylalanine	0.94 ± 0.19 a	2.77 ± 0.68 c	1.83 ± 0.23 b	9.52 ± 1.49 b	5.66 ± 0.72 a	4.46 ± 0.64 a
Ornithine	3.26 ± 0.25 a	49.27 ± 23.18 b	8.90 ± 1.41 a	33.74 ± 3.30 c	16.98 ± 1.40 a	25.49 ± 2.89 b
Lysine	2.42 ± 0.40 a	8.03 ± 0.87 c	4.13 ± 0.61 b	26.88 ± 3.35 b	19.89 ± 3.20 a	14.78 ± 1.31 a
Total amino acids	703.27 ± 41.79 a	866.04 ± 121.48 a	827.17 ± 63.60 a	2411.95 ± 135.64 a	2004.05 ± 237.31 a	2402.55 ± 208.62 a
Total amino acids without Pro	56.22 ± 7.14 a	139.27 ± 27.70 b	85.08 ± 11.11 a	239.91 ± 23.20 b	187.25 ± 21.38 a	159.17 ± 19.26 a

All parameters are listed with their standard deviation (n = 3). For each season and compound, different letters indicate significant differences between the samples ($p \leq 0.05$).

The total amino acids content did not differ between the wines, but the total amino acids without proline content increased in the MeJ treatment wines compared to those from the other two treatments (control and ACP-MeJ). Since proline is not among the amino acids preferred by yeasts as a nitrogen source during the wine fermentation, and it requires the presence of molecular oxygen for its metabolism, it is one of the most released amino acids by the yeast at the end of the alcoholic fermentation. Therefore, proline is the amino acid found in the highest concentration in all wines (Table 4), as also reported by other authors [29].

In 2020, treatments affected the amino acids content of wines to a different extent than in 2019. In general, wines from the control treatment had higher amino acids content than the treated wines, although this was not reflected in the total amino acids content. In addition, the total amino acids content without proline was higher in the control wines than in wines from the two elicitors (Table 4). Thus, MeJ treatment decreased the content of arginine, tyrosine, leucine, phenylalanine, ornithine and lysine in wines compared to the control one. ACP-MeJ reduced the content of aspartic and glutamic acids, alanine γ-aminobutyric acid, tyrosine, valine, methionine, isoleucine + tryptophan and lysine regarding the control wines, whereas increased the proline and ornithine content in comparison to the MeJ treatment (Table 4). In a study with Monastrell grapevines in rainfed and RDI regime, Pérez-Álvarez et al. [21] also did not observe the influence of ACP-MeJ in the total amino acids content of the musts. They suggested that, although the ACP nanoparticle has nitrates in its structure [19], which are a source of nitrogen for the plant, the coverage of the surface by the MeJ (ACP-MeJ) would not allow such an easy release of nitrogen. This would explain the little effect observed on the amino acids content of wines treated with ACP-MeJ compared to those from the MeJ treatment. Our work is pioneering in the study of the effects of these foliar applications of ACP-MeJ on wines composition. Therefore, although it is very likely that the wines are the reflection of the quality of the grapes, more studies are needed to increase knowledge of the influence on the wines' composition of this kind of plant elicitor, since wine is the product that reaches the consumer.

3.5. Wine Sensory Analysis

The sensorial evaluation (visual, odor and taste properties, as well as the total score given by tasters) of the control, MeJ and ACP-MeJ wines are shown in Table 5. Figure 3 shows a "spider web" diagram for the average scores of a) odor and b) taste attribute intensities of Tempranillo wines obtained from control and treated grapevines with MeJ and ACP-MeJ in the 2019 and 2020 seasons. Wines from the ACP-MeJ treatment were better evaluated by the tasters in their odor characteristics (genuineness and quality) in comparison to the wines from the other treatments (Table 5). In addition, these ACP-MeJ wines obtained the highest total score and harmony from the panelists. On the other hand, the tasters rated the 2019 wines higher than the 2020 wines, highlighting the attributes of odor genuineness and quality, as well as taste intensity (Table 5).

Table 5. Factor analysis of the sensory evaluation of the wines with the two factors studied: treatment (Control, MeJ, ACP-MeJ) and season (2019 and 2020).

		Treatment (T)			Season (S)		
		Control	**MeJ**	**ACP-MeJ**	**2019**	**2020**	**Interaction (T × S)**
Visual	**Clarity**	3.84 a	3.83 a	3.99 a	3.77 a	4.02 a	N.S.
	Color	7.75 a	7.59 a	7.93 a	7.75 a	7.78 a	N.S.
Odor	**Intensity**	5.81 a	5.69 a	5.73 a	5.87 a	5.62 a	N.S.
	Genuineness	3.65 a	3.74 a	4.14 b	4.09 b	3.64 a	*
	Quality	11.50 a	11.36 a	12.34 b	12.26 b	11.24 a	*
Taste	**Intensity**	5.68 a	5.55 a	5.99 a	5.95 b	5.56 a	N.S.
	Genuineness	3.68 a	3.74 a	4.11 a	3.87 a	3.85 a	N.S.
	Quality	14.86 a	14.71 a	15.47 a	15.35 a	14.71 a	N.S.
	Persistence	5.94 a	5.69 a	6.07 a	6.01 a	5.82 a	N.S.
Harmony		8.62 a	8.62 a	9.03 b	8.86 a	8.65 a	N.S.
Total rating		**71.34 a**	**70.55 a**	**74.67 b**	**73.67 b**	**70.89 a**	**N.S.**

For each parameter and factor, different letters indicate significant differences between samples ($p \leq 0.05$). Interaction: N.S., not significant ($p > 0.05$); *, $p \leq 0.05$.

Figure 3. Polar coordinate (spider web) plot of mean intensity ratings of sensory descriptors (odor and taste attributes) for control wines and from methyl jasmonate (MeJ) and apatite doped with methyl jasmonate (ACP-MeJ) treatments, in the two seasons (2019 and 2020). At the origin, intensity = 0; at the perimeter, intensity = 6. * indicates significant differences between treatments ($p \leq 0.05$).

Regarding odor attributes, in 2019 wines (Figure 3), no significant differences were observed between treatments. In 2020 wines, the only significant difference was the greater

perception of reduction by the tasters in the control wines. Concerning taste characteristics, in 2019, the control and ACP-MeJ wines were appreciated as more astringent than the MeJ wines. In 2020, the wines that were described as more astringent but at the same time more equilibrate were those from the ACP-MeJ treatment (Figure 3).

4. Conclusions

This is the first time that the effects of foliar applications of the elicitor methyl jasmonate (MeJ) doped in nanoparticles of calcium phosphate apatite (ACP-MeJ) on phenolic, nitrogen and volatile composition and sensory properties of Tempranillo wines have been studied. Thus, foliar applications of control, MeJ and ACP-MeJ were carried out in a Tempranillo vineyard during two seasons, and wines from those grapes were produced and analyzed. Although the vinifications generally homogenize the wines and it would seem that the effect of the elicitor could not be observed in the wines from the treated grapes, certain differences in the wine profiles can be noted in comparison to the control wines, having a positive impact on the taste and color properties, in which phenolic and volatile compounds are mainly involved. Thus, anthocyanins such as peonidin-3-*O*-glc, flavanols such as free-myricetin and free-quercetin, flavanols such as procyanidin B1, the hydroxybenzoic acid gallic acid, some hydroxycinnamics acids and stilbenes such as *cis*-piceid and *cis*-resveratrol increased their content in wines treated in comparison to the control ones, although the differences were greater with the MeJ wines than with the ACP-MeJ wines. The impact of the treatments also influenced the amino acids concentration, many of which were higher in the treated wines in 2019 but higher in 2020 in the control wine. In the case of volatile compounds, few were those that increased in the elicitor-treated wines compared to the control wine; however, the tasters rated all the wines as good, without detracting from the treated wines, even highlighting the ACP-MeJ wines in their overall rating.

In conclusion, applications of elicitors based on methyl jasmonate have an impact on the phenolic, nitrogen and aromatic composition of Tempranillo wines, affecting their quality and sensory perception by consumers.

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ORIGINAL PAPER

Influence of foliar treatments with methyl jasmonate and methyl jasmonate-doped nanoparticles on nitrogen composition of Tempranillo grapes during two vintages

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Abstract

Nanoparticles are emerging as a cutting-edge technology to improve crop agricultural input efficiency and reduce biotic and abiotic stresses. In viticulture, nanoparticles hold promise for the sustainable application of an elicitor (methyl jasmonate, MeJ), allowing a considerable dosage reduction. Herein, the influence of the foliar application of free MeJ (10 mM) and MeJ nanoformulation (ACP-MeJ, 1 mM MeJ) on Tempranillo grape amino acids content over two vintages (2019 and 2020) was evaluated. While both MeJ treatments provided a significant increase of the amino nitrogen and yeast assimilable nitrogen in the must in 2019, there were no significant differences on these parameters in 2020. In 2019, MeJ treatment enhanced the synthesis of most of the amino acids included in this study, while ACP-MeJ promoted the formation of six amino acids. Hence, the content of total amino acids, with and without proline, was higher after applying MeJ than in the control musts. However, these values were higher for control must than for MeJ samples in 2020. The multivariable analysis confirmed that the vintage factor had a more prominent effect on the overall parameters of the musts. This strong influence of the vintage could be related to the higher rainfall in 2020.

Keywords Amino acids · YAN · Must · Elicitor · Nanotechnology · Vineyard

Introduction

Nanomaterials have received an ever-increasing attention in the field of agrochemicals due to their exceptional properties, including their large surface area, higher chemical/thermal stability and tunable unique physicochemical characteristics (i.e., structure, solubility, surface reactivity) [1, 2]. Owing to these outstanding properties, nano-agrochemical delivery system has great potential for facilitating the uptake and translocation of nutrients in plants, improving the efficacy of agrochemicals, and consequently alleviating environmental pollution and promoting food security [3]. Recently, a novel nanoelicitor has been designed through the loading of biomimetic amorphous calcium phosphate nanoparticles, similar to those found in bone, with methyl jasmonate (ACP-MeJ) [4]. The nanoelicitor provided a sustainable release and protection against thermal degradation of MeJ, ensuring elicitor activity over longer period of times on the surface of the leaves and reducing by ten times the required dosage [4].

This nanoelicitor has been applied in the vineyard of two red grape varieties, Monastrell and Tempranillo, and the results, published to date, are collected in six scientific articles, focused on its effect on: (i) grape: Monastrell nitrogen composition [5] and Tempranillo phenolic composition [6]; (ii) wine: stilbenes content in Monastrell [4], volatile compounds in Monastrell [7] and a broad characterization of volatile, nitrogen and phenolic composition in Tempranillo [8]; and (iii) grape and wine: nitrogen composition in Monastrell [9].

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Regarding Monastrell grape variety, it has been observed that the application of MeJ, free or in nano-form, increased the total content of amino acids in grapes, although to a greater extent when used conventionally, i.e., as free [9]. However, Pérez-Álvarez et al. [5], when applying ACP-MeJ in this same grape variety, did not observe influence on the nitrogen composition of the grapes. As for Monastrell wine, the use of MeJ and ACP-MeJ in the vineyard increased the content of total stilbenes [4] and total amino acids [9], while the influence on the wine volatile composition depended on the family of compounds and the vintage: total acids increased in one of the vintages, and total alcohols and esters were not affected in any of the vintages, while terpenoids increased when applying conventional MeJ in all vintages [7].

Regarding the Tempranillo variety, the treatments in the vineyard with MeJ and ACP-MeJ improved the content of phenolic compounds in the grapes, the effect being dependent on the vintage, so that, in a vintage, MeJ increased the total content of anthocyanins and ACP-MeJ that of hydroxycinnamic acids (HCA); while, in the second vintage, the application of MeJ had no effect on the phenolic composition of the grapes and, however, ACP-MeJ increased the total content of flavonols, flavanols, gallic acid, and stilbenes [6]. Regarding the Tempranillo wine [8], its phenolic composition was little affected by the treatments with MeJ and ACP-MeJ carried out in the vineyard, since only the content of gallic acid and total HCA showed changes; furthermore, the total amino acids content was little affected; and volatile compounds decreased their concentration when applying both treatments in the first vintage, while in the second one, these compounds were not affected by ACP-MeJ, while esters and acids decreased again when applying MeJ, without effect on alcohols.

Therefore, the study of the nitrogen composition of Tempranillo grapes, after the application of MeJ and ACP-MeJ in the vineyard, is a key issue, since there are no previous studies on this topic, unlike in the Monastrell grape variety [5, 9]. Nitrogen compounds are essential for the correct development of alcoholic fermentation [10, 11]. Moreover, these compounds are precursors of the main fermentative volatile compounds [12, 13], and have been especially affected by the applications in the Tempranillo vineyard of MeJ and ACP-MeJ [8], although the same effect was not observed when the treatments were applied in Monastrell [7], despite the important difference in the nitrogen composition of the grapes [9]. For all these reasons, the aim of this work was to study the influence of MeJ and ACP-MeJ foliar treatments on Tempranillo grape amino acids content over two vintages.

Materials and methods

Foliar treatments and must samples

Tempranillo (*Vitis vinifera* L.) variety grown in Finca La Grajera, Logroño, La Rioja, Spain (42° 26′ 25″ North, Latitude; 2° 30′ 56″ West, Longitude; 456 m above sea level) in 2019 and 2020 vintages was used. Vineyard had grafted onto R-110 rootstock and trained to a vertical shoot positioned trellis system, had been planted in 1997. Climate data were recorded by the Agroclimatic Information Service of La Rioja (SIAR) installed close to field. The collected data were the rain accumulated from the beginning of April until 1st of September, being 247.8 L/m^2 in 2019 and 217.8 L/m^2 in 2020; and the average maximum, mean, and minimum temperatures, being 27.0 °C, 13.8 °C, and 3.7 °C, respectively, in 2019, and 26.3 °C, 13.8 °C, and 3.7 °C, respectively, in 2020.

The experiment involved the foliar application of free methyl jasmonate (MeJ) and amorphous calcium phosphate nanoparticles doped with this elicitor (ACP-MeJ). The synthesis and characterization of ACP-MeJ to determine the composition structure and morphology were carried out as previously described elsewhere [4]. To carry out the treatments, aqueous solutions were prepared with a concentration 10 mM of MeJ (according to previous works) [14, 15] and 1 mM of ACP-MeJ [5, 6], using Tween® 80 (Sigma-Aldrich, Madrid, Spain) as wetting agent (1 mL/L). Control plants were sprayed with Tween® 80 water solution. The foliar applications were carried out twice, at veraison and 7 days later on. The treatments were arranged in a complete randomized block design, with ten vines for replication and treatment, and were done in triplicate.

Grapes from all grapevines and treatments were picked at their optimum technological maturity, and they were destemmed and crushed to obtain the musts. Then, the general parameters were determined, and aliquots of each must sample were frozen at − 20° C for later analysis of nitrogen composition.

General enology parameters' determination

The must enological parameters were analyzed using the official methods of OIV [16]: ºBrix, probable alcohol, pH, and total acidity. Glucose, fructose, malic acid, and nitrogen fractions [ammonium nitrogen, amino nitrogen, and yeast assimilable nitrogen (YAN)] were determined using a Miura One enzymatic equipment (TDI, Barcelona, Spain).

As the treatments were performed in triplicate, the results of these parameters are shown as the average of three analyses (n = 3).

Analysis of amino acids in the musts by HPLC–DAD

The amino acids determination was performed by the method described by Garde-Cerdán et al. [17]. Briefly, the derivatization of nitrogen compounds was performed by reaction of 750 µL of methanol (Merck, Darmstadt, Germany), 1.75 mL of borate buffer 1 M (pH 9), 1 mL of sample, 30 µL of diethyl ethoxymethylenemalonate (DEEMM) (Sigma-Aldrich, Madrid, Spain), as reagent of derivatization, and 20 µL of 2-aminoadipic acid (internal standard) (Sigma-Aldrich). The derivatization was carried out in a tube over 30 min in an ultrasound bath. Then, the sample was heated at 75 °C for 2 h to degrade the excess DEEMM and other by-products.

The analyses were performed on a Shimadzu Nexera X2 Ultra High-Performance Liquid Chromatograph (UHPLC) (Shimadzu, Kyoto, Japan) equipped with an automatic liquid sampler, and a diode array detector (DAD). Chromatographic separation was performed in an ACE HPLC column (C18-HL) (Aberdeen, Scotland) particle size 5 µm (250 mm × 4.6 mm). Amino acids were eluted under the conditions described by Garde-Cerdán et al. [18]. Phase A, 25 mM acetate buffer, pH 5.8, with 0.4 g of sodium azide; phase B, 80:20 (v/v) mixture of acetonitrile and methanol (Merck). DAD monitored at 280, 269, and 300 nm was used to detection. The volume of sample injected was 50 µL. The target compounds were identified according to the retention times and the UV–Vis spectral characteristics of corresponding standards (Sigma-Aldrich) derivatizated. Quantification was carried out using the calibration graphs of the respective standards in 0.1 N HCl, which underwent the same process of derivatization that the samples.

The treatments were performed in triplicate, so the results of free amino acids correspond to the average of three analyses ($n = 3$).

Statistical analysis

The statistical elaboration of the data was performed using SPSS Version 21.0 statistical package for Windows (SPSS, Chicago, USA). General parameters and nitrogen compound data were processed using the variance analysis (ANOVA) ($p \leq 0.05$). Differences between samples were compared using the Duncan test at 95% probability level. Also, a multivariate factorial analysis (with treatment and vintage as factors) was performed considering enological parameters and nitrogen compounds in grapes. Discriminant analysis was performed to classify the different samples according to their nitrogen composition.

Table 1 General parameters and nitrogen fractions in grapes from control, methyl jasmonate (MeJ), and ACP-MeJ treatments, in 2019 and 2020 vintages

	2019			2020		
	Control	MeJ	ACP-MeJ	Control	MeJ	ACP-MeJ
Weight of 100 berries (g)	113.68 ± 11.07a	141.81 ± 27.18a	116.94 ± 4.62a	199.57 ± 7.27a	207.67 ± 40.39a	194.90 ± 20.65a
ºBrix	24.70 ± 0.72b	22.23 ± 1.17a	23.37 ± 0.49ab	22.30 ± 0.92a	22.17 ± 2.31a	22.37 ± 0.38a
Probable alcohol (% v/v)	14.63 ± 0.49b	12.92 ± 0.80a	13.71 ± 0.35ab	12.97 ± 0.63a	12.89 ± 1.58a	13.01 ± 0.26a
pH	3.83 ± 0.05a	3.78 ± 0.10a	3.82 ± 0.09a	3.76 ± 0.01a	3.70 ± 0.07a	3.73 ± 0.06a
Total acidity (g/L)*	4.61 ± 0.11a	5.20 ± 0.36b	5.13 ± 0.26ab	4.12 ± 0.33a	4.54 ± 1.08a	4.03 ± 0.21a
Glu + Fru (g/L)	249.86 ± 9.97b	215.50 ± 12.29a	231.40 ± 10.82ab	216.42 ± 10.70a	218.62 ± 26.56a	223.84 ± 2.98a
Glu (g/L)	120.18 ± 5.13b	102.88 ± 6.89a	110.89 ± 4.94ab	107.31 ± 4.54a	106.08 ± 12.84a	108.61 ± 2.98a
Fru (g/L)	129.68 ± 4.84b	112.62 ± 5.43a	120.51 ± 6.26ab	109.11 ± 6.53a	112.54 ± 13.76a	114.72 ± 0.98a
Malic acid (g/L)	2.24 ± 0.24a	2.54 ± 0.32a	2.51 ± 0.56a	1.21 ± 0.08a	1.54 ± 0.22a	1.39 ± 0.18a
Ammonium nitrogen (mg N/L)	78.00 ± 8.22a	106.34 ± 15.68a	101.40 ± 20.40a	121.16 ± 3.52a	101.66 ± 19.58a	114.66 ± 6.24a
Amino nitrogen (mg N/L)	118.51 ± 14.33a	202.11 ± 50.59b	175.71 ± 24.66ab	152.53 ± 14.33a	139.63 ± 35.64a	152.24 ± 5.50a
YAN (mg N/L)	196.51 ± 21.18a	308.45 ± 64.76b	277.11 ± 44.31ab	273.69 ± 17.69a	241.29 ± 55.05a	266.90 ± 11.62a

All parameters are listed with their standard deviation ($n = 3$). For each vintage and parameter, different letters indicate significant differences between the samples ($p \leq 0.05$)

Glu glucose, *Fru* fructose; *YAN* yeast assimilable nitrogen

*As g/L of tartaric acid

Results and discussion

General parameters in the musts

Table 1 shows the general enological parameters and nitrogen fractions in the control and in the samples from applications with free methyl jasmonate (MeJ) and MeJ loaded on ACP nanoparticles (ACP-MeJ), in 2019 and 2020 vintages. In the first vintage, there were no significant differences in the weight of 100 berries, while the control presented greater glucose and fructose content, which translated into higher ºBrix and probable degree, than the musts from the MeJ foliar application (Table 1). Wang et al. [19] found a reduction in ºBrix, glucose, and fructose after MeJ treatment in Gewürztraminer grape variety, pointing to an elicitor repressive effect on berries maturation. Must samples from ACP-MeJ treatment did not present significant differences with control and MeJ ones for these enological parameters. Regarding pH, total acidity, and malic acid, only total acidity was greater in the MeJ musts than in the control samples (Table 1). D'Onofrio et al. [20] observed that MeJ treatment diminished total acidity of Sangiovese grape variety. Regarding the nitrogen fractions, there was no difference between samples for ammonium nitrogen, while amino nitrogen and YAN were higher in the samples from the MeJ treatment than in the control one, and without significant differences with the ACP-MeJ samples (Table 1). In all cases, the samples had sufficient YAN content to avoid fermentation problems [21].

Nevertheless, in the second vintage, in any of the general parameters and nitrogen fractions of the musts were found significant differences (Table 1), in agreement with other works that have used this elicitor in free or conventional form in Tempranillo cultivar [14, 22, 23]. The vines on which the treatments were applied were the same in both vintages; thus, the different response in the general enological parameters and in the nitrogen fractions due to the vintage could be due to higher rainfall in August 2020, 32.9 L/m^2, compared to 11.5 L/m^2 in 2019.

Influence of the foliar MeJ and ACP-MeJ treatments on must amino acids' content

Table 2 shows the results of free amino acids content in control, MeJ and ACP-MeJ grapes, in both vintages, and Fig. 1 shows the concentration of total amino acids and total amino acids without proline in these samples.

Table 2 Amino acids content (mg/L) in musts from control, methyl jasmonate (MeJ), and ACP-MeJ treatments, in 2019 and 2020 vintages

	2019			2020		
	Control	MeJ	ACP-MeJ	Control	MeJ	ACP-MeJ
Aspartic acid	24.69 ± 0.90a	40.54 ± 6.24b	32.48 ± 5.48ab	17.04 ± 1.79a	17.10 ± 1.50a	20.28 ± 2.93a
Glutamic acid	95.87 ± 17.26a	114.57 ± 11.73a	90.96 ± 8.96a	115.61 ± 8.07a	124.81 ± 20.29a	114.71 ± 3.14a
Asparagine	2.22 ± 0.31a	4.48 ± 0.92b	3.69 ± 0.30b	15.53 ± 0.92ab	12.56 ± 2.67a	18.23 ± 1.78b
Serine	33.01 ± 6.06a	55.78 ± 6.96b	36.39 ± 2.95a	53.78 ± 3.80a	48.92 ± 0.16a	52.19 ± 3.13a
Glutamine	148.17 ± 14.23a	343.73 ± 32.35b	187.19 ± 18.97a	447.75 ± 84.95b	215.58 ± 15.22a	348.80 ± 33.82b
Histidine	39.75 ± 7.05a	93.35 ± 2.77c	52.89 ± 1.03b	74.20 ± 8.01b	51.00 ± 4.84a	70.69 ± 8.86b
Glycine	1.72 ± 0.21a	4.87 ± 0.60b	2.34 ± 0.48a	6.34 ± 0.74b	4.74 ± 0.43a	5.61 ± 0.32ab
Threonine + citrulline	53.08 ± 0.76a	129.83 ± 14.81b	73.14 ± 10.73a	8.93 ± 0.50c	6.82 ± 0.05a	8.13 ± 0.09b
Arginine	125.93 ± 0.84a	312.73 ± 55.68c	194.91 ± 6.94b	374.27 ± 29.28a	369.47 ± 86.98a	399.23 ± 81.49a
Alanine	31.30 ± 4.98a	65.99 ± 16.71b	34.69 ± 4.23a	74.74 ± 7.79a	71.24 ± 3.26a	75.64 ± 6.59a
γ-Aminobutyric acid	56.70 ± 11.14a	46.70 ± 6.62a	52.38 ± 0.85a	67.11 ± 7.22a	90.24 ± 13.61a	70.51 ± 12.81a
Proline	92.31 ± 2.08a	151.52 ± 15.75b	128.27 ± 17.87b	126.79 ± 3.98a	121.03 ± 15.98a	134.66 ± 4.04a
Tyrosine	7.65 ± 1.18a	16.76 ± 2.18b	9.38 ± 0.64a	16.77 ± 1.55a	15.59 ± 0.85a	17.19 ± 2.27a
Valine	18.93 ± 1.59a	55.43 ± 2.01b	26.42 ± 8.26a	54.84 ± 7.47c	28.21 ± 0.65a	43.88 ± 3.68b
Methionine	5.45 ± 0.45a	18.96 ± 1.57c	8.53 ± 0.90b	13.24 ± 2.13a	9.25 ± 2.19a	12.63 ± 1.48a
Cysteine	1.75 ± 0.39b	0.73 ± 0.15a	0.91 ± 0.27a	0.38 ± 0.03a	0.40 ± 0.01a	0.47 ± 0.05b
Isoleucine + tryptophan	26.30 ± 1.63a	70.52 ± 4.15b	35.28 ± 9.14a	69.08 ± 8.79b	43.93 ± 3.11a	57.35 ± 6.07b
Leucine	19.22 ± 2.29a	63.85 ± 0.07b	29.54 ± 9.60a	52.66 ± 8.26c	26.74 ± 3.65a	40.29 ± 3.68b
Phenylalanine	11.02 ± 0.41a	23.71 ± 2.55b	11.26 ± 1.99a	28.97 ± 3.24c	15.52 ± 1.42a	21.24 ± 2.15b
Ornithine	1.33 ± 0.26a	4.74 ± 0.01c	2.18 ± 0.15b	4.60 ± 0.17a	4.53 ± 0.22a	4.94 ± 0.61a
Lysine	1.51 ± 0.30a	4.13 ± 0.76b	2.00 ± 0.16a	4.47 ± 0.37a	5.58 ± 0.55b	4.31 ± 0.26a

All parameters are listed with their standard deviation ($n = 3$). For each vintage and compound, different letters indicate significant differences between the samples ($p \leq 0.05$)

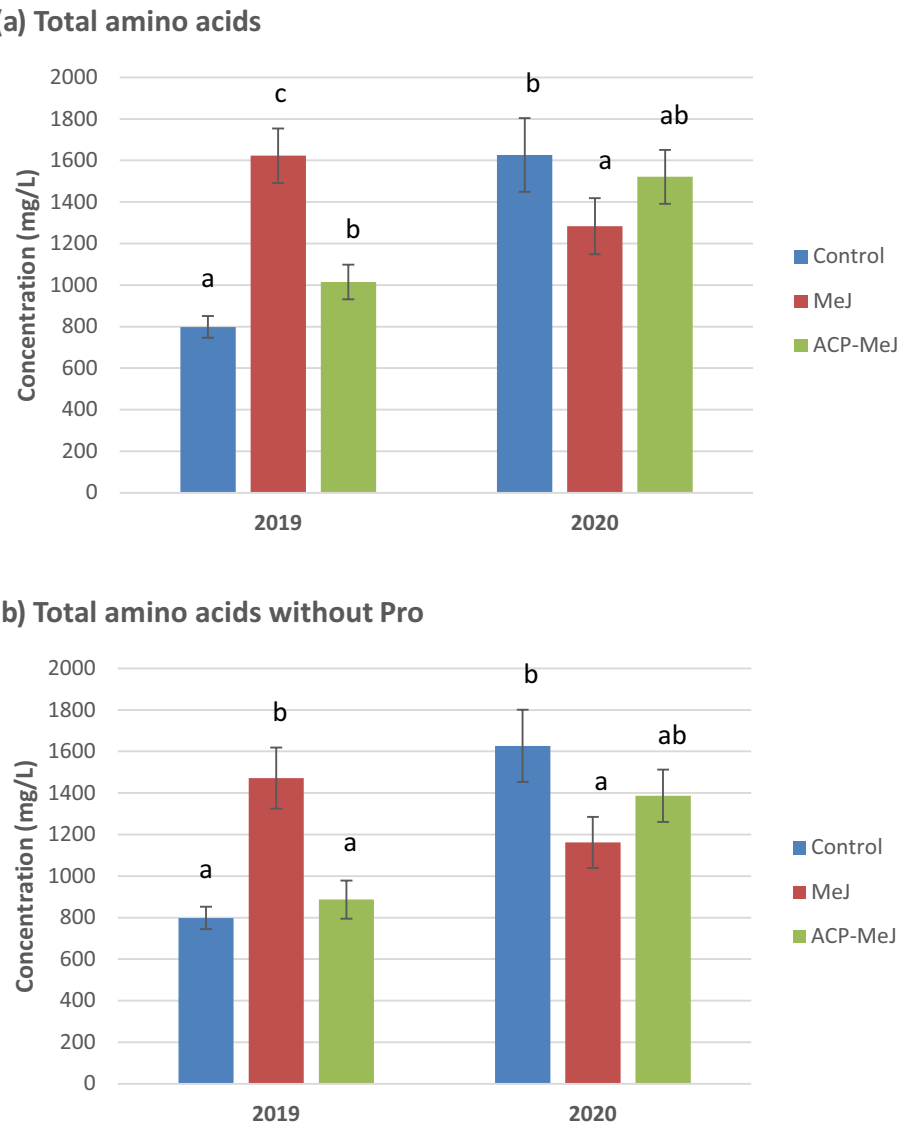

Fig. 1 Total amino acids and total amino acids without proline (mg/L) in musts from control, methyl jasmonate (MeJ), and nanoparticles doped with MeJ (ACP-MeJ) foliar treatments, in 2019 and 2020 vintages. All parameters listed with their standard deviation ($n = 3$). For each vintage and nitrogen parameter, different letters indicate significant differences between samples ($p \leq 0.05$)

In the first vintage, 2019, the concentration in the musts of all the amino acids, with the exception of two of them, increased with the application of MeJ; while the treatment with ACP-MeJ had less influence on the amino acid composition, since only affected to six of the amino acids present in the samples, increasing their content (Table 1). Cysteine was the only amino acid whose content decreased with both treatments. These results were reflected in a higher concentration of total amino acids in the musts, from both treatments, than in the control, being the increase greater when applying the conventional MeJ (Fig. 1a); while the content of total amino acids without proline was only affected by applying free MeJ (Fig. 1b).

The results observed in the second vintage, 2020, were very different from those found in the first one, 2019. Several amino acids were found in the musts in lower concentration when applying MeJ than in the control samples, i.e., glutamine, histidine, glycine, threonine + citrulline, valine, isoleucine + tryptophan, leucine, and phenylalanine, and only one of them, lysine, increased its content with this treatment (Table 1); thus, the content of total amino acids and total amino acids without proline was lower after applying MeJ than in the control musts (Fig. 1a and b). However, when the foliar treatments were carried out with ACP-MeJ, practically, all the amino acids were found in similar concentrations as in the control, except for threonine + citrulline,

valine, cysteine, leucine, and phenylalanine (Table 1), so the content of total amino acids and total amino acids without proline did not show significant differences with the control one (Fig. 1a and b).

The different response of the plant to the application of MeJ in both formats, free and nano, in each vintage could be due to several factors. First, the nitrogen needs of the plant, measured as nitrogen content in the berry, in 2019 were higher than in 2020, so that the plant responded more receptively in the first year of the trial, and it did in a more pronounced way when applying MeJ in a higher dosage, that is, in a conventional way than in nano-form (10 mM versus 1 mM) (Fig. 1). Second, MeJ is slowly released when applied in nano-form [4]; therefore, there could be a memory effect in the plants, so that in the second year, it behaved in a similar way to free MeJ (Fig. 1). This result was in agreement with Gil-Muñoz et al. [9], who also observed that, in the first year of application, the musts from the treatment with free MeJ had a higher content of amino acids than ACP-MeJ; while in the second year, the amino acid concentrations were lower, or similar, in ACP-MeJ than in MeJ. However, the application of free MeJ in the second year of our study was negative from the point of view of the must nitrogen composition respect to the control, which could be due, as indicated above, to the fact that the plants did not have the same nitrogen needs, since the content, in the control, of total amino acids in 2020 was practically double that in 2019 (Fig. 1).

The two most representative amino acids of grapes are arginine and proline, since they are two of the most abundant amino acids [24]. In addition, arginine is the best nitrogen source, after ammonium, for yeasts [17, 21], while proline is not metabolized by yeasts under typical vinification conditions, that is, in the absence of oxygen and in the presence of good nitrogen sources [25, 26]. Therefore, these two amino acids are related to assimilable and non-assimilable nitrogen, respectively, being the proline/arginine ratio a parameter that can indicate adequate or inadequate initial conditions, in terms of nitrogen available to yeasts during fermentation. Both treatments, MeJ and ACP-MeJ, decreased this ratio compared to the control in 2019, especially when applying free MeJ, while this parameter was not affected in 2020. Consequently, both treatments clearly improved the nitrogen available to the yeasts in 2019, which was the year with the lowest concentration of amino acids in the control must (Table 2 and Fig. 1).

Other important amino acids for being precursors of fermentative aromatic compounds, i.e., threonine, tyrosine, valine, methionine, isoleucine, tryptophan, leucine, and phenylalanine, that is, nitrogen compounds that determine the fermentative *bouquet* of wine and its organoleptic quality, were also affected by the treatments carried out in the vineyard, as previously mentioned (Table 2). Higher alcohols can be formed anabolically from sugars as well as catabolically from amino acids via the Ehrlich pathway [27]. In 2019, the content of alcohols in the control wines was higher than that of the wines from the MeJ and ACP-MeJ treatments [8], which was probably due to the fact that the sugar content was higher in the control must (Table 1), indicating that the main route of formation of these compounds was the anabolic pathway. Since there were no differences in sugar content between MeJ and ACP-MeJ must samples (Table 1), the differences, respect to the control wines, in the content of alcohols were higher in MeJ than in ACP-MeJ wines [8], probably due to the higher content of amino acids in the MeJ musts (Table 2). However, in 2020, as there were no differences in the sugar content between the control musts and those treated with MeJ and ACP-MeJ (Table 1), practically no differences were observed in the content of higher alcohols in the wines [8], and when there were differences, their content in MeJ wines was lower, since these musts had a lower content of several amino acids (Table 2). Given that several alcohols are precursors of acetate esters and the control wines had the highest alcohol content in 2019, since they had a greater amount of sugars (Table 1), what was said for higher alcohols corresponds to what was observed for esters [8].

Multivariable analysis

Table 3 shows the results of the factorial analysis (treatment, vintage, and their interaction) of the general parameters and nitrogen fractions of the musts. None of the parameters studied was affected by the treatment applied foliarly in the vineyard. Therefore, the vintage factor had the most impact on the overall parameters of the musts, so that, regardless of treatment, the weight of 100 berries and the ammonium nitrogen content were higher in 2020 than in 2019; while total acidity, fructose, and malic acid were higher in 2019 than in 2020 (Table 3). There was no interaction between the two factors for any of the general parameters studied, but did for nitrogen fractions.

Table 4 presents the results of the factorial analysis (treatment, vintage, and their interaction) of the must amino acids. The content in grapes of most of these compounds was affected by both factors. Regardless of vintage, the content of total amino acids and total amino acids without proline was higher in the samples treated with MeJ than in the control and in those from the treatments with ACP-MeJ, without significant differences between them (Table 4). This result was due to the fact that practically all the amino acids, with the exception of glutamic acid, asparagine, glutamine, GABA, valine, cysteine, and phenylalanine, in the case of the control, and aspartic acid, glutamic acid, asparagine, glutamine, arginine, GABA, and cysteine, in the case of ACP-MeJ, were in higher concentration in the MeJ musts

Table 3 Multifactor analysis of variance of general parameters and nitrogen fractions of the musts with the two factors studied: treatment (Control, MeJ, ACP-MeJ) and vintage (2019 and 2020) and their interaction (treatment × vintage)

	Weight of 100 berries (g)	°Brix	Probable alcohol (% v/v)	pH	Total acidity (g/L)	Glu + Fru (g/L)	Glu (g/L)	Fru (g/L)	Malic acid (g/L)	Ammonium nitrogen (mg N/L)	Amino nitrogen (mg N/L)	YAN (mg N/L)
Treatment (T)												
Control	156.63a	23.50a	13.80a	3.79a	4.37a	233.14a	113.74a	119.39a	1.73a	99.58a	135.52a	235.10a
MeJ	174.74a	22.20a	12.91a	3.74a	4.87a	217.06a	104.48a	112.58a	2.04a	104.00a	170.87a	274.87a
ACP-MeJ	155.92a	22.87a	13.36a	3.77a	4.58a	227.37a	109.75a	117.62a	1.95a	108.03a	163.97a	272.00a
Vintage (V)												
2019	124.14a	23.43a	13.75a	3.8a	4.98b	232.25a	111.32a	120.9b	2.43b	95.25a	165.44a	260.68a
2020	200.71b	22.28a	12.96a	3.7a	4.23a	219.46a	107.33a	112.12a	1.38a	112.49b	148.13a	260.63a
Interaction												
T×V	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	*	*	*

For each parameter and factor, different letters indicate significant differences between samples ($p \leq 0.05$). Interaction: N.S., not significant ($p > 0.05$); *$p \leq 0.05$

Glu glucose, *Fru* fructose, *YAN* yeast assimilable nitrogen

(Table 4). It should be noted that Pérez-Álvarez et al. [5] also found no differences due to the treatment factor between the control and the samples from the treatment with ACP-MeJ. As for the effect of the vintage, regardless of the treatment, it was observed that all amino acids, except histidine, proline, methionine, and leucine, showed significant differences depending on the vintage, and therefore also in the total content of amino acids, with and without proline (Table 4). It should be noted that practically all of them, with the exception of aspartic acid, threonine + citrulline, and cysteine, had a higher concentration in 2020 than in 2019, being the total content of these nitrogen compounds higher in the second vintage (Table 4). Given that the vines used for this trial were the same in both vintages and that the vineyard was not fertilized, the differences between vintages could be due to the aforementioned, that is, to the fact that in August 2020, it rained more than in 2019, allowing a higher absorption by the plant of the nitrogen available in the soil. The main soil environmental factor affecting the nutrient flow is the soil water potential. A lack of water makes the soil water potential drop; therefore, the nitrogen moves slowly, and its absorption and transport are reduced [28]. There was interaction between the two factors for all amino acids, except glutamic acid (Table 4).

Figure 2 shows the discriminant analysis carried out with the amino acids concentration of the different samples in 2019 (Fig. 2a), in 2020 (Fig. 2b), and considering both vintages (Fig. 2c). In 2019 and 2020, it was observed that Function 1 (99.6% and 96.8%, respectively) allows a very good separation of the samples according to the treatment performed (control, MeJ, ACP-MeJ). In both vintages, control and ACP-MeJ are quite separated from MeJ (Fig. 2a and b), but with opposite behavior, more amino acids in 2019 and less in 2020 in the samples from the MeJ application, according to the results obtained (Table 4 and Fig. 1). If the global study is considered (all treatments and both vintages), it can be observed the existence of four differentiated groups, separated by both Functions (Function 1: 70.2%; Function 2: 22.9%). Function 1 separates the samples by vintage, the 2020 samples on the right and the 2019 samples on the left, due to the higher content of nitrogen compounds in the second vintage; while Function 2 separates them by treatments, in both vintages, the MeJ is clearly differentiated from the control and ACP-MeJ (Fig. 1c), according to their nitrogen composition.

Conclusions

The effect of methyl jasmonate (MeJ) and methyl jasmonate-doped nanoparticle (ACP-MeJ) treatments on the nitrogen composition of Tempranillo grapes during two vintages were evaluated. The amino nitrogen and yeast assimilable nitrogen

Table 4 Multifactor analysis of variance of amino acids (expressed as mg/L)

	Treatment (T)			Vintage (V)		Interaction (T x V)
	Control	MeJ	ACP-MeJ	2019	2020	
Aspartic acid	20.87a	28.82b	26.38b	32.57b	18.14a	**
Glutamic acid	105.74a	119.69a	102.83a	100.46a	118.38b	N.S
Asparagine	8.88a	8.52a	10.96b	3.47a	15.44b	**
Serine	43.39a	52.35b	44.29a	41.73a	51.63b	***
Glutamine	297.81a	279.65a	268.00a	226.36a	337.28b	***
Histidine	56.98a	72.17b	61.79a	62.00a	65.30a	***
Glycine	4.03a	4.81b	3.97a	2.98a	5.56b	***
Threonine + citrulline	31.01a	68.32c	40.64b	85.35b	7.96a	***
Arginine	250.10a	341.10b	297.07ab	211.19a	380.99b	*
Alanine	53.02a	68.61b	55.16a	43.99a	73.87b	**
γ-Aminobutyric acid	61.91a	68.47a	61.45a	51.93a	75.95b	*
Proline	109.55a	136.27b	131.47b	124.04a	127.49a	**
Tyrosine	12.21a	16.18b	13.28a	11.27a	16.52b	***
Valine	36.88ab	41.82b	35.15a	33.59a	42.31b	***
Methionine	9.35a	14.11b	10.58a	10.98a	11.71a	***
Cysteine	1.06b	0.57a	0.69a	1.13b	0.42a	**
Isoleucine + tryptophan	47.69a	57.23b	46.32a	44.03a	56.79b	***
Leucine	35.94a	45.30b	34.91a	37.54a	39.89a	***
Phenylalanine	20.00b	19.62b	16.25a	15.33a	21.91b	***
Ornithine	2.96a	4.64c	3.56b	2.75a	4.69b	***
Lysine	2.99a	4.85b	3.15a	2.55a	4.79b	*
Total amino acids	1212.35a	1453.10b	1267.91a	1145.22a	1477.02b	***
Total amino acids without Pro	1102.80a	1316.82b	1136.44a	1021.19a	1349.52b	***

For each parameter and factor, different letters indicate significant differences between samples ($p \leq 0.05$). Interaction: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, and N.S., not significant ($p > 0.05$)

were higher in the grapes treated with MeJ and ACP-MeJ than in the control ones in 2019. However, in the second vintage, no significant differences were observed in any of the must general parameters and nitrogen fractions studied. With respect to the amino acids' content, MeJ treatment enhanced the concentration of all of them, except from glutamic acid and γ-aminobutyric acid, in 2019. ACP-MeJ treatment only increased the concentration of six of them, prompting to a total amino acid concentration lower than MeJ treatment (applying ten times higher MeJ dosage), but higher than control sample. Nevertheless, the content of total amino acids and total amino acids without proline was lower after applying MeJ treatments (MeJ and ACP-MeJ) than in the control musts in 2020. The multivariable analysis revealed that all amino acids, except histidine, proline, methionine, and leucine, show significant differences depending on the vintage and regardless of the treatment. A prominent effect of the vintage on the overall parameters of must could be related to the higher rainfall in 2020, considering that MeJ is an elecitor able to trigger plant defense responses against abiotic stress (i.e., drought).

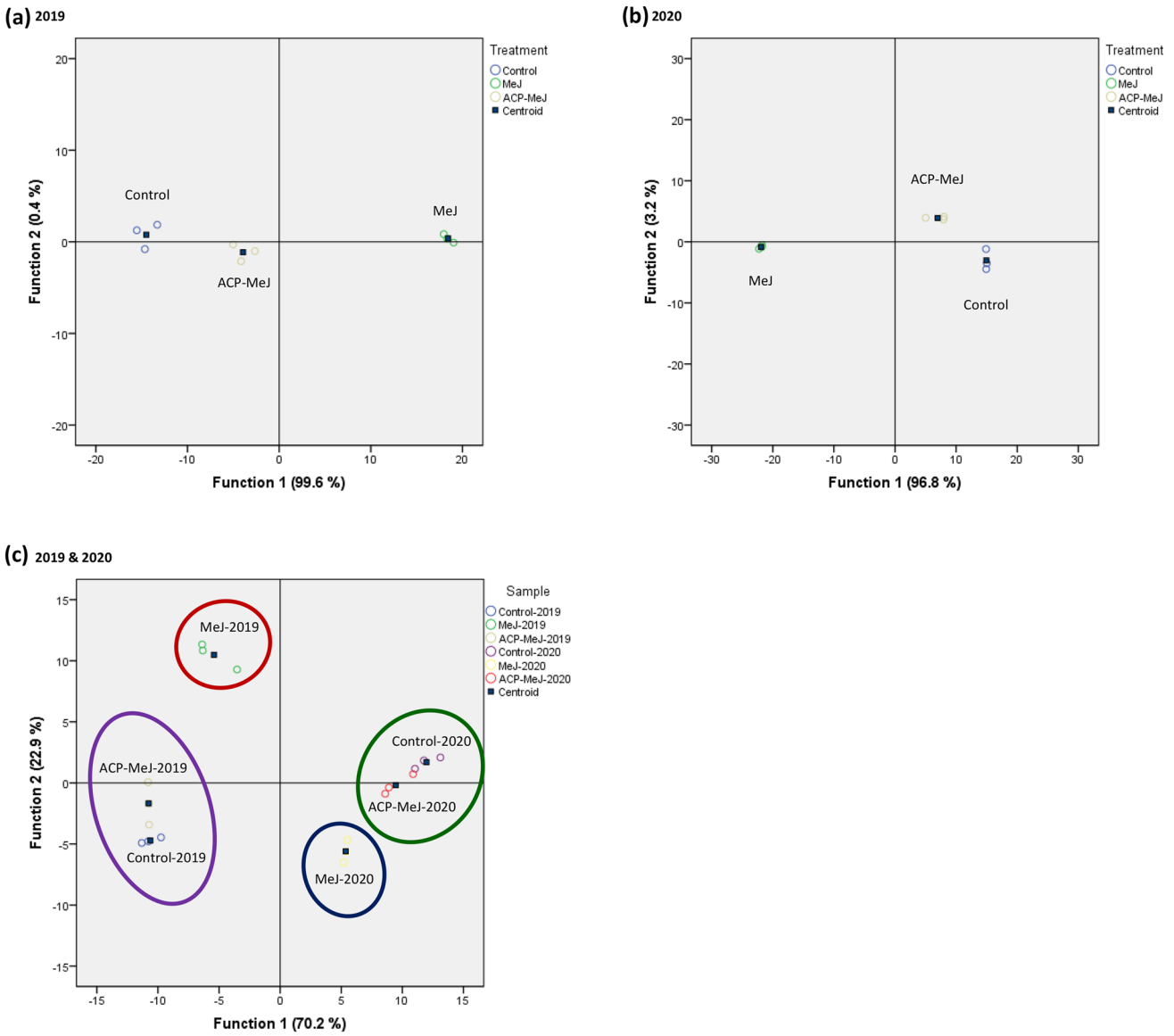

Fig. 2 Discriminant analysis of amino acids' content (mg/L) in musts from control, methyl jasmonate (MeJ), and nanoparticles doped with this elicitor (ACP-MeJ) treatments, in **a** 2019, **b** 2020, and **c** 2019 and 2020 vintages

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Data availability All data included in this manuscript are available upon request by contacting with the corresponding authors.

Declarations

Conflict of interest The authors declare no conflict of interest.

Compliance with ethics requeriments This article does not contain any studies with human or animal subjects.

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Foliar application of methyl jasmonate and methyl jasmonate supported on nanoparticles: Incidence on grape phenolic composition over two seasons

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ABSTRACT

Tools to address the mismatch between technological and phenolic maturity of grapes are needed. Application of elicitors could be an effective alternative. This work compares the effect of the application of methyl jasmonate (MeJ) in conventional form and, as a novelty, in the form of MeJ-doped nanoparticles (ACP-MeJ) on the phenolic composition of Tempranillo grapes. Results showed that, regardless of season, both treatments increased the grape total phenols content. In 2019, most of the anthocyanins, and to a lesser extent the flavanols, increased with the application of MeJ, and several hydroxycinnamic acids increased in the grapes treated with ACP-MeJ, with dose 10 times lower than those of the MeJ conventional. In 2020, anthocyanins were not affected by the treatments, but total flavanols, flavonols, hydroxybenzoic acid, and stilbenes increased after ACP-MeJ application. Thus, foliar application of ACP-MeJ could serve to increase grape phenolic composition, reducing maturity decoupling and the environmental impact.

1. Introduction

Viticulture is an agricultural activity of great global importance from an economic and social point of view. The wine sector is being affected by the consequences of the global warming. In this regard, since a few years, a mismatch has been observed between the so-called technological maturity and the phenolic maturity. Thus, the greater water stress and higher temperatures conditions resulting from climate change, have an impact on the synthesis of phenolic compounds by the plant, favoring the mismatch between the technological and the phenolic maturities, so that the quality of grapes and wines is impaired (Mira de Orduña, 2010; Rienth et al., 2021). Phenolic compounds are one of the most important groups of substances for the final quality of the wine. They are not only responsible for the color and stability of the wine, but also for the quality of the grapes and the wine itself (Trouillas et al., 2016; de Freitas et al., 2017), contributing significantly to their organoleptic characteristics and health properties. In this sense, in recent years, it has become evident that these compounds have a beneficial effect on the consumer's health, such as anti-cancer, anti-inflammatory, neuroprotective, or cardioprotective properties (Han et al., 2019; Hermosín-Gutiérrez et al., 2020; Koh et al., 2021; Pérez-Navarro et al., 2021).

Since grapevines are considered more vulnerable to the effects of climate change than other crops, one possible strategy to counteract these effects is the use of elicitors in the vineyard, substances that when applied exogenously trigger defensive mechanisms in the plant (Ruiz-García & Gómez-Plaza, 2013). In viticulture, one of the elicitors most widely used has been methyl jasmonate (MeJ), which could be a good alternative in sustainable agriculture, since it mainly improves the phenolic composition of the grapes (Gil-Muñoz et al., 2017; Portu et al., 2018a; Moro et al., 2020; Paladines-Quezada et al., 2021). When this elicitor is applied, the content of the grape phenolic compounds increases due mainly to the activation of the enzymes involved in their synthesis, mainly affecting enzymes specifically related to anthocyanins and stilbenes (Belhadj et al., 2008; Ju et al., 2022; Wang, Kumar et al., 2022). Therefore, the use of MeJ in the vineyard can be an alternative to mitigate the decoupling between technological maturity, mainly related to the content of sugars and acids, and phenolic maturity, determined by the amount of phenolic compounds, due to the climate change, but it is a product quite expensive for the winegrowers.

Hence, this work proposes the development of innovative tools based on the use of nanotechnology for a more efficient and sustainable agriculture. In recent decades, nanotechnology has had a great impact on

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many disciplines, with medicine being one of the most benefited (Manzoor et al., 2012). This strategy can also offer interesting opportunities in agriculture (DeRosa et al., 2010; Ur Rahim et al., 2021), topic in which it has been little explored. In the literature, there are not many practical studies that address the use of nanoparticles in agriculture (Pérez-de-Luque & Hermosín, 2013; Fellet et al., 2021; Fincheira et al., 2021). In addition, to the best of our knowledge, in viticulture there are few studies in which a nanometer-sized fertilizer was applied in the vineyard (Garde-Cerdán et al., 2021). Sabir et al. (2014) showed that a foliar-applied calcite product stimulated growth, accelerated ripening and increased vine yield. Moreover, Pérez-Álvarez et al. (2021a) and Gaiotti et al. (2021) reported that the treatments with amorphous calcium phosphate (ACP) nanoparticles doped with urea, improved the nitrogen composition of the grapes when were applied to the leaves or the soil, respectively. Whereas, Pérez-Álvarez et al. (2022) and Gil-Muñoz et al. (2021) observed very different behaviors when applying ACP nanoparticles doped with MeJ on the grapes amino acids composition, despite that they use the same grape variety and carried out the studies on the same two vintages. The first authors found practically no differences, while the second ones observed an increase in the content of nitrogen compounds. However, no work has been found that studies the effect of MeJ nanoparticle application in the vineyard on grapes phenolic composition. ACP nanoparticles protect and retain the MeJ, and, together with its slow release, increase the efficiency of this elicitor (Parra-Torrejón et al., 2021), potentially allowing the reduction of the elicitor quantity, and contributing to a more sustainable and economically viable viticulture.

Therefore, the aim of this work was to study the influence of foliar application of MeJ, in conventional and nano form (as a novelty), on grape phenolic composition throughout two consecutive seasons.

2. Materials and methods

2.1. Vineyard site, grapevine treatments and samples

Grapevines of Tempranillo (*Vitis vinifera* L.) cultivar grown in the experimental vineyard located in Finca La Grajera, Logroño, La Rioja, Spain (42°26′25.36′'North, Latitude; 2°30′56.41′'West, Longitude; and 456 m above sea level, altitude) were used during 2019 and 2020 seasons. Vines were planted in 1997, grafted onto R-110 rootstock and trained to a VSP (vertical shoot positioned) trellis system. Vine spacing was 2.80 m × 1.25 m. Climate data was recorded by an automatic meteorological station belonging to the Agroclimatic Information Service of La Rioja (SIAR) installed near to the experimental field. The collected data were the rain accumulated from the beginning of April until 1st of September, being 247.8 L/m^2 in 2019 and 217.8 L/m^2 in 2020; and the average maximum, mean and minimum temperatures, being 27.0 °C, 13.8 °C and 3.7 °C, respectively, in 2019, and 26.3 °C, 13.8 °C and 3.7 °C, respectively, in 2020.

Foliar applications of methyl jasmonate (MeJ) and nanoparticles doped with this elicitor (ACP-MeJ) were studied. To carry out the treatments, aqueous solutions were prepared with a MeJ concentration of 10 mM, according to previous works (Garde-Cerdán et al., 2016, 2018), and 1 mM of ACP-MeJ, according to Pérez-Álvarez et al. (2022), using Tween 80 as wetting agent (1 mL/L). The synthesis and detailed characterization of the ACP-MeJ were described in Parra-Torrejón et al. (2021) and Pérez-Álvarez et al. (2022). Control plants were sprayed only with a water solution of Tween 80. All treatments were applied to grapevine twice, at veraison and one week later. For each application, 200 mL/plant was sprayed over leaves. The treatments were performed in triplicate and were arranged in a complete randomized block design, with 10 vines for each replication and treatment.

Grapes from all grapevines and treatments were harvested at their optimum technological maturity, i.e., when the weight of 100 berries remained constant and the probable alcohol reached 13 (% v/v). A random set of 150 berries per replicate and treatment was collected and frozen at −20 °C until the analyses of grape phenolic compounds were carried out. Another set of 100 berries was separated and weighed to obtain the average berry weight. Then, grapes were crushed and in the must were determined the general parameters.

2.2. Determination of general parameters in must

The must enological parameters, °Brix, probable alcohol, pH, and total acidity, were analyzed using the official methods established by the OIV (2009). Glucose, fructose, malic acid and total phenols were determined using a Miura One enzymatic equipment (TDI, Barcelona, Spain), using the corresponding enzymatic kits provided by the TDI company for each parameter.

As the treatments were performed in triplicate, the results of these parameters are shown as the average of three analyses (n = 3).

2.3. Analysis of grape phenolic compounds by HPLC-DAD

2.3.1. Extraction of grape phenolics

Grape phenolic compounds were extracted according to Portu et al. (2015a). Briefly, about 50 g of each frozen grape sample were weighed and immersed into 50 mL of a mixture of methanol/water/formic acid (50:48.5:1.5, v/v/v). The mixture was then homogenized by an Ultra-Turrax T-18 (IKA, Staufen, Germany) at high speed (18,000 rpm) for 1 min. Then, samples were macerated in an ultrasonic bath (model DU-100, ArgoLab, Barcelona, Spain) for 10 min and were centrifuged at 3,640 × g at 10 °C for 10 min. The supernatant was separated and the resulting pellet was extracted again using the same volume of the solvent mixture (50 mL). At this point, the supernatants were combined, the volume annotated, and then samples were transferred to vials and stored at −20 °C until use.

2.3.2. Extract SPE clean-up for the analysis of non-anthocyanin phenolic compounds

According to Portu et al. (2015a), PCX SPE cartridges (500 mg, 6 mL; Bond Elut Plexa, Agilent, Palo Alto, CA) were used. Cartridges were placed in the extraction system (Visiprep^{TM} Vacuum Manifold, Sigma-Aldrich). First, grape phenolic extracts (3 mL) were diluted with 9 mL of 0.1 N HCl. The PCX SPE cartridges were conditioned using 5 mL of methanol and 5 mL of water. Then, the diluted samples were passed through the PCX SPE cartridges and washing was carried out with 5 mL of 0.1 N HCl and 5 mL of water. The non-anthocyanin phenolic compounds fraction was eluted with 3 × 5 mL of methanol. Then, the non-anthocyanin phenolic compounds fraction was dried in a centrifugal evaporator (miVac, Genevac ltd., Suffolk, UK) at 35 °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 flavanols).

2.3.3. Analysis of phenolic compounds by HPLC-DAD

Phenolic compounds were analyzed according to Portu et al. (2015a) using an Agilent 1260 Infinity II chromatograph, equipped with a diode array detector (DAD). Samples were filtered and injected on a Licrospher® 100 RP-18 reversed-phase column (250 × 4.0 mm; 5 μm packing; Agilent) with pre-column Licrospher® 100 RP-18 (4 × 4 mm; 5 μm packing; Agilent), both thermostated at 40 °C. A flow rate of 0.630 mL/min was established. For the analysis of anthocyanins, without SPE extraction, 10 μL of grape extract 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). For the analysis of non-anthocyanin phenolic compounds fractions, after SPE clean-up, the injection volume was 20 μL. Eluents were (A) acetonitrile/water/formic acid (3:88.5:8.5, v/v/v), (B) acetonitrile/water/formic acid (50:41.5:8.5, v/v/v), and (C) methanol/water/formic acid (90:1.5:8.5, v/v/v).

For the phenolic compounds identification, an ion trap ESI-MS/MS detector was used in both, positive and negative ion modes, setting the following parameters: dry gas N_2, 8 L/min; drying temperature, 325 °C; nebulizer, N_2, 50 psi; ionization and fragmentation parameters were optimized by direct infusion of appropriate standard solutions; scan range, 50–1200 *m/z*. Identification was based on spectroscopic data (UV–Vis and MS/MS) obtained from authentic standards or previously reported data (Castillo-Muñoz et al., 2009; Lago-Vanzela, Da-Silva, Gomes, García-Romero, & Hermosín-Gutiérrez, 2011). For quantification, DAD chromatograms were extracted at 520 nm (anthocyanins), 360 nm (flavonols), 320 nm (hydroxycinnamic acids and stilbenes), and 280 nm (gallic acid and flavanols) and the calibration graphs of the respective standards ($R^2 > 0.99$) were used. When a standard was not available, quantification was made according to the calibration graph of the most similar compound. Hence, malvidin-3-*O*-glucoside was used for anthocyanins, quercetin-3-*O*-glucoside was used for flavonols, *trans*-caftaric acid was used for free hydroxycinnamic acids and the corresponding tartaric esters, catechin was used for procyanidins B1 and B2, epicatechin was used for epigallocatechin, and *trans*-piceid and *trans*-resveratrol were used for their respective *cis* isomers. Concentrations were expressed as mg/kg. The validation data of the HPLC method were the following: variation coefficient (%) for retention time of commercially standards varied from 0.09 to 0.72; the detection limit (mg/L) ranged from 0.099 to 0.711; the quantification limit (mg/L) changed from 0.292 to 2.370; the variation coefficient (%) for concentration varied from 1.66 to 6.67. The response factor (mg/area units) was also calculated ranging from $3.99E^{-6}$ to $1.00E^{-4}$. The variation coefficients were obtained from 10 consecutive analyses.

Since field treatments were performed in triplicate, the results for phenolic compounds are the average of the analyses of three samples (n = 3).

2.4. Statistical analysis

The statistical elaboration of the data was performed using SPSS Version 21.0 statistical package for Windows (SPSS, Chicago, USA). General parameters and phenolic compounds data were processed using the variance analysis (ANOVA) ($p \leq 0.05$). The differences between means were compared using the Duncan test. Moreover, the effect of foliar treatment, seasons and their interaction was analyzed using a multifactor analysis (MANOVA) and post hoc Duncan's multiple range test. Discriminant analysis were carried out on phenolic compounds data in order to classify them according to the treatments and the season.

3. Results and discussion

3.1. Effect of MeJ and ACP-MeJ foliar applications on the musts general parameters

Table 1 shows the enological parameters in the samples from control and vines treated with methyl jasmonate (MeJ) and with nanoparticles doped with this elicitor (ACP-MeJ), in 2019 and 2020 seasons.

In the first season, 2019, in the weight of 100 berries, there were no significant differences between control and treated samples. Meanwhile, the control samples showed higher concentrations of the two major sugars in the grapes, glucose (p-value = 0.028) and fructose (p-value = 0.026), which translated into higher °Brix (p-value = 0.032) and probable degree (p-value = 0.031) than the grapes from the MeJ treatment. Wang, VanderWeide et al. (2022) also observed a decrease in °Brix, glucose and fructose values when MeJ was applied to the Gewürztraminer cultivar, indicating a repressive effect of the elicitor on grape ripening. This could indicate that the MeJ treatment allows a slight delay in the technological maturation of the berries and, therefore, its application would bring the two maturities of the grapes closer together. Meanwhile, grapes from the ACP-MeJ treatment did not show significant differences with either the control or MeJ samples for these parameters, i.e. glucose, fructose, °Brix, and probable alcohol (Table 1). Regarding to the acidity parameters, i.e. pH, total acidity and malic acid, only the total acidity content was higher in the MeJ samples than in the control ones, and neither the control nor the MeJ samples presented significant differences with ACP-MeJ. D'Onofrio, Matarese, and Cuzzola (2018) found that the MeJ application to Sangiovese vines decreased the total acidity content of the grapes. There was a significant increase in total phenols content when both treatments with MeJ were applied, in conventional form (MeJ) and in nano size (ACP-MeJ) (p-value = 0.029), with no significant differences between them (Table 1), despite the fact that the elicitor concentration was reduced by one tenth when was applied in the nano size form (ACP-MeJ) compared to the conventional form (MeJ). These results are particularly relevant since the foliar application of this elicitor in the vineyard contributed to decrease the sugar content and increase the content of phenolic compounds in the grapes. Therefore, this elicitor can contribute to reduce the problem of decoupling between technological and phenolic maturity increased by the climate change, as mentioned above.

However, in the second season, no significant differences were observed in any of the must general parameters studied (Table 1), which is in agreement with other studies that have applied this elicitor in conventional form in Tempranillo cultivar (Portu et al., 2015b, 2016). The vines on which the foliar applications were carried out were the same in both seasons. Thus, the different response observed in the general parameters due to the season, could be conditioned by a higher rainfall in August 2020 (32.9 L/m^2) *versus* 2019 (11.5 L/m^2) season. The higher availability of water content for vineyards in 2020, may have led

Table 1
General parameters in musts from control, methyl jasmonate (MeJ) and nanoparticles doped with this elicitor (ACP-MeJ) treatments, in 2019 and 2020 seasons.

	2019			2020		
	Control	**MeJ**	**ACP-MeJ**	**Control**	**MeJ**	**ACP-MeJ**
Weight of 100 berries (g)	113.68 ± 11.07 a	141.81 ± 27.18 a	116.94 ± 4.62 a	199.57 ± 7.27 a	207.67 ± 40.39 a	194.90 ± 20.65 a
°Brix	24.70 ± 0.72b	22.23 ± 1.17 a	23.37 ± 0.49 ab	22.30 ± 0.92 a	22.17 ± 2.31 a	22.37 ± 0.38 a
Probable alcohol (% v/v)	14.63 ± 0.49b	12.92 ± 0.80 a	13.71 ± 0.35 ab	12.97 ± 0.63 a	12.89 ± 1.58 a	13.01 ± 0.26 a
pH	3.83 ± 0.05 a	3.78 ± 0.10 a	3.82 ± 0.09 a	3.76 ± 0.01 a	3.70 ± 0.07 a	3.73 ± 0.06 a
Total acidity (g/L)*	4.61 ± 0.11 a	5.20 ± 0.36b	5.13 ± 0.26 ab	4.12 ± 0.33 a	4.54 ± 1.08 a	4.03 ± 0.21 a
Glu + Fru (g/L)	249.86 ± 9.97b	215.50 ± 12.29 a	231.40 ± 10.82 ab	216.42 ± 10.70 a	218.62 ± 26.56 a	223.84 ± 2.98 a
Glu (g/L)	120.18 ± 5.13b	102.88 ± 6.89 a	110.89 ± 4.94 ab	107.31 ± 4.54 a	106.08 ± 12.84 a	108.61 ± 2.98 a
Fru (g/L)	129.68 ± 4.84b	112.62 ± 5.43 a	120.51 ± 6.26 ab	109.11 ± 6.53 a	112.54 ± 13.76 a	114.72 ± 0.98 a
Malic acid (g/L)	2.24 ± 0.24 a	2.54 ± 0.32 a	2.51 ± 0.56 a	1.21 ± 0.08 a	1.54 ± 0.22 a	1.39 ± 0.18 a
Total phenols (mg/L)	1185.33 ± 72.31 a	1306.57 ± 61.35b	1351.40 ± 27.32b	541.60 ± 64.02 a	603.07 ± 73.82 a	582.70 ± 66.02 a

*As g/L of tartaric acid; Glu: glucose; Fru: fructose. All parameters are listed with their standard deviation (n = 3). For each season and parameter, different letters indicate significant differences between the samples ($p \leq 0.05$).

an increase in berry size (weight of 100 grapes, Table 1) and therefore a greater dilution of the compounds in grapes.

3.2. Influence of the foliar MeJ and ACP-MeJ treatments on grape phenolic compounds

Table 2 shows the results of anthocyanins content in control, MeJ and ACP-MeJ grapes. In 2019, of the five non-acylated anthocyanins studied, the concentration of two of them (cyanidin-3-glc (p-value = 0.000), and peonidin-3-glc (p-value = 0.036)) increased in the grapes after the application on the vineyard of conventional MeJ. However, the application of this elicitor in nano form (ACP-MeJ) had no significant effect on the content of any of these five non-acylated anthocyanins. The total content of non-acylated anthocyanins was higher in the MeJ grapes than in the control, and neither the control nor the MeJ samples showed significant differences with the ACP-MeJ samples. It should be noted that a higher incidence of the treatments was observed in the acylated anthocyanins (Table 2): of the 12 compounds studied, eight showed significant differences between treatments. The application of conventional MeJ increased the concentration of peonidin-3-acglc (p-value = 0.023), cyanidin-3-cmglc (p-value = 0.014), petunidin-3-cmglc (p-value = 0.016), and peonidin-3-cmglc (p-value = 0.002) in grapes compared to the control, whereas the application of MeJ in nano form (ACP-MeJ) increased the content of petunidin-3-cmglc (p-value = 0.016), and malvidin-3-*trans*-cmglc (p-value = 0.053) compared to the control. In addition, the concentration of malvidin-3-cfglc (p-value = 0.019) was higher in the MeJ samples than in those of the ACP-MeJ treatment, without differences with those of the control treatment. Malvidin-3-*cis*-cmglc content was higher in ACP-MeJ samples compared to those treated with MeJ in conventional form (p-value = 0.050), and without differences with those of the control treatment. Consequently, the total content of acylated anthocyanins was higher in grapes from the both elicitor treatments than in the control (Table 2) (p-value = 0.001), with no significant differences between them, despite the fact that the dose was 10 times higher when MeJ was applied conventionally than in the nano form. Regarding to the total anthocyanins in 2019 samples, their concentration was higher in the grapes of the MeJ treatments than in the control samples (p-value = 0.052), but without significant differences with the application of this elicitor in nano form (ACP-MeJ). As the aforementioned regarding to the must general parameters (Table 1), in the second season (2020), there were hardly any significant differences in the anthocyanins content between vineyard treatments (Table 2). Only two non-acylated anthocyanins, the same as in the 2019 season, cyanidin-3-glc (p-value = 0.048) and peonidin-3-glc (p-value = 0.033), showed differences between samples, increasing their content when MeJ was applied conventionally compared to the control samples and without significant differences with ACP-MeJ samples in the case of the cyaniding-3-glc concentration. These results highlight the great importance of the season in the effect of this elicitor on the anthocyanin composition of the grapes, as also observed other authors (Portu et al., 2018a; Paladines-Quezada et al., 2021). Thus, this reinforces the previously mentioned idea of the higher dilution of compounds found when berry size increases, decreasing the skin to pulp ratio, in those vines with higher water availability, as also observed by Pérez-Álvarez, Intrigliolo, Almajano, Rubio-Bretón, and Garde-Cerdán (2021b) for the total phenolic compounds in their study with Monastrell vines under two different irrigation regimes. Anthocyanins are the most important phenolic compounds both, quantitatively and qualitatively, in red grape varieties, being responsible for many of their organoleptic properties and health benefits (Han et al., 2019; Pérez-Álvarez et al., 2019; Hermosín-Gutierrez et al., 2020).

Table 3 shows the content of flavonols, flavanols, hydroxybenzoic and hydroxycinnamic acids and stilbenes from control, MeJ and ACP-MeJ grapes. Unlike what was observed for the enological parameters and anthocyanins, for these five families of phenolic compounds there were numerous significant differences in their concentration due to the treatments in both seasons, 2019 and 2020.

In 2019, foliar treatment with MeJ decreased the content in grapes of two flavonols (quercetin-3-glcU (p-value = 0.039), and kaempferol-3-gal (p-value = 0.052)) and ACP-MeJ treatment decreased the concentration of quercetin3-glc (p-value = 0.033) in samples compared to those of the control. Ruiz-García et al. (2012) also observed that some of these compounds (quercetin-3-glc and isorhamnetin-3-glc) decreased their concentration in Monastrell grapes after the application of this elicitor (in conventional form), i.e. methyl jasmonate, in the vineyard. Therefore, the total concentration of flavonols was lower in MeJ grapes than in the control, and with no significant differences with those of the ACP-MeJ treatment (Table 3). However, in 2020, the effects on the

Table 2
Anthocyanins content (mg/kg) in grapes from control, methyl jasmonate (MeJ) and nanoparticles doped with this elicitor (ACP-MeJ) treatments, in 2019 and 2020 seasons.

	2019			2020		
	Control	**MeJ**	**ACP-MeJ**	**Control**	**MeJ**	**ACP-MeJ**
Delphinidin-3-glc	123.77 ± 12.34 a	148.36 ± 10.88 a	127.91 ± 17.62 a	50.58 ± 1.69 a	57.09 ± 7.80 a	64.49 ± 12.46 a
Cyanidin-3-glc	25.83 ± 3.66 a	44.14 ± 3.96b	21.92 ± 1.75 a	8.44 ± 0.34 a	10.95 ± 1.16b	8.82 ± 1.53 ab
Petunidin-3-glc	85.84 ± 6.28 a	97.26 ± 16.47 a	90.58 ± 10.06 a	47.93 ± 1.59 a	53.03 ± 5.03 a	54.44 ± 7.45 a
Peonidin-3-glc	45.73 ± 3.95 a	57.46 ± 3.93b	44.90 ± 6.50 a	19.77 ± 0.91 a	24.72 ± 1.14b	20.06 ± 2.98 a
Malvidin-3-glc	215.76 ± 8.13 a	240.95 ± 29.77 a	229.20 ± 3.01 a	169.84 ± 0.91 a	178.61 ± 13.92 a	177.62 ± 21.44 a
Total non-acylated	**496.94 ± 32.57 a**	**588.20 ± 58.37b**	**514.51 ± 36.71 ab**	**296.57 ± 7.58 a**	**324.41 ± 16.54 a**	**325.43 ± 44.16 a**
Delphinidin-3-acglc	10.42 ± 0.75 a	9.93 ± 0.57 a	9.84 ± 0.36 a	6.66 ± 0.12 a	6.93 ± 0.66 a	6.99 ± 0.77 a
Cyanidin-3-acglc	3.84 ± 0.02b	3.84 ± 0.01b	3.62 ± 0.05 a	3.60 ± 0.02 a	3.62 ± 0.07 a	3.57 ± 0.05 a
Petunidin-3-acglc	6.86 ± 0.27 a	6.79 ± 0.20 a	7.01 ± 0.12 a	5.57 ± 0.11 a	5.69 ± 0.39 a	5.79 ± 0.37 a
Peonidin-3-acglc	4.48 ± 0.08 a	4.97 ± 0.26b	4.52 ± 0.12 a	3.85 ± 0.04 a	4.02 ± 0.17 a	3.92 ± 0.04 a
Malvidin-3-acglc	11.71 ± 0.26 a	12.10 ± 0.15 a	12.54 ± 0.92 a	10.53 ± 0.42 a	10.37 ± 0.89 a	11.13 ± 0.57 a
Delphinidin-3-cmglc	16.28 ± 0.68 a	18.09 ± 1.21 a	18.18 ± 1.42 a	14.31 ± 0.38 a	14.62 ± 1.78 a	15.79 ± 2.46 a
Cyanidin-3-cmglc	6.21 ± 0.28 a	7.87 ± 0.68b	6.32 ± 0.55 a	5.38 ± 0.17 a	5.79 ± 0.42 a	5.29 ± 0.20 a
Petunidin-3-cmglc	12.97 ± 0.26 a	14.34 ± 0.55b	14.60 ± 0.64b	12.47 ± 0.25 a	12.57 ± 0.99 a	12.34 ± 2.55 a
Peonidin-3-cmglc	8.27 ± 0.06 a	10.45 ± 0.58b	8.75 ± 0.42 a	7.42 ± 0.07 a	8.08 ± 0.26 a	7.46 ± 1.10 a
Malvidin-3-*cis*-cmglc	4.55 ± 0.08 ab	4.44 ± 0.14 a	4.84 ± 0.22b	4.66 ± 0.21 a	4.53 ± 0.36 a	4.81 ± 0.08 a
Malvidin-3-*trans*-cmglc	36.74 ± 2.11 a	40.27 ± 2.57 ab	43.32 ± 2.92b	51.03 ± 0.75 a	48.42 ± 4.48 a	50.02 ± 9.71 a
Malvidin-3-cfglc	4.21 ± 0.02 ab	4.55 ± 0.30b	3.95 ± 0.09 a	10.90 ± 1.42 a	9.95 ± 1.19 a	8.98 ± 2.01 a
Total acylated	**126.54 ± 1.10 a**	**137.63 ± 2.22b**	**137.49 ± 2.72b**	**136.37 ± 1.96 a**	**134.58 ± 5.10 a**	**136.10 ± 15.14 a**
Total anthocyanins	***623.48 ± 32.23 a***	***725.83 ± 58.85b***	***652.00 ± 36.21 ab***	***432.94 ± 9.42 a***	***458.99 ± 21.21 a***	***461.52 ± 59.20 a***

Nomenclature abbreviations: glc, glucoside; acglc, acetylglucoside; cmglc, *trans-p*-coumaroylglucoside; cfglc, caffeoylglucoside.
All parameters are listed with their standard deviation (n = 3). For each season and compound, different letters indicate significant differences between the samples ($p \leq 0.05$).

Table 3
Flavonols, flavanols, phenolic acids and stilbenes content (mg/kg) in grapes from control, methyl jasmonate (MeJ) and nanoparticles doped with this elicitor (ACP-MeJ) treatments, in 2019 and 2020 seasons.

	2019			2020		
	Control	**MeJ**	**ACP-MeJ**	**Control**	**MeJ**	**ACP-MeJ**
Flavonols						
Myricetin-3-glcU	27.15 ± 2.47 a	23.86 ± 3.76 a	25.87 ± 3.65 a	16.26 ± 0.70 a	16.07 ± 0.98 a	20.85 ± 2.44b
Myricetin-3-gal	35.08 ± 3.48 a	34.24 ± 2.26 a	35.41 ± 2.17 a	22.16 ± 1.58 a	23.29 ± 2.31 a	27.66 ± 4.98 a
Myricetin-3-glc	181.66 ± 15.36 a	179.26 ± 19.81 a	179.48 ± 10.88 a	82.27 ± 4.81 a	83.68 ± 8.37 a	133.03 ± 13.42b
Quercetin-3-glcU	164.18 ± 15.66b	122.47 ± 19.81 a	151.87 ± 20.40 ab	24.60 ± 1.67 a	30.61 ± 2.69 a	29.03 ± 4.84 a
Quercetin-3-glc	172.29 ± 14.90b	157.59 ± 4.20 ab	145.09 ± 14.83 a	32.82 ± 0.70 a	36.32 ± 6.50 a	49.76 ± 8.42b
Laricitrin-3-glc	33.29 ± 3.44 a	30.37 ± 3.59 a	32.37 ± 3.53 a	30.31 ± 1.31 a	37.37 ± 4.05 a	34.33 ± 6.13 a
Kaempferol-3-gal	2.48 ± 0.23b	1.89 ± 0.03 a	2.50 ± 0.42b	0.46 ± 0.04 a	0.52 ± 0.06 a	0.56 ± 0.10 a
Kaempferol-3-glcU + 3-glc	15.99 ± 1.83 a	14.55 ± 1.72 a	14.73 ± 2.10 a	2.17 ± 0.35 a	3.23 ± 0.28b	3.54 ± 0.01b
Isorhamnetin-3-glc	12.17 ± 1.18 a	12.88 ± 0.33 a	11.63 ± 1.37 a	3.54 ± 0.21 a	3.86 ± 0.54 a	6.93 ± 0.70b
Syringetin-3-glc	21.88 ± 1.52 a	21.59 ± 2.39 a	21.78 ± 0.62 a	12.03 ± 0.94 a	14.02 ± 1.07b	20.08 ± 0.16c
Total flavonols	***666.15 ± 33.09b***	***598.69 ± 31.38 a***	***620.73 ± 22.57 ab***	***226.61 ± 5.16 a***	***248.96 ± 13.50 a***	***325.77 ± 25.72b***
Flavanols						
Catechin	63.31 ± 3.37b	48.32 ± 5.31 a	60.44 ± 9.50 ab	11.06 ± 0.31 a	13.25 ± 3.05 a	19.72 ± 3.03b
Epicatechin	39.10 ± 3.85 a	35.22 ± 1.24 a	36.38 ± 5.23 a	11.09 ± 0.43 a	14.28 ± 1.66b	27.73 ± 1.88c
Epicatechin-3-gallate	14.12 ± 2.12b	10.90 ± 1.23 a	14.31 ± 1.09b	8.24 ± 0.76 ab	9.77 ± 2.04b	6.37 ± 0.38 a
Epigallocatechin	4.42 ± 0.32b	2.93 ± 0.49 a	2.80 ± 0.25 a	8.25 ± 0.91 a	9.30 ± 1.37 a	8.78 ± 1.71 a
Procyanidin B1	28.28 ± 3.57 a	25.17 ± 3.86 a	35.69 ± 2.83b	10.26 ± 1.10 a	10.61 ± 0.24 a	9.15 ± 0.55 a
Procyanidin B2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total flavanols	***149.24 ± 9.65b***	***122.53 ± 11.31 a***	***149.61 ± 16.72b***	***48.90 ± 1.71 a***	***57.20 ± 5.14 a***	***71.76 ± 5.80b***
Hydroxybenzoic acid						
Gallic acid	6.00 ± 0.80 a	5.18 ± 0.35 a	6.07 ± 0.32 a	5.20 ± 0.57 a	6.49 ± 0.80 ab	7.27 ± 1.06b
Hydroxycinnamic acids (HCAs)						
trans-Caftaric acid	6.54 ± 0.09 a	5.81 ± 1.21 a	11.71 ± 0.93b	1.51 ± 0.07 a	1.58 ± 0.19 ab	1.94 ± 0.28b
trans + *cis*-Coutaric acids	4.62 ± 0.40b	1.87 ± 0.23 a	7.14 ± 1.50c	0.17 ± 0.03 a	0.29 ± 0.00b	0.49 ± 0.05c
trans-Fertaric acid	1.78 ± 0.19b	0.81 ± 0.07 a	2.48 ± 0.41c	1.34 ± 0.23 a	1.57 ± 0.16 a	1.65 ± 0.30 a
Caffeic acid	0.43 ± 0.05b	0.31 ± 0.04 a	0.26 ± 0.02 a	0.26 ± 0.03 a	0.27 ± 0.03 a	0.30 ± 0.03 a
p-Coumaric acid	0.36 ± 0.09 a	0.36 ± 0.00 a	0.35 ± 0.07 a	0.14 ± 0.01 a	0.19 ± 0.03b	0.17 ± 0.02 ab
Ferulic acid	2.27 ± 0.15 a	1.85 ± 0.34 a	1.86 ± 0.14 a	10.56 ± 1.65 a	12.14 ± 0.92 a	9.99 ± 1.54 a
Total HCAs	***15.99 ± 0.86b***	***11.00 ± 1.08 a***	***23.79 ± 2.76c***	***13.98 ± 1.36 a***	***16.05 ± 1.12 a***	***14.54 ± 1.65 a***
Stilbenes						
trans-Piceid	12.75 ± 1.06 a	12.43 ± 1.48 a	11.53 ± 1.17 a	5.37 ± 0.38 a	5.56 ± 0.59 a	8.89 ± 1.71b
cis-Piceid	1.70 ± 0.24 a	1.60 ± 0.27 a	1.71 ± 0.13 a	1.13 ± 0.09 a	1.26 ± 0.19 a	2.02 ± 0.23b
trans-Resveratrol	0.63 ± 0.05 a	0.58 ± 0.10 a	0.52 ± 0.09 a	0.11 ± 0.02b	0.12 ± 0.02b	0.07 ± 0.01 a
cis-Resveratrol	0.35 ± 0.03 a	0.40 ± 0.06 a	0.37 ± 0.02 a	0.20 ± 0.02 a	0.27 ± 0.05 a	0.39 ± 0.08b
Total stilbenes	***15.43 ± 1.30 a***	***15.01 ± 1.70 a***	***14.12 ± 1.21 a***	***6.82 ± 0.46 a***	***7.21 ± 0.79 a***	***11.36 ± 1.79b***

Nomenclature abbreviations: glcU, glucuronide; gal, galactoside; glc, glucoside.
All parameters are listed with their standard deviation (n = 3). For each season and compound, different letters indicate significant differences between the samples ($p \leq 0.05$). n.d.: not detected.

composition of this family of phenolic compounds was very different, since the application of the elicitor in nano form (ACP-MeJ) favored the synthesis of most of these compounds, and consequently their total content in the grapes was higher than in the control and in the samples from the conventional MeJ treatment (Table 3). This result is relevant since flavonols are of great importance in the color stability of red wines due to their copigmentation reactions with anthocyanins (Escribano-Bailón et al., 2018).

Regarding to the flavanols, in 2019, the application of MeJ caused that the content of most of these compounds were lower than in the control grapes, with smaller differences when the elicitor was applied in nano form; thus, the total flavanols content was higher in control and ACP-MeJ samples than in MeJ (Table 3). This trend was not repeated in 2020, a year in which it was observed that when MeJ was applied in nano form, the content of catechin (p-value = 0.013) and epicatechin (p-value = 0.000) in grapes increased, making higher the total flavanols content than in the control and MeJ samples (Table 3) (p-value = 0.002). This result does not match with that found by other authors after foliar application of this elicitor in Tempranillo and Graciano vineyards in several seasons (Portu et al., 2015b, 2016, 2018a), who observed hardly any influence on the composition of flavanols in the grapes. These phenolic compounds are mainly responsible for astringency and they influence the evolution of the wine color (Pérez-Navarro et al., 2019).

Flavonols and flavanols are closely related to anthocyanins as they share most of their biosynthetic pathway. The fact that in 2019 the anthocyanin content increased when MeJ was applied but the flavonols and flavanols content decreased seems to indicate that the application of this elicitor preferentially induced the activation of enzymes related to anthocyanin synthesis to the detriment of enzymes related to flavonols and flavanols synthesis. The absence of differences in the content of these three groups of phenolic compounds when MeJ was applied in 2020 could be due to the fact that in 2019, 1 month has passed between the first application and the harvest; whereas in 2020, this period was 1 month and 20 days. This longer period of time could have decreased the effect of the elicitor on the phenolic composition of the grapes, as observed by Gómez-Plaza, Bautista-Ortín, Ruiz-García, Fernández-Fernández, and Gil-Muñoz (2017), since the differences between the control and MeJ treatment decreased with the passage of the weeks after treatment. Regarding to the results observed when applying ACP-MeJ on the content in grapes of these three groups of phenolic compounds, it seems that in 2019 the treatment was not sufficient to cause modifications in their synthesis pathway, unlike what was observed when applying MeJ. Meanwhile, in 2020, the synthesis pathway of flavonols and flavanols was favored, which could indicate a memory effect in plants, since the elicitor in nano form is slowly released in the plants.

The only hydroxybenzoic acid found in the samples was gallic acid, whose content in grapes was not modified by the treatments in 2019, while in 2020 the application of MeJ in nano form (ACP-MeJ) favored its synthesis compared to the control (Table 3). Regarding to the hydroxycinnamic acids (HCAs), as observed with the other families of phenolic compounds, the effects on their content in grapes were not the same in both seasons, with the exception of *trans*-caftaric acid, whose

Table 4
Multifactor analysis of variance of general parameters of the musts with the two factors studied: treatment (Control, MeJ, ACP-MeJ) and season (2019 and 2020) and their interaction (treatment × season).

	Weight of 100 berries (g)	°Brix	Probable alcohol (% v/v)	pH	Total acidity (g/L)	Glu + Fru (g/L)	Glu (g/L)	Fru (g/L)	Malic acid (g/L)	Total phenols (mg/L)
Treatment (T)										
Control	156.63 a	23.50a	13.80 a	3.79 a	4.37 a	233.14 a	113.74 a	119.39 a	1.73 a	863.47 a
MeJ	174.74 a	22.20 a	12.91 a	3.74 a	4.87 a	217.06 a	104.48 a	112.58 a	2.04 a	954.82b
ACP-MeJ	155.92 a	22.87 a	13.36 a	3.77 a	4.58 a	227.37 a	109.75 a	117.62 a	1.95 a	967.05b
Season (S)										
2019	124.14 a	23.43 a	13.75 a	3.81 a	4.98b	232.25 a	111.32 a	120.94b	2.43b	1281.10b
2020	200.71b	22.28 a	12.96 a	3.73 a	4.23 a	219.46 a	107.33 a	112.12 a	1.38 a	575.79 a
Interaction										
T × S	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

For each parameter and factor, different letters indicate significant differences between samples ($p \leq 0.05$). Interaction: N.S., not significant ($p > 0.05$).

concentration increased when MeJ was applied in nano form (ACP-MeJ) compared to the control, in both years (Table 3). In 2019, the content of *trans* + *cis*-coutaric acids (p-value = 0.001) and *trans*-fertaric acid (p-value = 0.001) increased when MeJ was applied in nano form (ACP-MeJ) but decreased when this elicitor was applied conventionally (MeJ); while in 2020, only *trans* + *cis*-coutaric acids were affected by the treatments (p-value = 0.000), increasing their content in grapes when both elicitor treatments were applied (Table 3). The content of caffeic acid (p-value = 0.004) decreased in grapes in 2019 with both treatments, and the concentration of *p*-coumaric acid increased compared to the control when MeJ was applied conventionally in 2020 (Table 3). As a consequence of all this, in 2019 the lowest concentration of total HCAs was found in grapes from the MeJ treatment, and the highest in ACP-MeJ samples, being intermediate those of the control ones (p-value = 0.000); while, in 2020, no significant differences were observed between samples for the total of these phenolic compounds (Table 3). Again, this result does not match with that found by other authors after foliar application of this elicitor in Tempranillo and Graciano vineyards in several seasons (Portu et al., 2015b, 2016, 2018a), who observed hardly any influence on the composition of hydroxybenzoid and hydroxycinnamic acids in grapes. It should be pointed out that *p*-coumaric and ferulic acids are precursors of ethylphenols, 4-ethylphenol and 4-ethylguaiacol, respectively, harmful compounds to wine quality (Chatonnet et al., 1992; Garde-Cerdán et al., 2010).

Finally, in 2019, no effect was observed on individual or total stilbenes content in grapes; while in 2020, the concentration of all stilbenes was affected by the treatments performed in the vineyard (Table 3). The application of MeJ in nano form (ACP-MeJ) favored the synthesis of *trans*- and *cis*-piceids (p-values = 0.011 and 0.002, respectively) and *cis*-resveratrol (p-value = 0.015) but decreased the synthesis of *trans*-resveratrol (p-value = 0.031), compared to the control and MeJ treatments. Consequently, the content of total stilbenes was higher in the grapes treated with MeJ in nano form (ACP-MeJ) than in the control and MeJ samples (p-value = 0.005), highlighting again the fact of the high difference of the elicitor concentration between both treatments. Portu et al. (2018b) reported that the effect of foliar application of MeJ in the vineyard was influenced by the variety, finding an increase in stilbenes content in Graciano and Tempranillo grapes, but not in Garnacha. Grapes and wines are the major dietary sources for humans of these compounds, which possess a great range of biological activities, potentially beneficial for human health (Koh et al., 2021), among the more recent research lines, stilbenes are gaining considerable interest as potential anti-obesity agents (Benbouguerra et al., 2021).

3.3. Multivariable analysis

Table 4 shows the results of the factorial analysis (treatment, season, and their interaction) of the general parameters of the musts. Regardless of the season, the only parameter that was affected by the treatments in the vineyard was the grapes total phenols content, which was higher in the both MeJ treatments samples than in the control ones. It should be noted again that the effect of applying the elicitor conventionally (MeJ) or in nano form (ACP-MeJ) led to the same result, despite the significant difference in MeJ concentration (10 mM *versus* 1 mM, respectively). The season factor had more impact than the treatment factor on the overall parameters of the musts, so that, regardless of treatment, the weight of 100 berries was higher in 2020 than in 2019, while total acidity, fructose, malic acid, and total phenols content was higher in 2019 than in 2020 (Table 4). There was no interaction between the two factors for any of the overall parameters studied.

Table 5 presents the results of the factorial analysis (treatment, season, and their interaction) of the grapes phenolic compounds. The content in grapes of most of these compounds was affected by both factors, treatment and season. Of the 44 phenolic compounds studied (17 anthocyanins, 10 flavonols, 6 flavanols, 1 hydroxybenzoic acid, 6 hydroxycinnamic acids, and 4 stilbenes), 23 were affected by the

Table 5
Multifactor analysis of variance of grape phenolic compounds (expressed as mg/kg).

	Treatment (T)			Season (S)		
	Control	MeJ	ACP-MeJ	2019	2020	Interaction (T × S)
Anthocyanins						
Delphinidin-3-glc	87.18 a	102.73b	96.20 ab	133.35b	57.39 a	N.S.
Cyanidin-3-glc	17.14 a	27.55b	15.37 a	30.63b	9.41 a	***
Petunidin-3-glc	66.89 a	75.16 a	72.51 a	91.23b	51.80 a	N.S.
Peonidin-3-glc	32.75 a	41.09b	32.48 a	49.37b	21.51 a	N.S.
Malvidin-3-glc	192.80 a	209.78 a	203.41 a	228.64b	175.36 a	N.S.
Total non-acylated	**396.75 a**	**456.30b**	**419.97 ab**	**533.22b**	**315.47 a**	N.S.
Delphinidin-3-acglc	8.54 a	8.42 a	8.42 a	10.06b	6.86 a	N.S.
Cyanidin-3-acglc	3.72b	3.72b	3.60 a	3.77b	3.60 a	**
Petunidin-3-acglc	6.21 a	6.24 a	6.40 a	6.89b	5.68 a	N.S.
Peonidin-3-acglc	4.16 a	4.50b	4.22 a	4.66b	3.93 a	*
Malvidin-3-acglc	11.12 a	11.23 a	11.84 a	12.12b	10.68 a	N.S.
Delphinidin-3-cmglc	15.29 a	16.36 a	16.98 a	17.52b	14.91 a	N.S.
Cyanidin-3-cmglc	5.79 a	6.83b	5.81 a	6.80b	5.49 a	*
Petunidin-3-cmglc	12.72 a	13.45 a	13.47 a	13.67b	12.46 a	N.S.
Peonidin-3-cmglc	7.85 a	9.27b	8.11 a	9.16b	7.65 a	N.S.
Malvidin-3-*cis*-cmglc	4.61 ab	4.49 a	4.83b	4.61 a	4.67 a	N.S.
Malvidin-3-*trans*-cmglc	43.89 a	44.34 a	46.67 a	40.11 a	49.82b	N.S.
Malvidin-3-cfglc	7.55 a	7.25 a	6.46 a	4.23 a	9.94b	N.S.
Total acylated	**131.46 a**	**136.11 a**	**136.79 a**	**133.89 a**	**135.69 a**	N.S.
Total anthocyanins	***528.21 a***	***592.41b***	***556.76 ab***	***667.10b***	***451.15 a***	N.S.
Flavonols						
Myricetin-3-glcU	21.70 a	19.96 a	23.36 a	25.63b	17.72 a	N.S.
Myricetin-3-gal	28.62 a	28.76 a	31.53 a	34.91b	24.37 a	N.S.
Myricetin-3-glc	131.97 a	131.47 a	156.25b	180.13b	99.66 a	**
Quercetin-3-glcU	94.39b	76.54 a	90.45 ab	148.17b	28.08 a	**
Quercetin-3-glc	102.55 a	96.95 a	97.43 a	158.32b	39.63 a	**
Laricitrin-3-glc	31.80 a	33.87 a	33.35 a	32.01 a	34.00 a	N.S.
Kaempferol-3-gal	1.47b	1.21 a	1.53b	2.29b	0.52 a	*
Kaempferol-3-glcU + 3-glc	9.08 a	8.90 a	9.14 a	15.09b	2.98 a	N.S.
Isorhamnetin-3-glc	7.85 a	8.37 ab	9.28b	12.23b	4.78 a	***
Syringetin-3-glc	16.95 a	17.80 a	20.93b	21.75b	15.37 a	***
Total flavonols	***446.38 ab***	***423.83 a***	***473.25b***	***628.52b***	***267.11 a***	***
Flavanols						
Catechin	37.19b	30.78 a	40.08b	57.36b	14.68 a	*
Epicatechin	25.10 a	24.75 a	32.05b	36.90b	17.70 a	***
Epicatechin-3-gallate	11.18 a	10.33 a	10.34 a	13.11b	8.13 a	**
Epigallocatechin	6.34 a	6.11 a	5.79 a	3.39 a	8.78b	N.S.
Procyanidin B1	19.27 a	17.89 a	22.42b	29.72b	10.01 a	**
Procyanidin B2	n.d.	n.d.	n.d.	n.d.	n.d.	–
Total flavanols	***99.07 ab***	***89.87 a***	***110.69b***	***140.46b***	***59.29 a***	*
Hydroxybenzoic acid						
Gallic acid	5.60 a	5.83 ab	6.67b	5.75 a	6.32 a	*
Hydroxycinnamic acids (HCAs)						
trans-Caftaric acid	4.02 a	3.70 a	6.83b	8.02b	1.68 a	***
trans + *cis*-Coutaric acids	2.39b	1.08 a	3.82c	4.54b	0.32 a	***
trans-Fertaric acid	1.56b	1.19 a	2.06c	1.67 a	1.52 a	***
Caffeic acid	0.35b	0.29 a	0.28 a	0.33b	0.28 a	***
p-Coumaric acid	0.25 a	0.27 a	0.26 a	0.36b	0.17 a	N.S.
Ferulic acid	6.42 a	6.99 a	5.93 a	1.99 a	10.90b	N.S.
Total HCAs	***14.99 a***	***13.52 a***	***19.17b***	***16.93b***	***14.86 a***	***
Stilbenes						
trans-Piceid	9.06 a	9.00 a	10.21 a	12.24b	6.61 a	**
cis-Piceid	1.42 a	1.43 a	1.86b	1.67 a	1.47 a	**
trans-Resveratrol	0.37 a	0.35 a	0.30 a	0.58b	0.10 a	N.S.
cis-Resveratrol	0.28 a	0.34 ab	0.38b	0.37b	0.29 a	*
Total stilbenes	***11.12 a***	***11.11 a***	***12.74 a***	***14.85b***	***8.47 a***	**

Nomenclature abbreviations: glc, glucoside; acglc, acetylglucoside; cmglc, *trans-p*-coumaroylglucoside; cfglc, caffeoylglucoside; glcU, glucuronide; gal, galactoside. For each parameter and factor, different letters indicate significant differences between samples ($p \leq 0.05$). Interaction: *, $p \leq 0.05$, **, $p \leq 0.01$, ***, $p \leq 0.001$, and N.S., not significant ($p > 0.05$).

treatment factor (8 anthocyanins, 5 flavonols, 3 flavanols, 1 hydroxybenzoic acid, 4 hydroxycinnamic acids, and 2 stilbenes), and 38 by the season factor (16 anthocyanins, 9 flavonols, 5 flavanols, 5 hydroxycinnamic acids, and 3 stilbenes). Regardless of the season, the content of total non-acylated anthocyanins and total anthocyanins was higher in the MeJ grapes than in the control ones, and without differences with the ACP-MeJ samples, while the total content of acylated anthocyanins did not show significant differences between the samples (Table 5). On the other hand, the total concentration of flavonols and flavanols was higher in the samples from the application of MeJ in nano form (ACP-MeJ) than when this elicitor was applied conventionally (MeJ); neither of the two treatments showed significant differences with the control (Table 5). As for non-flavonoids compounds (hydroxybenzoic acid, hydroxycinnamic acids, and stilbenes), foliar applications had no effect on the total content of stilbenes; treatment with MeJ in nano form (ACP-MeJ) increased the content of HCAs in the grapes compared to the control and the conventional application (MeJ), and the content of the only hydroxybenzoic acid found in the grapes increased when ACP-MeJ was applied compared to the control, but without differences with MeJ (Table 5). With regard to the season factor, practically all the phenolic

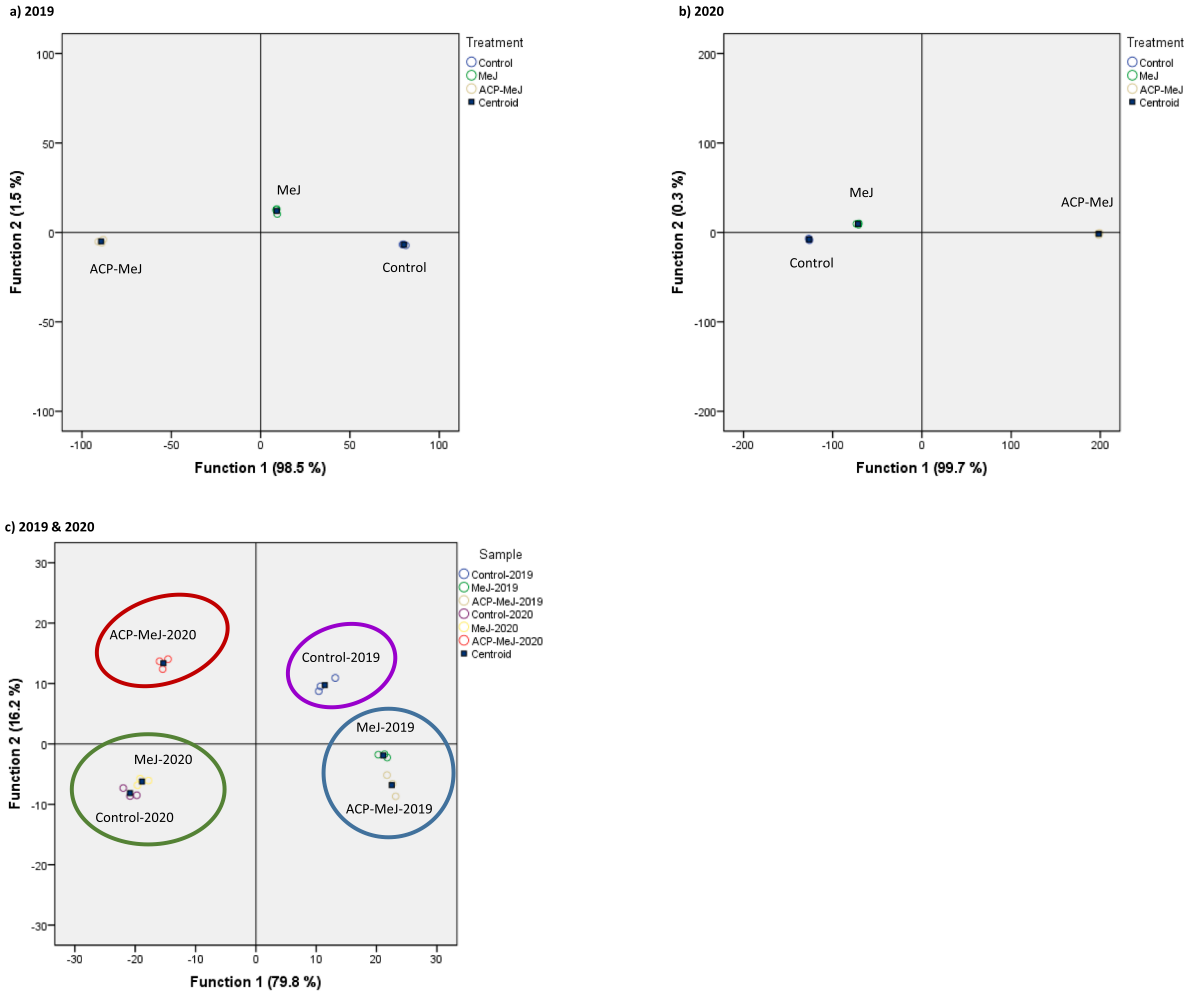

Fig. 1. Discriminant analysis of phenolic compounds content (mg/kg) in grapes from control, methyl jasmonate (MeJ) and nanoparticles doped with this elicitor (ACP-MeJ) treatments, in (a) 2019, (b) 2020, and (c) 2019 & 2020 seasons.

compounds found in the grapes showed higher concentrations in 2019 than in 2020, so that the total content of each of the families studied, with the exception of hydroxybenzoic acid, was significantly higher in the first year of the study than in the second, regardless of the treatment performed. Numerous interactions were observed between the two factors studied (treatment and season), mainly in the non-anthocyanins compounds, probably because of the treatments performed had little effect on the anthocyanin content in 2020 (Table 2), while for the other phenolic compounds, the treatments affected, in different ways, their content in the grapes in both vintages (Table 3).

Fig. 1 shows the discriminant analysis carried out with the phenolic composition of the different samples in 2019 (Fig. 1a), in 2020 (Fig. 1b) and considering both seasons (Fig. 1c). In 2019, it is observed that Function 1 (98.5 %) allows a very good separation of the samples according to the treatment performed (control, MeJ, ACP-MeJ). The variables with more weight in the discrimination, in both functions, were caffeic acid, cyanidin-3-acglc, and *p*-coumaric acid. Moreover, in 2020, the ACP-MeJ sample appears perfectly separated from the control and MeJ by Function 1 (99.7 %). In this case, the variables that determined the separation of the samples were: *cis*-piceid, epicatechin, and gallic acid (Function 1), and peonidin-3-glc, *cis*-piceid, and malvidin-3-acglc (Function 2). If the global study is considered (all treatments and both seasons), it can be observed the existence of four differentiated groups, separated by both Functions (Function 1: 79.8 %; Function 2: 16.2 %). Caffeic acid, *trans*-caftaric acid, and cyanidin-3-glc (Function 1), and epicatechin, isorhamnetin-3-glc, and *trans*-caftaric acid (Function 2) were the variables with more weight in the separation of the samples. Function 1 separates the samples by season, while Function 2 separates them by treatments. In 2019, the control is clearly differentiated from the treated samples (MeJ and ACP-MeJ) according to their phenolic composition, whereas in 2020, ACP-MeJ samples appears perfectly separated from the control and MeJ ones, according to their phenolic content (Fig. 1c). This fact could be due to what has been aforementioned, since in 2020 there was practically no effect of the treatments on the anthocyanins content, while the concentration of the rest of the phenolic compounds was more similar between the control and MeJ than with ACP-MeJ.

4. Conclusions

Methyl jasmonate (MeJ) application in the vineyard differentially affected phenolic compounds in Tempranillo grapes depending on season. However, both MeJ treatments, conventional and nano, increased the content of some anthocyanins, flavonols, flavanols and non-flavonoid compounds compared to the control treatment and besides, decreased the grapes sugar content. Therefore, the applications of this elicitor in the vineyard could be a good tool to achieve an increase of phenolic compounds in the grapes, improving not only the organoleptic properties, color stability, and healthy characteristics for which they are responsible in grapes, so, improving grape quality, but also allowing to

bring closer the grape technological and phenolic maturities. Besides, the application of the elicitor in nano form (ACP-MeJ), allows to reduce both, the economical and the environmental impact of this technique in the vineyard. Nevertheless, as the results were not completely equivalent between the two seasons under study, it would be desirable to carry out more trials to determine the effect of these foliar treatments under different edaphoclimatic and varietal conditions in order to obtain a more solid response to their effect on the grape phenolic compounds content.

CRediT authorship contribution statement

T. Garde-Cerdán: Conceptualization, Supervision, Project administration, Funding acquisition, Investigation, Methodology, Writing – original draft. **I. Sáenz de Urturi:** Formal analysis, Investigation, Writing – review & editing. **P. Rubio-Bretón:** Investigation, Formal analysis, Writing – review & editing. **S. Marín-San Román:** Investigation, Formal analysis, Writing – review & editing. **E. Baroja:** Investigation, Conceptualization, Writing – review & editing. **G.B. Ramírez-Rodríguez:** Methodology, Writing – review & editing. **J.M. Delgado-López:** Funding acquisition, Conceptualization, Methodology, Writing – review & editing. **E.P. Pérez-Álvarez:** Funding acquisition, Conceptualization, Methodology, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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ORIGINAL RESEARCH ARTICLE

Foliar applications to vines of methyl jasmonate and nanoparticles doped with methyl jasmonate: impact on grape and wine polysaccharide composition

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ABSTRACT

Polysaccharides in wine play important roles in the stabilization and in the sensory properties of wines. Elicitor application constitutes an interesting field of research since it is indirectly involved in the accumulation in grape cell walls of molecules like callose, lignin, phenolic compounds and glycoproteins. Currently, biomimetic calcium phosphate (ACP) nanoparticles are successfully used in viticulture for the controlled delivery of bioactive molecules, such as elicitors. The aim of this study was to compare the effect of the application of two different elicitors on both grape and wine of Tempranillo polysaccharide composition. Methyl jasmonate (MeJ) and nanoparticles doped with MeJ were applied to the canopy at veraison and one week later in two vintages. In the grape extracts, the foliar treatments did not increase the content of monosaccharides or that of the main pectin families; therefore, the elicitors did not reinforce the cell walls of the Tempranillo grape. The extractability and solubility of the pectic families of the grape cell walls into the wine depended on the type of family and the climate of the vintages.

KEYWORDS: Elicitors, methyl jasmonate, ACP nanoparticles, monosaccharides, grape and wine Tempranillo

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INTRODUCTION

Applications to the grapevine of suitable elicitors and combinations of different stress stimuli can activate structural and biochemical response mechanisms (Lijavetzky *et al.*, 2008; Benhamou, 1996). Grapevine responds to these stressors by activating an array of mechanisms similar to the defense responses to pathogen infections or environmental stresses (Apolinar-Valiente *et al.*, 2018). The biochemical changes in the grape and leaves before a pathogen infection in the grapevine involve the accumulation of phenolic compounds and pathogenesis-related (PR) proteins (Lijavetzky *et al.*, 2008). The structural grapevine defense response consists of a reinforcement of the mechanical properties of the grape cell wall. These properties are associated with the sequential deposition of newly formed molecules including callose, lignin, phenolic compounds, and glycoproteins (Benhamou, 1996). Apolinar-Valiente *et al.* (2018) observed a notable reinforcement of the skin cell wall in response to the application of four different elicitors to Monastrell grapes, one of which being methyl jasmonate (MeJ). Nevertheless, the extent of the reinforcement of the cell wall probably depends on the composition and morphology of the skin cell wall material, which is different for each grape crop (Apolinar-Valiente *et al.*, 2016). The analysis of the composition and morphology of Monastrell skin cell walls has shown that its skin is thicker than Syrah and Cabernet-Sauvignon (Ortega-Regules *et al.*, 2008). In red winemaking, the skin cell walls form a hydrophobic barrier to the diffusion of phenolic compounds, thus majorly controlling extractability (Goulao *et al.*, 2012).

Type-I cell walls, according to Carpita and Gibeaut (1993), is composed of approximately 90 % polysaccharides (McNeil *et al.*, 1984) from three major classes that form its structural elements: cellulose, matrix cross-linking glycans (hemicelluloses) and pectic polysaccharides. Several authors describe the pectocellulosic portion as one of the main constituents of the grape cell wall (Osete-Alcaraz *et al*., 2022; Gao *et al*., 2015). The extractability of cell wall polysaccharides from grapes to wine depends on several factors, such as the type of grape tissue used in winemaking, and the respective polysaccharides solubility and stability towards enzymatic activity and ethanol content (Vidal *et al*., 2001).

The composition of berry and yeast cell walls is the main variable influencing the initial amount and nature of wine polysaccharides; however, due to their propensity to interact with other macromolecules, like proanthocyanidins (Riou *et al*., 2002), with volatile molecules (Chalier *et al.*, 2007), colour and foam (Guadalupe *et al*., 2010; Martínez-Lapuente *et al*., 2013; Martínez-Lapuente *et al*., 2019), polysaccharides continuously change and evolve over time during fermentation and ageing (Guadalupe and Ayestarán, 2007).

The major wine polysaccharides that come from the pectocellulosic portion of the grape cell walls are rich in arabinose and galactose, PRAG, (arabinogalactans type I, AG-I and arabinogalactans type II joined to protein, AGP), rhamnogalacturonans (rhamnogalacturonans type I, RG-I and rhamnogalacturonans type II, RG-II) and homogalacturonans (HL), in contrast to mannoproteins (MP) from yeast cell walls (Martínez-Lapuente *et al*., 2019). Ayestarán *et al*. (2004) identified that the composition of Tempranillo wines was 45 % MP, 37 % AGP and 15 % RG-II, and Vidal *et al*. (2003) observed that the red wines from Carignan noir wines were of 42 % AGP, 35 % MP, 19 % RG-II and 4 % RG-II.

Polysaccharides in wine play important roles in the stabilization and in the sensory properties of wines. From a stabilization perspective AGP/PRAG and MP have been shown to be strong inhibitors of the aggregation of tannins and prevent the formation of large colloids, whereas RG-II dimers form co-aggregates with tannins (Riou *et al*., 2002) and reduce the precipitation of tannin-protein complexes (Maury *et al.*, 2016). From a sensory perspective, polysaccharides affect all aspects of wine mouthfeel, such as astringency, viscosity and hotness, and aroma (Villamor *et al.*, 2013; Villamor and Ross, 2013) and clarity (De Iseppi *et al*., 2021).

MeJ is an elicitor that triggers the synthesis of secondary metabolites. Portu *et al.* (2016) demonstrated that foliar treatments carried out with this elicitor increased the Tempranillo grape and wine anthocyanins, while Paladines-Quezada *et al.* (2019) observed increases in the fresh skins of Monastrell, but not in the wines. These results showed that MeJ induces the phenolic biosynthesis in the grape and that the extension of the reinforcement of the skin cell wall depends, among other factors, on the grape variety. It is likely that, in the case of Tempranillo treated with MeJ, the reinforcement of the skin cell wall was not so intense as to hinder the extractability of anthocyanins and other components of the cell wall material.

Nanotechnology has been considered as a potential strategy for shifting to sustainable agriculture, since it enables time-controlled, targeted and self-regulated agrochemical delivery (Garde-Cerdán *et al.*, 2021). Thus, crops can be treated in a more efficient and sustainable way by maintaining high yields and quality while reducing the dosage and thus the environmental and economic impact (Pérez-Álvarez *et al.*, 2021). Biomimetic calcium phosphate nanoparticles, such as nanocrystalline apatite (Ap) or its precursor amorphous calcium phosphate (ACP), have inspired great scientific and technological interest in their potential use in agriculture due to their rich composition in important plant nutrients (P and Ca), as well as their biocompatibility, high surface reactivity and pH-dependent solublity (Ramírez-Rodríguez *et al.*, 2020). They have been successfully used for the controlled delivery of plant nutrients and bioactive molecules, including elicitors (Pérez-Álvarez *et al.*, 2022). In fact, ACP nanoparticles have been found to provide protective action against thermal degradation and the sustainable and gradual release of the MeJ, resulting in a prolonged supply of the resistance-inductor elicitor via the leaves and in efficiency enhancement (Parra-Torrejón *et al.*, 2022).

Considering the importance of all the above-mentioned aspects, the aim of this work was to study, in two vintages, the effect of conventional MeJ and nanoparticles doped with MeJ on Tempranillo grape and wine polysaccharide composition.

MATERIALS AND METHODS

1. Vineyard site, grapevine treatments and grape samples

During the 2019 and 2020 vintages, the same vines of the Tempranillo variety (*Vitis vinifera* L.) grown in the experimental vineyard located at Finca La Grajera, Logroño, La Rioja, Spain (42º26'25.36''North, Latitude; 2º30'56.41''West, Longitude; and 456 meters above sea level, altitude) were used. Vines were planted in 1997, grafted onto R-110 rootstock and trained to a VSP (vertical shoot positioned) trellis system. Vine spacing was 2.80 m x 1.25 m. Foliar applications of MeJ and ACP-MeJ were studied. To carry out the treatments, aqueous solutions were prepared with a concentration of 10 mM of MeJ according to Garde-Cerdán *et al*. (2016) and Garde-Cerdán *et al*. (2018), and 1 mM of ACP-MeJ according to Gil-Muñoz *et al*. (2021) and Pérez-Álvarez *et al*. (2022), using Tween 80 as a wetting agent (1 mL/L). The control plants were sprayed only with a water solution of Tween 80. All treatments were applied twice: at veraison and 7 days later. For each application, 200 mL/plant was sprayed over the leaves. The treatments were performed in triplicate and were arranged in a complete randomised block design, with 10 vines for each replication and treatment (Figure 1S).

The meteorogical data were obtained from the Agroclimatic Information Service of La Rioja (SIAR); we selected the station located about 5 km from the place where the vineyard was located. The collected data were: the rain accumulated from the beginning of April until 1 September (247.80 L/m2 in 2019 and 217.80 L/m2 in 2020), global radiation (5,651.42 MJ/m2 in 2019 and 5,298.25 MJ/m2 in 2020) and the average maximum, mean and minimum temperatures, (27.05 °C, 13.83 °C and 3.70 °C respectively in 2019, and 26.3 °C, 13.8 °C and 3.7 °C respectively in 2020.) The plots were managed according to the viticultural practices of the region.

2. Harvest and vinification

Berries from different vines were randomly sampled in the rows where the treatments were carried out and when they reached 13 % of potential ethanol content, all the trials were harvested on the same day, in this way we can know the effect of the treatments on the grape composition. A random set of 100 berries per replicate and treatment was separated and weighed to obtain the average berry weight, and then the 100 berries were frozen at -20 °C until the analyses of grape polysaccharides were carried out. The remaining grapes were destemmed and crushed, and oenological parameters were determined in the musts. Grape samples were named control, MeJ and ACP-MeJ grapes. Must samples were named control, MeJ and ACP-MeJ musts.

To evaluate the influence of elicitor application on wine quality, the grapes were vinified in 25 L tanks. Potassium metabisulfite was added to the samples to give a final total SO_2 concentration of 50 mg/L. Alcoholic fermentation, carried out at 20 +/- 2 ºC, was induced by inoculating the commercial *Saccharomyces cerevisiae* strain Safoeno SC22 (Fermentis, Marcq-en-Barœul, France) (20 g/hL). Caps were punched down daily and fermentation activity was followed by determining must temperature and the density decrease. When the alcoholic fermentation was finished i.e. when sugar concentration was lower than 2.5 g/L, the solid parts were removed and placed in 12 L tanks. Then, malolactic fermentation was induced by inoculating the commercial *Oenococcus oeni* strain VINIFLORA® CH16 (CHR Hansen, Hoersholm, Denmark) (1 g/hL). Malolactic fermentation was carried out under a controlled temperature of 20 ºC, and its development was monitored by analysing L-malic and L-lactic content. Once it had finished, wine general parameters were analysed and aliquots of each wine were frozen and stored at −20 °C for wine polysaccharides analysis. Wine samples were named control, MeJ and ACP-MeJ wines.

3. Oenological parameters of musts and wines

The must oenological parameters, ºBrix, probable alcohol, pH, and total acidity, were analysed using the official methods established by the OIV (OIV, 2009). Glucose, glucose+fructose, malic acid, lactic acid and total phenols were determined using Miura One enzymatic equipment (TDI, Barcelona, Spain). Wines were analysed for alcoholic degree, pH, total acidity, volatile acidity, colour intensity (CI) and total polyphenol index (TPI) (OIV, 2009). Malic and lactic acids and total phenols were analysed by the Miura One equipment (TDI). Total anthocyanin content was analysed according to Ribéreau-Gayon and Stonestreet (1965). As the treatments were performed in triplicate, the results of these parameters are shown as the average of three analyses (n = 3).

4. Analysis of soluble polysaccharides from grapes and wines

4.1. Procedure for the extraction of soluble polysaccharides from grapes

After defrosting, the grapes were homogenised using an UltraTurrax at 18,000–20,000 rpm in static conditions to achieve total grape homogenisation. Thereafter, 1 g of homogenates were taken for the extraction with the following parameters: 2.5 g/L Tartaric acid, pH = 1, 1:4 solid to liquid ratio, and 18 h of extraction time (Canalejo *et al.*, 2021). The extractions were performed while stirring in a thermostatic ultrasonic bath at 22 ºC and 35 kHz.

4.2. Precipitation of total soluble grape and wine polysaccharides

Polysaccharides from wine samples (2 mL) and grape extracts were recovered in the supernatants by precipitation after sample concentration as described (Guadalupe *et al.*, 2012). Total polysaccharides were then precipitated by

adding four volumes of cold 96 % ethanol containing 0.3 M HCl and kept for 20 h at 4 ºC. Thereafter, the samples were centrifuged (33,000 x g for 20 min), the supernatants discarded, and the pellets dissolved in ultrapure water and freeze-dried. The freeze-dried precipitates contained polysaccharides from grapes and wine. The precipitation of polysaccharides was performed in triplicate in each sample.

4.3. Identification and quantitation of monosaccharides by GC-MS

The monosaccharide composition of extracted grape polysaccharides and wine was determined by GC-MS of their trimethylsilyl-ester O-methyl glycosyl-derivates obtained after acidic methanolysis and derivatization following the methodology described by Guadalupe *et al*., 2012 and Ayestarán *et al*., 2004. 100 µL of myo-inositol (**1** mg mL^{-1}) was added to the extracts as internal standard, and freeze-dried. Thereafter, they were treated with 1 mL of the methanolysis reagent (MeOH anhydrous containing CH_3COCl 0.5 M) and the reaction was conducted in nitrogen atmosphere at 80 ºC for 16 h in order to hydrolyse neutral and acidic monosaccharides to their corresponding methyl glycosides. After removing the excess of reagent with a stream of nitrogen, the conversion of the methyl glycosides to their trimethylsilyl (TMS) derivates was performed by adding 0.5 mL of a mix of pyridine: hexamethyldisilazane: trimethylchlorosilane (10:2:1 v/v). The reaction was carried out at 80 ºC for 30 min and the reagent was removed using a stream of nitrogen gas. Finally, the derivatized residues were extracted with 1 mL of hexane. GC-MS was performed with 2 µL of these solutions and the samples were analysed in triplicate. Standard carbohydrates were used as patterns for identification quantitation.

GC was made on an Agilent 7890A gas chromatograph (Agilent Technologies, Waldbronn, Germany) coupled to a 5975C VL quadrupole mass detector (MS). Samples were injected in triplicate. The chromatographic column was a Teknokroma fused silica capillary column (30 m x 0.25 mm x 0.25 µm) of phase 5 % phenyl – 95 % methylpolysiloxane. The oven program started at an initial temperature of 120 ºC which was increased at a rate of 1 ºC/min to 145 ºC, and then to 180 ºC at a rate of 0.9 ºC/min and finally to 230 ºC at 40 ºC/min. The GC injectors were equipped with a 3.4 mm I.D. and were maintained at 250 ºC with a 1:20 split ratio. The carrier gas was helium (99.996 %) at a flow rate of 1 mL/min. Ionisation was performed by electron impact (EI) mode at 70 eV. The temperatures used were 150 ºC for the MS Quad, 230 ºC for the MS Source, and 250 ºC for the transfer line.

The total monosaccharides components of the precipitated polysaccharides were called TMS. The content of each polysaccharide family in the samples was estimated from the concentration of individual glycosyl residues which are characteristic of structurally identified must and wine polysaccharides (Ayestarán *et al.*, 2004; Doco *et al.*, 1999). The content of total polysaccharide families (TPF) was estimated from the sum of PRAG, MP or Mannans, RG-II and HL.

5. Statistical analyses

Analyses of variance (ANOVA) and multivariate analysis of variance (MANOVA) were performed using the SPSS 15.0 for Windows (SPSS Statistics, Chicago, USA).

RESULTS AND DISCUSSION

1. Effect of elicitor foliar applications on must and wine oenological parameters

Table 1 shows two different behaviours of the effect of foliar applications on the oenological parameters of the must in each vintage. In 2019, significant differences were observed between the control and MeJ musts in terms of ºBrix, probable alcohol, total acidity, glucose+fructose, glucose, fructose and total phenols; meanwhile the values of these parameters for the must from the ACP-MeJ treatment were similar to those of the control and MeJ must, with the exception of total phenols, which was only similar to the must from the MeJ treatment. This result indicates that the application of ACP-MeJ induced polyphenol synthesis in grapes with the same effectiveness as MeJ, even though the MeJ dose in the apatite nanoparticle doping was one-tenth lower than the dilution application of MeJ. The application of MeJ probably had the effect of reducing the º Brix content and the probable degree of the grape in the 2019 vintage compared to the control. In contrast to 2019, no significant differences in the content of any general parameters were observed in 2020 among the treatments applied (Table 1).

Nevertheless, the weight of 100 berries, pH and malic acid did not show any significant differences between control must and the grapes treated with the foliar elicitors in 2019. These results indicate that in both vintages the dilution effect was not observed, as the higher the grape weight, the lower was the observed ºBrix.

The multivariante analysis of variance results indicate that the application of elicitors only affected the total phenols, with the MeJ and ACP-MeJ musts showing similar and significantly higher values than the control must (Table 2). Portu *et al.* (2015) observed that foliar application of MeJ induced anthocyanin synthesis in grapes, and Ruiz-García *et al.* (2012) found that MeJ-treated grapes had higher anthocyanin content than control grapes. It is noteworthy that foliar application of ACP-MeJ had the same effectiveness in terms of polyphenol synthesis as the MeJ application, despite the difference in dosage. This effect is due to the advantages of the nano-MeJ system, as discussed above. However, there are few studies on the influence of ACP-MeJ application on grape composition.

In the musts, the seasonal factor significantly influenced the weight of 100 berries, total acidity, fructose, malic acid and total phenols (Table 2). The value of the weight of 100 berries was higher in 2020 than in 2019 samples, while the content of malic acid and total phenols in 2019 was 1.8 and 2.2 times higher than in 2020 (Table 2). These seasonal differences

TABLE 1. General parameters in grapes and wines from the control, methyl jasmonate (MeJ) and nanoapatite doped with MeJ (ACP-MeJ) foliar treatments in 2019 and 2020 seasons.

	2019			2020		
	Grapes			Grapes		
	Control	MeJ	ACP-MeJ	Control	MeJ	ACP-MeJ
Weight of 100 berries (g)	113.68 ± 11.07 a	141.81 ± 27.18 a	116.94 ± 4.62 a	199.57 ± 7.27 a	207.67 ± 40.39 a	194.90 ± 20.65 a
°Brix	24.70 ± 0.72 b	22.23 ± 1.17 a	23.37 ± 0.49 ab	22.30 ± 0.92 a	22.17 ± 2.31 a	22.37 ± 0.38 a
Probable alcohol (% v/v)	14.63 ± 0.49 b	12.92 ± 0.80 a	13.71 ± 0.35 ab	12.97 ± 0.63 a	12.89 ± 1.58 a	13.01 ± 0.26 a
pH	3.83 ± 0.05 a	3.78 ± 0.10 a	3.82 ± 0.09 a	3.76 ± 0.01 a	3.70 ± 0.07 a	3.73 ± 0.06 a
Total acidity (g/L)*	4.61 ± 0.11 a	5.20 ± 0.36 b	5.13 ± 0.26 ab	4.12 ± 0.33 a	4.54 ± 1.08 a	4.03 ± 0.21 a
Glu+Fru (g/L)	249.86 ± 9.97 b	215.50 ± 12.29 a	231.40 ± 10.82 ab	216.42 ± 10.70 a	218.62 ± 26.56 a	223.84 ± 2.98 a
Glu (g/L)	120.18 ± 5.13 b	102.88 ± 6.89 a	110.89 ± 4.94 ab	107.31 ± 4.54 a	106.08 ± 12.84 a	108.61 ± 2.98 a
Fru (g/L)	129.68 ± 4.84 b	112.62 ± 5.43 a	120.51 ± 6.26 ab	109.11 ± 6.53 a	112.54 ± 13.76 a	114.72 ± 0.98 a
Malic acid (g/L)	2.24 ± 0.24 a	2.54 ± 0.32 a	2.51 ± 0.56 a	1.21 ± 0.08 a	1.54 ± 0.22 a	1.39 ± 0.18 a
Total phenols (mg/L)	1185.33 ± 72.31 a	1306.57 ± 61.35 b	1351.40 ± 27.32 b	541.60 ± 64.02 a	603.07 ± 73.82 a	582.70 ± 66.02 a
	Wines			Wines		
	Control	MeJ	ACP-MeJ	Control	MeJ	ACP-MeJ
Alcoholic degree (% v/v)	13.97 ± 0.31 b	12.57 ± 0.25 a	12.93 ± 0.64 a	12.47 ± 0.70 a	12.18 ± 1.59 a	12.42 ± 0.12 a
pH	3.96 ± 0.07 a	3.90 ± 0.10 a	3.97 ± 0.08 a	3.66 ± 0.08 a	3.70 ± 0.04 a	3.70 ± 0.09 a
Total acidity (g/L)*	4.27 ± 0.10 b	4.08 ± 0.06 ab	3.96 ± 0.15 a	4.43 ± 0.59 a	4.38 ± 0.23 a	4.26 ± 0.17 a
Volatile acidity (g/L)**	0.23 ± 0.02 a	0.28 ± 0.03 b	0.24 ± 0.02 a	0.22 ± 0.02 b	0.18 ± 0.01 a	0.21 ± 0.02 b
Malic acid (g/L)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lactic acid (g/L)	1.32 ± 0.10 a	1.36 ± 0.07 a	1.36 ± 0.13 a	0.86 ± 0.07 a	1.14 ± 0.15 b	0.99 ± 0.13 ab
Total phenols (mg/L)	2440.83 ± 123.16 a	2160.37 ± 221.12 a	2300.20 ± 236.75 a	1116.63 ± 106.69 a	1263.07 ± 224.95 a	1231.77 ± 75.81 a
Total anthocyanins (mg/L)	1117.33 ± 69.97 ab	1225.67 ± 98.64 b	1019.67 ± 97.01 a	130.99 ± 20.13 a	158.53 ± 18.35 a	155.49 ± 11.41 a
Colour intensity (CI)	18.27 ± 1.03 b	17.53 ± 1.81 ab	15.06 ± 0.80 a	6.05 ± 0.55 a	7.70 ± 2.13 a	7.12 ± 0.53 a
Total polyphenol index (TPI)	70.83 ± 3.47 a	66.43 ± 7.95 a	64.55 ± 5.79 a	36.82 ± 4.05 a	41.04 ± 8.69 a	40.39 ± 2.33 a

*As g/L of tartaric acid. **As g/L of acetic acid. All parameters are listed with their standard deviation (n = 3). For each season and parameter, different letters indicate significant differences among the samples ($p \leq 0.05$). Glu = glucose. Fru = fructose. n.d. = not detected.

were probably due to the accumulated rainfall and the global radiation being higher in 2019 than in 2020. It is interesting to note that, in 2020, the precipitation in August (the month in which the treatments in the vineyard began) was triple that in 2019 (SIAR). These data of SIAR probably explain the greater weight of the berries in 2020.

Table 1 also shows the wine oenological parameters. In 2019, MeJ and ACP-MeJ wines had a significantly lower

alcohol content than control wines. The MeJ and control wines showed no significant differences in the values of total phenols, total anthocyanins, CI, and TPI. ACP-MeJ wines presented a significantly lower CI than control and a lower total anthocyanin than MeJ wines. On the contrary, the differences found in 2019 were not observed in 2020. In 2020, only significant differences in volatile acidity and lactic acid were observed among the wines.

The application of elicitors only significantly affected the total anthocyanin and lactic acid of wines (Table 2). The total anthocyanin in ACP-MeJ wine was significantly lower than in MeJ wine (Table 2). In contrast to our previous work (Portu *et al.*, 2015), no significant differences were observed in the total anthocyanin between the control and MeJ wines (Table 2); nevertheless, the absolute value of this parameter was higher in the MeJ wine. The different climatic conditions had a strong influence on grape ripening and, consequently, on the oenological parameters of the wine. Except for total acidity, the 2019 wines showed significantly higher values for all parameters than the 2020 wines (Table 2). The values of total phenols, total anthocyanins, CI, TPI of 2019 wines were 1.9, 7.6, 2.4 and 1.7 times higher respectively than those of 2020 wines.

2. Effect of elicitors on the glycosyl residue composition of grape and wine polysaccharides

Table 3 shows the concentration of the monosaccharide composition of cellulose, xyloglucans, mannoproteins, mannans and pectic polysaccharides from grapes and wines. Glucose is the main component of major structural polysaccharides from the grape cell walls, such as cellulose and hemicellulosic xyloglucans, arabinoglucans and mannans. In 2019, glucose was the major glycosyl residue detected in the grapes (28.6 % of total monosaccharides (TMS)), and there were no significant differences among the treatments. In contrast, glucose was not the major glycosyl residue detected in the grapes in 2020, and the control showed a significantly higher content (9.3 % with respect to TMS) than the MeJ and ACP-MeJ grapes, with no significant differences between them (6.6 % with respect to TMS). The control had a significantly higher glycosyl residue content (9.3 % with respect to TMS) than the MeJ and

TABLE 2. Factorial analysis of the general parameters of the grapes and wines taking into account two factors: treatment (Control, MeJ, and ACP-MeJ) and season (2019 and 2020).

	Grapes									
	Weight of 100 berries (g)	°Brix	Probable alcohol (% v/v)	pH	Total acidity (g/L)*	Glu+Fru (g/L)	Glu (g/L)	Fru (g/L)	Malic acid (g/L)	Total phenols (mg/L)
Treatment (T)										
Control	156.63 a	23.50 a	13.80 a	3.79 a	4.37 a	233.14 a	113.74 a	119.39 a	1.73 a	863.47 a
MeJ	174.74 a	22.20 a	12.91 a	3.74 a	4.87 a	217.06 a	104.48 a	112.58 a	2.04 a	954.82 b
ACP-MeJ	155.92 a	22.87 a	13.36 a	3.77 a	4.58 a	227.37 a	109.75 a	117.62 a	1.95 a	967.05 b
Season (S)										
2019	124.14 a	23.43 a	13.75 a	3.81 a	4.98 b	232.25 a	111.32 a	120.94 b	2.43 b	1281.10 b
2020	200.71 b	22.28 a	12.96 a	3.73 a	4.23 a	219.46 a	107.33 a	112.12 a	1.38 a	575.79 a
Interaction (T x S)										
T x S	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Wines									
	Alcoholic degree (% v/v)	pH	Total acidity (g/L)*	Volatile acidity (g/L)**	Malic acid (g/L)	Lactic acid (g/L)	Total phenols (mg/L)	Total anthocyanins (mg/L)	Colour intensity (CI)	Total polyphenol index (TPI)
Treatment (T)										
Control	13.22 a	3.81 a	4.35 a	0.23 a	n.d.	1.09 a	1778.73 a	624.16 ab	12.16 a	53.83 a
MeJ	12.38 a	3.80 a	4.23 a	0.23 a	n.d.	1.25 b	1711.72 a	692.10 b	12.61 a	53.74 a
ACP-MeJ	12.68 a	3.83 a	4.11 a	0.23 a	n.d.	1.18 ab	1765.98 a	587.58 a	11.09 a	52.47 a
Season (S)										
2019	13.16 b	3.94 b	4.10 a	0.25 b	n.d.	1.35 b	2300.47 b	1120.89 b	16.95 b	67.27 b
2020	12.36 a	3.68 a	4.36 a	0.21 a	n.d.	1.00 a	1203.82 a	148.33 a	6.95 a	39.42 a
Interaction (T x S)										
T x S	N.S.	N.S.	N.S.	**	-	N.S.	N.S.	*	*	N.S.

*As g/L of tartaric acid. **As g/L of acetic acid. For each parameter and factor, different letters indicate significant differences among the samples ($p \leq 0.05$). Interaction: N.S., not significant ($p > 0.05$); *, $p \leq 0.05$; **, $p \leq 0.001$.

ACP-MeJ, with no significant differences between them (6.6 % with respect to TMS). Therefore, no differences were found in terms of glucose content in the grape samples treated with elicitors, and it is likely that the cell wall remodelling in response to the application of elicitors in the two studied vintages was not significant. It is known that following a pathogen or elicitor attack, plants often deposit a cell wall rich in callose (appositions at sites of attempted pathogen or elicitor penetration) accumulate phenolic compounds and various toxins in the wall, and/or synthesise lignin-like polymers to reinforce the wall (Benhamou, 1996). Callose is a polysaccharide that contains a high proportion of glucose bound to 1,3-β. Lignin is a rigid, hydrophobic polymer usually presents in the secondary cell wall of vasculature (Apolinar-Valiente *et al.*, 2018). The xylose residues were thus components of xyloglucans. The xylose content did not show significant differences between the control and treated grapes, but its content in 2020 was double that of 2019 (Table 3). The source of mannose content has been attributed to the mannans and hemicelluloses in the grape pericarp (Arnous and Meyer, 2009; Minjares-Fuentes *et al.*, 2016). As with xylose content, mannose content did not differ significantly between treatments and, in the 2020 season, its concentration was approximately twice as high as in 2019 (Table 3).

Galactose, arabinose, rhamnose and glucuronic acid are components of pectic polysaccharides that are rich in arabinose and galactose (PRAG), such as galacturonans, galactans, arabinogalactans, arabinogalactan proteins and arabinans (Vidal *et al.*, 2000). Another pectic domain is the homogalacturonan (HL), which is composed of galacturonic acid (Ayestarán *et al.*, 2004). In both seasons, galactose, arabinose and galacturonic acid were the major monosaccharydes of the grapes, with no significant differences in their content between the treated grapes and the control (Table 3). These results confirm that the foliar application of both elicitors did not result in any significant changes in content of the major pectic monosaccharides in the grape cell walls. Paladines-Quezada *et al.* (2019) observed that the exogenous application of MeJ and benzothiadiazole during veraison caused significant changes in the content of uronic acids in grape skin cell walls (such as galacturonic acid), which was present in different proportions depending on the grape variety and season; indicating that the response to the application of these elicitors being dependent on variety and weather. Weather dependence was also observed in the concentration of these glycosyl residues in the present study. The galactose content was three times higher in 2020 than in 2019, arabinose was more than double and galacturonic acid was approximately four times higher (Table 3). Another pectic zone is rhamnogalacturonan type II, whose marker monosaccharides are minor carbohydrates, such as 2-*O*-methyl xylose, 2-*O*-methyl fucose, aceric acid, apiose, DHA and Kdo.

Similar to the rest of the pectic monosaccharides, the content of the markers did not show significant differences between treatments, except for Kdo in both vintages and Api in 2020, but their contents were low (Table 3). Weather dependence was also observed in the content of these glycosyl residue markers, which increased approximately four-fold in 2020.

The application of MeJ and ACP-MeJ did not affect the content of cellulose monosaccharides, xyloglucans, mannans and pectic polysaccharides constituents of the grape cell wall in either season.

Different characteristic ratios were calculated to elucidate the sugar structure from grape: Arabinose to Galactose (Ara/Gal), Rhamnose to Galacturonic acid (Rha/GalA) and Arabinose + Galactose to Rhamnose (Ara + Gal)/Rha (Table 3). The Ara/Gal ratio is characteristic of PRAG-like structures, and higher values of this ratio indicate higher contents in arabinose or structures rich in arabinose that arise from the pectic framework (Vidal *et al.*, 2003). The Rha/GalA ratio could be an indicator of the relative richness of polysaccharides as homogalacturonans *versus* rhamnogalacturonan-like structures (Arnous and Meyer, 2009). In all samples, Ara/Gal and Rha/GalA values were < 0.45, indicating that the samples contained a lower content of arabinose-rich polysaccharides and a majority of homogalacturonan-type structures. Except for the 2019 season, the Ara/Gal of ACP-MeJ sample was significantly higher than the control and MeJ. The Ara + Gal/Rha ratios were used to estimate the relative importance of neutral side chains in the rhamnogalacturonan backbone, since most of the arabinose and galactose content is associated with the pectin pilose regions (Apolinar-Valiente *et al.*, 2015a; Apolinar-Valiente *et al.*, 2015b). These proportions were significantly higher in the 2020 season in the ACP-MeJ and MeJ samples compared to the control grape (Table 3), which could indicate that the rhamnogalacturonan-like structures in these grapes carried more neutral side chains. In this season the response of the foliar application of elicitors was probably the modification of the pectin structure of the pilose regions.

The content of total monosaccharides (TMS) was more than one hundred times higher in the wines than in the grapes (Table 3). It is known that maceration assisted by grape endogenous enzymes and/or the presence of ethanol causes the extraction of polysaccharides from the cell wall of the grape, and their solubilisation determines the amount of TMS in the produced wine. In 2019, the ACP-MeJ wines contained significantly higher TMS content than the rest of the wines, while the MeJ wines had the lowest TMS value in 2020 (Table 3).

The TMS content was double in 2019 wines than in 2020 wines, even though the TMS values of the grapes in 2019 were half those of 2020 (Table 3). The extractability of grape cell wall monosaccharides, total phenols, total anthocyanins, colour intensity and total polyphenol index to wine (Table 2) was significantly higher in the 2019 wines. These results were probably due to the lower weight of the set of 100 berries in this season (Table 2), being berries with lower must volume and size. This implied a higher skin-to-must ratio in the 2019 berries. The polysaccharides from the skin cell walls probably contributed more to TMS content in 2019

TABLE 3. Glucosyl composition (mg/g) of polysaccharides from Tempranillo grapes and wines from the control, methyl jasmonate (MeJ) and nanoapatite doped with MeJ (ACP-MeJ) treatments in the 2019 and 2020 seasons.

	2019			2020		
	Grapes			Grapes		
	Control	MeJ	ACP-MeJ	Control	MeJ	ACP-MeJ
2-OMeFuca	0.04 ± 0.00 a	0.04 ± 0.00 a	0.05 ± 0.00 b	0.14 ± 0.02 a	0.13 ± 0.01 a	0.11 ± 0.04 a
2-OmeXyla	0.02 ± 0.00 a	0.02 ± 0.00 a	0.02 ± 0.00 a	0.08 ± 0.00 a	0.07 ± 0.00 a	0.07 ± 0.02 a
Apia	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a	0.04 ± 0.00 b	0.03 ± 0.00 b	0.01 ± 0.00 a
Kdoa	0.03 ± 0.00 b	0.02 ± 0.01 a	0.02 ± 0.00 a	0.04 ± 0.00 b	0.04 ± 0.00 b	0.03 ± 0.00 a
Araa	1.59 ± 0.01 a	1.48 ± 0.43 a	1.86 ± 0.20 a	4.74 ± 0.35 a	4.69 ± 0.50 a	4.11 ± 0.85 a
Rhaa	0.60 ± 0.08 a	0.94 ± 0.60 a	0.57 ± 0.02 a	1.66 ± 0.14 a	1.40 ± 0.08 a	1.25 ± 0.37 a
Fuca	0.02 ± 0.00 a	0.03 ± 0.01 a	0.02 ± 0.00 a	0.05 ± 0.00 a	0.05 ± 0.00 a	0.05 ± 0.01 a
Gala	5.72 ± 0.24 a	4.87 ± 1.67 a	5.69 ± 0.39 a	16.34 ± 0.90 a	16.09 ± 2.93 a	15.40 ± 4.20 a
GalAa	2.81 ± 0.04 a	2.73 ± 0.49 a	2.96 ± 0.74 a	11.32 ± 0.59 a	9.44 ± 0.66 a	8.79 ± 3.76 a
GluAa	0.53 ± 0.09 a	0.50 ± 0.03 a	0.57 ± 0.04 a	1.52 ± 0.16 b	1.61 ± 0.20 b	1.16 ± 0.12 a
Glca	5.91 ± 1.10 a	3.74 ± 1.79 a	5.31 ± 0.61 a	3.93 ± 0.75 b	2.08 ± 0.12 a	2.58 ± 0.59 a
Xyla	0.36 ± 0.08 a	0.33 ± 0.06 a	0.28 ± 0.01 a	0.84 ± 0.07 a	0.80 ± 0.20 a	0.86 ± 0.26 a
Mana	0.72 ± 0.00 a	0.75 ± 0.06 a	0.72 ± 0.12 a	1.46 ± 0.23 a	1.53 ± 0.34 a	1.21 ± 0.41 a
TMSa	18.38 ± 1.14 a	15.46 ± 2.60 a	18.07 ± 1.06 a	42.16 ± 1.40 a	37.94 ± 3.09 a	35.63 ± 5.76 a
Ara/Gal	0.28 ± 0.01 a	0.31 ± 0.02 a	0.40 ± 0.04 b	0.29 ± 0.01 a	0.29 ± 0.02 a	0.29 ± 0.00 a
Rha/GalA	0.21 ± 0.03 a	0.33 ± 0.16 a	0.26 ± 0.01 a	0.15 ± 0.01 a	0.15 ± 0.00 a	0.15 ± 0.00 a
(Ara+Gal)/Rha	12.36 ± 1.24 a	8.15 ± 3.61 a	12.60 ± 1.31 a	12.74 ± 0.36 a	14.76 ± 1.59 b	15.59 ± 0.56 b
	Wines			Wines		
	Control	MeJ	ACP-MeJ	Control	MeJ	ACP-MeJ
AceAa	0.01 ± 0.00 a	0.07 ± 0.09 a	0.01 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
2-OMeFuca	19.37 ± 5.68 a	26.81 ± 8.58 a	20.58 ± 0.02 a	5.38 ± 0.56 b	0.70 ± 0.31 a	1.25 ± 0.29 a
2-OmeXyla	9.29 ± 4.09 a	8.58 ± 3.01 a	10.60 ± 0.49 a	3.28 ± 0.53 b	0.53 ± 0.12 a	0.57 ± 0.15 a
Apia	3.88 ± 2.29 a	3.07 ± 2.38 a	3.73 ± 0.38 a	1.51 ± 0.32 c	0.45 ± 0.10 b	0.02 ± 0.00 a
Kdoa	11.61 ± 5.02 a	9.11 ± 4.56 a	7.02 ± 9.57 a	1.42 ± 0.03 a	1.55 ± 1.23 a	1.10 ± 0.33 a
Araa	323.71 ± 116.87 a	334.70 ± 67.78 a	390.80 ± 6.66 a	206.14 ± 45.21 a	116.47 ± 32.89 a	181.35 ± 52.36 a
Rhaa	161.22 ± 73.57 a	156.73 ± 46.78 a	194.45 ± 6.71 a	43.00 ± 2.59 b	19.41 ± 4.08 a	52.17 ± 5.14 c
Fuca	7.56 ± 2.28 a	7.34 ± 0.66 a	8.67 ± 0.16 a	1.84 ± 0.29 c	0.54 ± 0.11 a	1.21 ± 0.20 b
Gala	1103.55 ± 427.71 ab	676.78 ± 204.13 a	1693.11 ± 368.07 b	623.81 ± 75.30 ab	575.14 ± 84.18 a	827.32 ± 162.28 b
GalAa	641.24 ± 73.45 a	679.81 ± 148.13 a	768.58 ± 1.16 a	68.28 ± 4.30 b	29.84 ± 4.71 a	55.63 ± 17.66 b
GluAa	35.15 ± 17.42 a	28.97 ± 17.54 a	52.32 ± 1.08 a	24.96 ± 7.64 a	15.62 ± 2.50 a	23.36 ± 5.86 a
Glca	178.68 ± 53.51 a	126.75 ± 46.11 a	202.33 ± 5.43 a	78.06 ± 13.99 ab	55.52 ± 1.73 a	97.27 ± 14.99 b
Xyla	22.77 ± 7.24 ab	14.07 ± 7.88 a	30.01 ± 1.00 b	9.84 ± 2.06 a	9.91 ± 1.80 a	10.43 ± 2.78 a
Mana	542.37 ± 171.67 a	670.90 ± 206.84 a	785.59 ± 16.86 a	582.66 ± 108.83 a	476.87 ± 73.48 a	554.77 ± 80.43 a
TMSa	3060.41 ± 487.14 a	2743.69 ± 337.13 a	4167.80 ± 368.71 b	1650.18 ± 140.87 b	1302.54 ± 116.71 a	1806.46 ± 190.13 b
Ara/Gal	0.30 ± 0.01 a	0.51 ± 0.05 b	0.24 ± 0.05 a	0.33 ± 0.03 b	0.20 ± 0.03 a	0.22 ± 0.02 a
Rha/GalA	0.24 ± 0.09 a	0.23 ± 0.02 a	0.25 ± 0.01 a	0.63 ± 0.00 a	0.65 ± 0.04 a	0.99 ± 0.23 b
(Ara+Gal)/Rha	9.11 ± 0.86 b	6.50 ± 0.21 a	10.68 ± 1.56 b	19.24 ± 1.65 a	35.85 ± 1.54 b	19.19 ± 2.23 a

[A]aceA = aceric acid; 2-OmeFuc = 2-O-CH3-fucose; 2-OmeXyl = 2-O-CH3-xylose; Api = apiose; Ara = arabinose; Rha = rhamnose; Fuc = fucose; Xyl = xylose; Man = mannose; Gal = galactose; GalA = galacturonic acid; Glc = glucose; GluA = glucuronic acid; Kdo = 2-keto-3-deoxyoctonate ammonium salt; TMS = Total monosaccharides. All parameters are listed with their standard deviation (n = 3). For each season and compound, different letters indicate significant differences among the samples ($p \leq 0.05$).

wines than those from pulp. The cell walls of the skin are rich in polysaccharides, because the cells are smaller, more compact and have thicker walls than the cells of the pulp (Apolinar-Valiente *et al.*, 2018). Other authors also point out that the extractability of polysaccharides increases with grape maturity (Gil *et al.*, 2012; Martínez-Lapuente *et al.*, 2016). However, the ºBrix of the grapes in both seasons did not show any significant differences (Table 2), and the total acidity of the grapes in 2019 was significantly higher than in 2020 (Table 2). The ºBrix/total acidity ratio was 4.7 in 2019 and 5.3 in 2020, but this small difference does not explain the double TMS value of the 2019 wines compared to those of 2020.

In most of the 2019 and 2020 wines, galactose was the monosaccharide with the highest levels (Table 3). In both seasons, the galactose content was similar between control and MeJ wines, and between control and ACP-MeJ; meanwhile it was significantly higher in ACP-MeJ wines than that in MeJ wines. Xylose, a monosaccharide present in low levels, was the only glycosylated residue that showed significantly lower values in MeJ wines than in the control and in ACP-MeJ wines in the 2019 season, while the latter wines showed similar values. In 2020, the monosaccharides with the lowest levels in MeJ wines, rhamnose, fucose and galacturonic acid, were lower than in the control and in ACP-MeJ wines, and the glucose content of MeJ wines was only significantly lower than in ACP-MeJ wines. A very limited number of monosaccharides with significant differences in their content between control and treated wines were observed in both seasons. These results suggested that there was no reinforcement of skin cell wall due to the action of these elicitors and, therefore, the extraction of the monosaccharides from the cell wall of Tempranillo grapes to the wines was not affected. In contrast, Apolinar-Valiente *et al.* (2018) concluded that the application of methyl jasmonate, benzothiadiazole, chitosan from fungi, and chitosan from seafood elicitors in the clusters of the vineyard of Monastrell grapes affected the extraction of monosaccharides in the wines. In the 2019 and 2020 wines, the major monosaccharides differed: in terms of glycosylated residues, galacturonic acid and mannose showed the second highest levels in the 2019 and 2020 wines respectively, and mannose and arabinose the third highest levels in the 2020 and 2019 wines respectively.

A previous study demonstrated that the mannoprotein concentration in wines increased in the last stages of fermentation (Guadalupe and Ayestarán, 2007). The origin of mannose residues in wines is attributed to yeast mannoproteins (Guadalupe and Ayestarán, 2007; Apolinar-Valiente *et al.*, 2018). In the present study, the mannose content did not show any significant differences in the 2019 and 2020 between control and treated wines (Table 3). These results indicated that the application of these elicitors did not degrade the cell walls of the yeasts.

To improve the knowledge of the structure of polysaccharide sugars from wine, the ratios arabinose to galactose (Ara/Gal),

TABLE 4. Polysaccharides families (mg/g) from Tempranillo grapes and wines from the control, methyl jasmonate (MeJ) and nanoapatite doped with MeJ (ACP-MeJ) treatments in the 2019 and 2020 seasons.

	2019			2020		
	Grapes			Grapes		
	Control	MeJ	ACP-MeJ	Control	MeJ	ACP-MeJ
RG-II[a]	0.44 ± 0.02 c	0.35 ± 0.03 a	0.39 ± 0.01 b	1.23 ± 0.07 b	1.07 ± 0.06 ab	0.88 ± 0.17 a
Mannans[a]	0.72 ± 0.00 a	0.75 ± 0.06 a	0.72 ± 0.12 a	1.46 ± 0.23 a	1.53 ± 0.34 a	1.21 ± 0.41 a
PRAG[a]	8.10 ± 0.44 a	7.46 ± 1.97 a	8.32 ± 0.56 a	23.09 ± 1.11 a	22.78 ± 3.24 a	21.03 ± 4.95 a
HL[a]	2.44 ± 0.15 a	2.37 ± 0.58 a	2.55 ± 0.66 a	10.02 ± 0.62 a	8.31 ± 0.89 a	7.78 ± 3.56 a
TPF[a]	11.70 ± 0.47 a	10.93 ± 2.06 a	11.99 ± 0.87 a	35.80 ± 1.29 a	33.69 ± 3.38 a	30.89 ± 6.11 a
	Wines			Wines		
	Control	MeJ	ACP-MeJ	Control	MeJ	ACP-MeJ
RG-II[a]	176.64 ± 35.38 a	190.58 ± 41.79 a	167.58 ± 38.38 a	46.34 ± 1.31 b	12.91 ± 3.58 a	11.80 ± 1.64 a
MP[a]	542.37 ± 171.67 a	670.90 ± 206.84 a	785.59 ± 16.86 a	582.66 ± 108.83 a	476.87 ± 73.48 a	554.77 ± 80.43 a
PRAG[a]	1468.67 ± 466.28 ab	982.66 ± 218.70 a	2166.07 ± 393.97 b	854.90 ± 29.56 ab	721.02 ± 121.14 a	1075.55 ± 225.59 b
HL[a]	466.91 ± 91.56 a	438.48 ± 109.69 a	583.39 ± 51.62 a	19.89 ± 0.78 a	23.52 ± 1.88 a	30.72 ± 3.85 b
TPF[a]	2654.58 ± 506.50 a	2282.62 ± 323.09 a	3702.79 ± 399.54 b	1503.79 ± 79.80 ab	1191.96 ± 124.98 a	1672.19 ± 300.48 b

[a]RG-II, Rhamnogalacturonan type II, MP/mannans, Mannoproteins or mannans, PRAG, Polysaccharides rich in Arabinose and Galactose, HL, Homogalacturonans, TPF, Total Polysaccharides Families. All parameters are listed with their standard deviation (n = 3). For each season and compound, different letters indicate significant differences among the samples ($p \leq 0.05$).

rhamnose to galacturonic acid (Rha/GalA) and arabinose plus galactose to rhamnose (Ara+Gal/Rha) were calculated.

The Ara/Gal ratio is characteristic of the PRAG-like structures de los vinos (Doco *et al*., 2003; Vidal *et al*., 2003). With the exception of the 2019 MeJ wine, the Ara/Gal ratio was < 0.45 in all the wines, indicating that they contained a lower content of arabinose-rich polysaccharides. The relative richness of the wines polysaccharides in homogalacturonans versus rhamnogalacturonans can be deduced from the Rha/GalA ratio (Arnous and Meyer, 2009). The Rha/GalA ratio was higher in 2020 than in 2019 wines, indicating lower contentes of homogalacturonan-like structures in the 2020 wines. The Ara + Gal/Rha ratio estimate the relative importance of the neutral side-chains to the rhamnogalacturonan backbone (Apolinar-Valiente *et al.*, 2015b). In both vintages, the Ara + Gal/Rha ratio did not show any significant differences between the control and the ACP-MeJ wines, and this value was significantly higher in 2019 for the control and ACP-MeJ than for the MeJ wines. However, the Ara + Gal/Rha ratios of the 2020 MeJ wines were the highest. This indicates that the rhamnogalacturonan-like structures in these 2020 MeJ wines may carry more neutral lateral chains.

3. Effect of elicitors on grape and wine polysaccharide families

The concentration of the different polysaccharide families of the grapes and wines is presented in Table 4, and the results obtained are in agreement with the observations described in the previous section.

The total content of polysaccharide families (TPF) of the grapes in each vintage did not show any significant differences between the control and the grape samples treated with the elicitors. The TPF content was approximately three times lower in the grapes in 2019 than in 2020. In the grapes of both seasons, polysaccharides rich in arabinose and galactose (PRAG) were the major family (64 %−69 % of TPF), followed by homogalacturonans (20 %−28 %) and, at much lower levels, mannans (3.5 %−7.0 %) and rhamnogalacturonan type II (2.8 %−3.8 %).

TABLE 5. Multifactor analysis of variance of monosaccharides and polysaccharides (expressed as mg/g) in Tempranillo grapes and wines from the control, methyl jasmonate (MeJ) and nanoapatite doped with MeJ (ACP-MeJ) treatments.

Part 1/2

	Grapes					
	Treatment (T)			Season (S)		
	Control	MeJ	ACP-MeJ	2019	2020	Interaction (T x S)
AceA[a]	0.00 a	0.00 a	0.00 a	0.00 b	0.00 a	N.S.
2-OMeFuc[a]	0.09 a	0.08 a	0.08 a	0.04 a	0.13 b	N.S.
2-OmeXyl[a]	0.05 a	0.05 a	0.05 a	0.02 a	0.07 b	N.S.
Api[a]	0.02 b	0.02 b	0.01 a	0.01 a	0.03 b	***
Ara[a]	3.16 a	3.08 a	2.99 a	1.64 a	4.51 b	N.S.
Rha[a]	1.13 a	1.17 a	0.91 a	0.70 a	1.44 b	N.S.
Fuc[a]	0.04 a	0.04 a	0.04 a	0.02 a	0.05 b	N.S.
Xyl[a]	0.60 a	0.56 a	0.57 a	0.32 a	0.83 b	N.S.
Man[a]	1.09 a	1.14 a	0.96 a	0.73 a	1.40 b	N.S.
Gal[a]	11.03 a	10.48 a	10.54 a	5.43 a	15.94 b	N.S.
GalA[a]	7.07 a	6.08 a	5.88 a	2.84 a	9.85 b	N.S.
Glc[a]	4.92 b	2.91 a	3.94 ab	4.99 a	2.86 b	N.S.
GluA[a]	1.02 b	1.05 b	0.86 a	0.53 a	1.43 b	**
Kdo[a]	0.04 c	0.03 b	0.02 a	0.02 a	0.04 b	*
TMS[a]	30.27 a	26.70 a	26.85 a	17.30 a	38.58 b	N.S.
Ara/Gal	0.28 a	0.30 a	0.30 a	0.30 a	0.28 a	**
Rha/GalA	0.18 a	0.24 a	0.17 a	0.25 b	0.15 a	N.S.
(Ara+Gal)/Rha	12.55 ab	11.46 a	14.47 b	11.26 a	14.39 b	*
RG-II[a]	0.83 b	0.71 a	0.64 a	0.39 a	1.06 b	*
Mannans[a]	1.09 a	1.14 a	0.96 a	0.73 a	1.40 b	N.S.
PRAG[a]	15.60 a	15.12 a	14.67 a	7.96 a	22.30 b	N.S.
HL[a]	6.23 a	5.34 a	5.17 a	2.45 a	8.70 b	N.S.
TPF[a]	23.75 a	22.31 a	21.44 a	11.54 a	33.46 b	N.S.

	Wines					
	Treatment (T)			Season (S)		
	Control	MeJ	ACP-MeJ	2019	2020	Interaction (T x S)
AceA[a]	0.01 a	0.03 a	0.00 a	0.03 a	0.00 a	N.S.
2-OMeFuc[a]	12.37 a	13.76 a	10.91 a	22.25 b	2.44 a	N.S.
2-OmeXyl[a]	6.28 a	4.56 a	5.59 a	9.49 b	1.46 a	N.S.
Api[a]	2.69 a	1.76 a	1.88 a	3.56 b	0.66 a	N.S.
Ara[a]	264.92 a	225.59 a	286.08 a	349.74 b	167.99 a	N.S.
Rha[a]	102.11 a	88.07 a	123.31 a	170.80 b	38.19 a	N.S.
Fuc[a]	4.70 a	3.94 a	4.94 a	7.86 b	1.20 a	N.S.
Xyl[a]	16.30 ab	11.99 a	20.22 b	22.28 b	10.06 a	*
Man[a]	562.52 a	573.88 a	670.18 a	666.29 a	538.10 a	N.S.
Gal[a]	863.68 ab	625.96 a	1260.22 b	1157.82 b	675.43 a	N.S.
GalA[a]	354.76 a	354.83 a	412.11 a	696.55 b	51.25 a	N.S.
Glc[a]	128.37 ab	91.13 a	149.80 b	169.25 b	76.95 a	N.S.
GluA[a]	30.05 ab	22.30 a	37.84 b	38.81 b	21.31 a	N.S.
Kdo[a]	6.51 a	5.33 a	4.06 a	9.25 b	1.35 a	N.S.
TMS[a]	2355.29 a	2023.12 a	2987.13 b	3323.96 b	1586.40 a	*
Ara/Gal	0.31 b	0.35 b	0.23 a	0.35 a	0.25 a	***
Rha/GalA	0.44 a	0.44 a	0.62 b	0.24 a	0.75 b	*
(Ara+Gal)/Rha	14.17 a	21.17 b	14.94 a	8.76 a	24.76 b	***
RG-II[a]	111.49 a	101.74 a	89.77 a	178.32 b	23.68 a	N.S.
MP[a]	562.52 a	573.88 a	670.18 a	666.29 a	538.10 a	N.S.
PRAG[a]	1161.78 a	851.84 a	1620.81 ab	1539.13 b	883.82 a	N.S.
HL[a]	243.40 a	231.00 a	307.06 a	496.26 b	24.71 a	N.S.
TPF[a]	2079.19 a	1737.29 a	2687.49 ab	2880.00 b	1455.98 a	*

AaceA = aceric acid; 2-OmeFuc = 2-O-CH3-fucose; 2-OmeXyl = 2-O-CH3-xylose; Api = apiose; Ara = arabinose; Rha = rhamnose; Fuc = fucose; Xyl = xylose; Man = mannose; Gal = galactose; GalA = galacturonic acid; Glc = glucose; GluA = glucuronic acid, Kdo = 2-keto-3-deoxyoctonate ammonium salt; TMS = Total monosaccharides; RG-II = Rhamnogalacturonan type II; MP/mannans = Mannoproteins or mannans; PRAG = Polysaccharides rich in Arabinose and Galactose; HL = Homogalacturonans; TPF = Total Polysaccharides Families. For each parameter and factor, different letters indicate significant differences between among the samples ($p \leq 0.05$). Interaction: N.S., not significant ($p > 0.05$); *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

In both seasons, the only family that showed significant differences among treatments was RG-II. Different behaviour was observed between the two seasons. The RG-II content of the 2019 MeJ grape was significantly lower than the control grape, as was that of the ACP-MeJ grape; however, the RG-II content of ACP-MeJ grape was significantly higher than that of MeJ grape. In 2020, the RG-II content of the ACP-MeJ grape was significantly lower than the control.

On the other hand, no significant differences were observed in either season between the content of the major families in the control grapes and the MeJ and ACP-MeJ-treated grapes. These results indicate that elicitor treatments did not lead to the strengthening of the grape cell wall as an active defense mechanism (Benhamou, 1996). The higher content of polysaccharide and TPF families in the 2020 grapes than in 2019 can be attributed to the differences in climate of the vintages.

Endogenous enzyme-assisted maceration of the grapes and/or the action of ethanol resulted in the extraction and solubilisation of the content of all pectic families (PRAG, HL and RG-II) in the wines obtained, but in greater quantity in 2019 than in 2020. As previously discussed, 2019 Tempranillo berries were smaller in size, which implies a higher skin-to-must ratio. In both vintages, the extractability of the main pectic family PRAG was significantly higher in the ACP-MeJ wines than in the MeJ wines, and these wines had similar PRAG content to the control wines. These results indicate that the cell walls of the elicitor-treated grapes were hydrolysed by the endogenous pectolytic enzymes of the grapes and/or solubilized by the action of ethanol without difficulty during maceration. In contrast, in Monastrell wines obtained from grapes treated with methyl jasmonate, benzothiadiazole, chitosan from fungi, and chitosan from seafood elicitors, other authors (Apolinar-Valiente *et al.*, 2018) have observed a lower PRAG content, which may have resulted from the greater difficulty in extracting it from the

skin cell walls and, therefore, from an increase in the grape skin "rigidity". This effect of the elicitors was not observed in the Tempranillo ACP-MeJ and MeJ wines in either study seasons.

In the 2019 wines, homogalacturones were the second most abundant family and RG-II was the least abundant, while in 2020 both families were found in lower amounts and at a similar percentage with respect to TPF (from 0.7 % to 3.0 %). In the wines of both seasons, the RG-II and HL content did not show significant differences among treatments, except in the 2020 ACP-MeJ wines, which showed significantly higher HL content than the control and MeJ wines. The extraction and solubilisation of polysaccharide families differed depending on the polysaccharide family and the season's meteorology, a factor that determines the conditions of grape ripening and berry weight. The mannoproteins showed similar values between the wines of each vintage. This was expected since the same yeast strain was used in all vinifications. It was the only polysaccharide family that did not depend on the season.

4. Principal factors of variability of the content of wine monosaccharides and polysaccharide families

A multivariate analysis of variance (MANOVA) was conducted on the grape and wine samples to analyse the effect of treatment, T, (control, MeJ and ACP-MeJ) and season, S, (2019 and 2020) on the wine monosaccharides and polysaccharide families (Table 5).

The factor treatment and treatment x season accounted for a small fraction of the observed variation, whereas the season effect was the dominant factor of variation for most of the monosaccharide and polysaccharide concentration of grapes and wines (Table 5). Except for the Ara/Gal rathio, the season had a great effect on the average concentration of monosaccharides and polysaccharides in grapes and wines, confirming the higher content in the 2020 grape. While in 2019 wines the concentration of the monosaccharides and families of polysaccharides of the grape was higher than that of the 2020 wines, confirming the effect of the vintage. It should be noted that the MP content of the wines was independent of the effect of the vintage.

Regarding the treatment, the ACP-MeJ grapes showed a higher (Ara+Gal)/Rha ratio than the MeJ and control grapes. However, the RG-II content was lower in the grapes treated with the elicitors. When the grapes were treated with ACP-MeJ, the resulting wines showed higher contents of galactose, glucose, galacturonic acid, TMS and Rha/GalA.

CONCLUSION

The effect of the foliar application of the elicitors, the conventional MeJ and the new ACP-MeJ, in two vintages on the polysaccharide composition of Tempranillo grapes and wines was not as expected. The contents of the PRAG, RG-II, HL families and total polysaccharides showed that the extractability and solubility of the cell wall of Tempranillo grapes treated with MeJ and ACP-MeJ to wine was not altered in either vintage. The reinforcement of grape cell walls by the action of these elicitors was not observed in the results of the main pectic families (PRAG, HL) and total polysaccharide families in the grapes, except for the minority (RG-II), which showed different behavior in both vintages. The results show that the extractability and solubility of the pectic families of the Tempranillo grape cell walls in the wine depended on the type of family and the climatology of the vintage, which determines the ripening conditions of the grapes and the weight of the grapes. The content of mannoproteins in the wines was independent of the vintage and the application of the elicitors.

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Year, watering regime and foliar methyl jasmonate doped nanoparticles treatments: Effects on must nitrogen compounds in Monastrell grapes

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ABSTRACT

In viticulture, the application of elicitors to the grapevines is increasing although their effect on the amino acids content is little studied. In this work, nanoparticles of methyl jasmonate (Ap-MeJ) were applied for the first time to Monastrell grapevines during two seasons (2019 and 2020) to increase its profitability and nitrogen plant uptake and to reduce environmental impact (less quantity) and increase its economic viability. In addition, rainfed plants were compared with grapevines under regulated deficit irrigation (RDI) watering regime, since global climatic conditions are limiting water use and quality. Results showed that season was the factor that most affected to the amino acids content (values of 12 from the 21 free amino acids determined were higher in 2019 and 4 in 2020), followed by the watering regime (higher amino acids content in musts from rainfed than in RDI water status). Foliar treatments had little impact on grape enological parameters, as well as on the amino acids concentration (only content of Pro was higher in control than in Ap-MeJ musts). This preliminary study could not confirm the elicitor effect of MeJ loaded on nanoparticles on must amino acid content and further research work should be performed in order to optimize dose of application.

1. Introduction

Nitrogen is a major nutrient for plants involved in many vital physiological processes and participates in the composition of key metabolites, such as proteins, amino acids, enzymes, chlorophyll, etc. Nitrogen uptake and amino acids synthesis are required for protein and enzyme synthesis, which in turn, are necessary for the photosynthetic activity and other biochemical pathways related to plant growth and development (Verdenal et al., 2021). In grapes, the main nitrogen form are amino acids, representing around 25–30 % of total nitrogen, and ammonium (Garde-Cerdán and Ancín-Azpilicueta, 2008). Grape nitrogen composition plays a key role on must and final wine quality due to amino acids are precursor of important volatile compounds in wine (Hernández-Orte et al., 2002) and biogenic amines (Smit and du Toit, 2013). Besides, the grape nitrogen content, influences the yeast growth, fermentation kinetics, flavor metabolism and the formation of secondary metabolites, especially higher alcohols and esters (Bell and Henschke, 2005; Garde-Cerdán and Ancín-Azpilicueta, 2008). The profile and content of amino acids in grapes are influenced by different factors such as viticultural and soil management, environmental conditions and grape variety (Pérez-Álvarez et al., 2019).

Leaves can take up nutrients through their cuticle and stomata and, in contrast to root uptake, leaf uptake is non-selective (Eichert, 2013). The foliar application of different compounds that acts as elicitors is a new technique for improving the grape quality. Elicitors are substances that, when applied exogenously, trigger all the defensive mechanisms of the plants (Ruíz-García and Gómez-Plaza, 2013; Delaunois et al., 2014). Therefore, their use can increase grapevine resistance to both, biotic and abiotic stresses; thus, it is known that elicitors induce plant resistance to pathogens and trigger enhancements in the biosynthesis of specific compounds such as phenolic compounds (Ruíz-García et al., 2013; Portu et al., 2016) and aromatic compounds (Garde-Cerdán et al., 2018). Chemical elicitors, such as methyl jasmonate (MeJ), simulate the action of signal molecules (such as salicylic acid or jasmonic acid or their derivatives) or simulate the attack of a pathogen. These molecules interact with receptors in the plant activating the defensive response and a

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hypersensitization reaction (Ruíz-García, 2014). Both, jasmonic acid and its derivative, methyl jasmonate, are natural plant growth regulators that modulate chlorophyll degradation and anthocyanin biosynthesis (Ruíz-García et al., 2013; Portu et al., 2015, 2016, 2017). Methyl jasmonate is mainly involved in plant responses triggered by wounding and insects feeding, and is implicated in resistance to pathogens (Gozzo, 2003). Authors such as Garde-Cerdán et al. (2016) and Gutiérrez--Gamboa et al. (2017, 2018), suggested that methyl jasmonate foliar application modified the amino acids concentrations, improving the quality of grapes. Although elicitors are being widely used in agriculture, their use is expensive, particularly that of methyl jasmonate. In order to reduce the amount of needed elicitors, the nanoparticles have been proposed as delivery nano-systems providing slow release kinetic and protection against degradation and thus, improving elicitor efficiency, respect to the conventional elicitor treatment (Parra-Torrejón et al., 2021).

Nanoparticles are materials of small size (1–100 nm), which have a large surface area and can be used for targeted and controlled release of nutrients, more efficiently and with less lost, increasing their availability to plants (Pérez-Álvarez et al., 2021a). In agriculture, the use of calcium phosphate nanoparticles (mainly hydroxyapatite, HA, or its precursor phase, amorphous calcium phosphate, ACP) have gained a special interest, such as nanofertilizer, due to its composition (mainly calcium and phosphorus, two important plant nutrients), high surface reactivity, capability to be doped with foreign ions, its biodegradability and biocompatibility (Ramírez-Rodríguez et al., 2020a). Recent works in agrochemical field demonstrated the potential of these nanoparticles for the controlled delivery of plant nutrients and the increase of crop productivity (Liu and Lal, 2014, 2015; Kottegoda et al., 2017; Ramírez-Rodríguez et al., 2020a, b; Carmona et al., 2021). On Tempranillo grapevines, Pérez-Álvarez et al. (2021a) reported that the concentration of amino acids was greater after applied nanoparticles of urea (0.4 kg N/ha) than the control plants and those treated with a commercial urea solution at 3 kg N/ha and similar values than those applied with a commercial urea solution at 6 kg N/ha. Thus, they stated the considerable reduction of nitrogen dosage while maintaining the quality of the harvest after using nanotechnology for nitrogen fertilization, thereby mitigating the environmental impact.

In recent years, the rise of temperatures and the change in the global dynamics of the rainfalls, have highlighted the need for viticultural research to face new climatic challenges, especially, in semi-arid areas (Schultz and Jones, 2010). Thus, in the current climate scenario, water scarcity would be even more limiting factor in the not-too-distant future for vine cultivation, not only because grape growers will need to apply water to reduce water stress, but it will also be important to improve the grapevine water status to counteract possible stress from extreme heat events (Myers, 1988). Therefore, an efficient water management in vineyards is considered a fundamental tool for controlling vegetative growth, the sustainability, yield production and grape composition (Romero et al., 2010; Munitz et al., 2017).

Deficit irrigation (DI) is the most widely used technique and development to reduce water consume maintaining the quality of the grapes without major yield losses (Mirás-Avalos and Intrigliolo, 2017). Regulated deficit irrigation (RDI) is a variant of DI based on the principle that the grapevine sensitivity to water stress varies according to its phenological stage. The RDI strategy promotes a mild water stress based on the evapotranspiration (ETc) estimated by the crop throughout the vegetative cycle or in previously established phenological periods. Therefore, RDI is a standard practice in Mediterranean viticulture that allows to save water and achieve different objectives such as reducing vine vigor and berry size, increasing anthocyanin concentration or improving grape quality (Zarrouk et al., 2012; Romero et al., 2010, 2013, 2016a,b; Niculcea et al., 2013, Niculcea et al., 2014; Buesa et al., 2017).

Based on the above mentioned, these two agricultural tools (elicitor application and irrigation) have a key impact on the grape composition and quality. However, the possible interaction between these two techniques, whether synergistic or antagonistic, has never been addressed. Consequently, the aim of this work was to evaluate the consequences of foliar application of methyl jasmonate loaded on nanoparticles on the must amino acids content from Monastrell grapevines under two different water conditions, rainfed and RDI, over two consecutive vintages, 2019 and 2020.

2. Materials and methods

2.1. Synthesis of Ap-MeJ nanoelicitor

Amorphous calcium phosphate nanoparticles (Ap), with similar physico-chemical properties than the precursor phase of bone mineral (Delgado-López et al., 2012, 2014), were synthesized through a simple and green route previously reported (Ramírez-Rodríguez et al., 2020a). Briefly, 2 L of an aqueous solution containing 0.2 mol/L $Ca(NO_3)_2$, and 0.2 mol/L Na_3Cit was mixed with an equal volume of a solution containing 0.12 mol/L K_2HPO_4 and 0.1 mol/L Na_2CO_3, appearing instantaneously a white precipitate. The mixture was then kept at room temperature during 5 min. Subsequently, the precipitate was collected and repeatedly washed with ultrapure water by centrifugation (3700 rpm, 15 min). The nanoparticles were then dispersed in 2 L of ultrapure water, vigorously shake with vortex and then, 5 mL of methyl jasmonate (MeJ) (Sigma-Aldrich, Madrid, Spain) was added to the nanoparticle suspension. After 24 hours under agitation, MeJ-doped Ap nanoparticles (Ap-MeJ) were collected by centrifugation (3700 rpm, 15 min) and stored at 4 ºC until the treatments. Nanoparticles contained 6 % w/w of MeJ, as estimated by UV–Vis (Parra-Torrejón et al., 2021).

2.2. Site location and experimental design

The experimental research was undertaken during the 2019 and 2020 seasons on a commercial vineyard located in Fuente-Álamo, Albacete, Southeastern of Spain (Lat: 38°43′43.3″N; Long: 1°28′12.6″, elevation: 820 m.a.s.l.). The Monastrell (*Vitis vinifera* L.) (syn. Mourvedre) grapevines, grafted on 1103-P rootstock, were planted in 2007 in North-South rows orientation and trained to a double Guyot system on a vertical trellis. The site has a continental Mediterranean climate, with hot and dry summers (temperatures close to 40ºC in July and August) and with an average annual rainfall around 450 mm (falling 527 mm and 459 mm annual rainfall in 2019 and 2020, respectively) of which about 60% falls during grapevine dormant period.

In 2019, three foliar treatments were applied: control, nanoparticles (Ap) and nanoparticles doped with methyl jasmonate (Ap-MeJ) at 1 mM concentration. Due to the situation caused by the COVID-19 pandemic, in 2020, the time to synthesize the nanoparticles was reduced and, therefore, the Ap treatment was not available for application in the vineyard. Thus, in 2020, two foliar treatments were applied: control and Ap-MeJ at 1 mM concentration. To carry out the treatments, aqueous solutions with nanoparticles were prepared, using Tween 80 (Sigma-Aldrich) as wetting agent (0.1% v/v). Control plants were sprayed with water solution of Tween 80 alone. For each treatment, 200 mL/plant were sprayed over leaves (100 mL per each side of the plant wall). The foliar treatments were applied twice, at veraison and one week later.

The foliar applications were carried out under two water deficit conditions: non-irrigated grapevines (rainfed) and regulated deficit irrigation strategy (RDI), where grapevines were watered at 30 % of the estimated crop evapotranspiration (ETc). RDI vines were irrigated with water form a well, which had an average electrical conductivity of 1.8 dS/m and the irrigation began when the grapevines stem water potential (Ψs) reached values of -0.8 MPa (prior to veraison) and finished after harvest. In order to estimate the ETc, the $ETc = ETo \times Kc$ equation was used, being calculated the reference evapotranspiration (ETo) daily with the Penman-Monteith equation FAO56 (Allen et al., 1998), using the climatic data provided by a meteorological station located nearby, and the crop coefficient (Kc = 0.15–0.3 depending on the vine phenological

stage, according to Monastrell values referenced by Romero et al., (2013). The water was applied through a drip irrigation system with one emitter for each linear meter of pipe and with a nominal flow rate of 3.8 L/h. In general, two or three weekly irrigations of about 3.3 mm each were applied with a total of 12–20 irrigations per year and an irrigation volume of 137.4 mm and 134 mm in 2019 and 2020, respectively.

The experimental field treatments were applied in quadruplicate and were arranged in a complete randomized block design. Each replicate involved four consecutive rows of vines, randomly distributed in the vineyard. The same number of vines was used for each of the two water regimes: three vines for each of the two elicitor treatments and six vines for the control. This scheme was followed in each of the four replicates per treatment.

2.3. Grape samples, enological parameters and nitrogen fractions

Grapes were harvested (October 7th, 2019 and October 10th, 2020) at their optimum technological maturity, i.e. when the weight of 100 berries was constant and the probable alcohol was around 13 % (v/v). A random set of 250 representative grapes, per treatment and replicate, were collected. From them, 100 grapes were counted and weighed and another set of 50 grapes were frozen and stored at -20 °C until the analysis of must amino acids composition was carried out. The remaining grapes were destemmed and crushed. In the must obtained, enological parameters such as ºBrix, probable alcohol, pH, and total acidity were analysed by methodology established by OIV (2016). Besides, malic and tartaric acids, glucose (Glu) and glucose + fructose (Glu +Fru), and amino and ammonium nitrogen content was determined by enzymatic test on the autoanalyser Miura One (Tecnología Difusión Ibérica (TDI), Barcelona, Spain). The yeast assimilable nitrogen (YAN) was calculated by sum of these two last parameters. The fructose content in grapes was obtained by difference between Glu + Fru and Glu.

Since the treatments were performed in quadruplicate, the results were shown as the average of four analyzes (n = 4).

2.4. Analysis of amino acids by HPLC-DAD

The content of amino acids in the samples was analysed by the method described by Garde-Cerdán et al. (2009). The analyses were performed using a Shimadzu Nexera X2 Ultra High-Performance Liquid Chromatograph (UHPLC) (Shimadzu, Kyoto, Japan) equipped with an automatic liquid sampler, and a diode array detector (DAD). Each must sample was centrifuged at 4000 rpm for 10 minutes at 20 °C and then the supernatant was used to derivatization reaction. The derivatization of amino acids was performed by reaction of 1.75 mL of 1 M borate buffer (pH 9), 750 μL of methanol, 1 mL of sample (previously centrifuged), and 30 μL of derivatization reagent diethyl ethoxymethylenemalonate - DEEMM (Sigma-Aldrich). The derivatization reaction was carried out in an ultrasonic bath for 30 minutes at 20 °C. Then, the samples were heated for 2 h at 75 °C, to degradation of excess DEEMM and reagent by-products.

The derivatized samples were filtered through 0.22 μm PVDF filter (Proquinorte, Bilbao, Spain) and transferred to screw vials; the volume of the sample injected was of 50 μL. Separations were performed in an ACE C18-HL (50 mm × 4.6 mm; I.D. 5 μm) column (Aberdeen, Scotland) at 20 °C. Two eluents were used as mobile phases: eluent A: 25 mM acetate buffer with 0.02 % sodium azide at pH 5.8; and eluent B: 80:20 (v/v) mixture of acetonitrile and methanol. The flow rate was of 0.9 mL/min, and the eluents proportion was the same as by Garde-Cerdán et al. (2009). For detection, a photodiode array detector monitored at 280, 269, and 300 nm was used.

In these conditions, aspartic acid (Asp), glutamic acid (Glu), asparagine (Asn), serine (Ser), glutamine (Gln), histidine (His), glycine (Gly), isoleucine + tryptophan (Ile+Trp), arginine (Arg), alanine (Ala), γ-aminobutyric acid (GABA), proline (Pro), tyrosine (Tyr), valine (Val), methionine (Met), cysteine (Cys), leucine (Leu), phenylalanine (Phe), ornithine (Orn), and lysine (Lys) were separated, identified and quantified.

The amino acids were identified according to the retention time of corresponding standards (Sigma-Aldrich) and were quantified using their respective calibration curve ($R^2 > 0.98$). Each standard solution was prepared in HCl (0.1 N) and analysed after the derivatization process in the same conditions of samples.

Since the treatments were performed in quadruplicate, the results of amino acids content were expressed as the average of four analyses (n = 4).

2.5. Statistical analysis

The statistical elaboration of the data was performed using the variance analysis (ANOVA) by SPSS Version 21.0 statistical package for Windows (SPSS, Chicago, IL). Significant differences between means were determined using the Duncan test at $p \leq 0.05$. Multifactor analysis and post-hoc Duncan's multiple range test were performed to determine the statistically significant effect of parameters results between treatments and vintages ($p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$). Since in 2020 there were only two treatments, multifactorial statistical analysis across elicitors treatments (T), water status (W) and seasons (S) factors and their interactions (T × W, T x S, W x S, T x W x S) were performed without Ap-2019 data. A discriminant analysis with the amino acids content in the samples was also performed with the SPSS statistical software.

3. Results and discussion

3.1. Effect of the foliar treatments on enological parameters and nitrogen fractions

Neither the weight of 100 berries nor the most of the other enological parameters determined in musts showed differences in either of the two years studied (Table 1). Only in 2020, grapes from vines under the RDI water regime and treated with the Ap-MeJ treatment showed higher total acidity and malic acid content than those from the control treatment. Besides, the nitrogen fractions of the must (amino nitrogen (N), ammonium N and YAN) were not affected by treatments under the rainfed or RDI water regime in any of the two years (Table 1). Thus, the multifactorial analysis indicates that there was hardly any interaction between the three factors studied (treatments, water status and vintage) for these parameters; only the malic acid content was affected by the interaction between the applications of the elicitor and the water status regime (Table 2). pH values were modified by the foliar applications, decreasing significantly in the vines treated with Ap-MeJ respect to the control ones. The vines that were irrigated (RDI) produced berries with higher weight and malic acid content than the rainfed, although the latter water regime increased the content of ammonium N and YAN presented in musts. However, in all cases, the YAN content was lower than 140 mg N/L, the threshold proposed by Bell and Henschke (2005) as the minimum amount of N required for a successful fermentation. This could represent more problems in the fermentation kinetics and aroma production of the wines from the irrigated treatment (that are further away from the aforementioned threshold value) than those from vines under the rainfed regime. On the other hand, values of weight of 100 berries, ºBrix, probable alcohol, pH, total acidity, tartaric acid, Glu-and Fru and their sum (Glu+Fru) were influenced by season factor, being all of them higher in 2020 than in 2019 (Table 2). Although both vintages had the same average temperature (20.1ºC) during the growing period of the vine (April to 10th October), the precipitation and reference evapotranspiration (ETo) during this period were higher in 2019 (372 mm and 916 mm, respectively) than in 2020 (166 mm and 882 mm, respectively), which could make the differences between the water regimes more decisive in this second vintage than in 2019.

This is a pioneering study of the effect of foliar application of MeJ

Table 1
Monastrell enological parameters and nitrogen fractions from control grapevines and from grapevines treated with nanoparticles (Ap) and nanoparticles doped with MeJ (Ap-MeJ), under non irrigated (rainfed) and regulated deficit irrigation (RDI) conditions, in 2019 and 2020 seasons.

	2019						2020			
	Rainfed			RDI			Rainfed		RDI	
	Control	Ap	Ap-MeJ	Control	Ap	Ap-MeJ	Control	Ap-MeJ	Control	Ap-MeJ
Weight of 100 berries (g)	149.4 ± 15.9a	144.1 ± 8.3a	153.9 ± 23.4a	185.1 ± 18.6a	166.3 ± 7.8a	171.2 ± 6.9a	173.2 ± 17.0a	166.2 ± 24.7a	195.4 ± 11.3a	192.7 ± 29.6a
ºBrix	22.1 ± 1.1a	20.9 ± 1.9a	21.2 ± 2.4a	20.3 ± 2.5a	20.3 ± 1.3a	19.2 ± 1.2a	23.4 ± 1.5a	22.6 ± 1.2a	22.8 ± 1.3a	22.2 ± 0.5a
Probable alcohol (%, v/v)	12.9 ± 0.8a	12.0 ± 1.3a	12.2 ± 1.6a	11.6 ± 1.7a	11.4 ± 1.1a	10.9 ± 0.8a	13.7 ± 1.0a	13.1 ± 0.8a	13.3 ± 0.9a	12.9 ± 0.3a
pH	3.7 ± 0.1a	3.5 ± 0.09a	3.5 ± 0.06a	3.5 ± 0.1a	3.5 ± 0.06a	3.5 ± 0.06a	3.7 ± 0.05a	3.7 ± 0.07a	3.7 ± 0.1a	3.6 ± 0.07a
Total acidity* (g/L)	5.5 ± 0.3a	5.6 ± 0.5a	5.5 ± 0.3a	5.4 ± 0.7a	5.6 ± 0.6a	5.6 ± 0.3a	4.6 ± 0.5a	4.4 ± 0.7a	4.5 ± 0.2a	4.8 ± 0.1b
Tartaric acid (g/L)	2.7 ± 0.2a	3.1 ± 0.5a	3.4 ± 0.4a	2.7 ± 0.7a	3.0 ± 0.3a	3.2 ± 0.2a	5.7 ± 0.6a	6.5 ± 0.7a	5.5 ± 0.04a	5.7 ± 0.2a
Malic acid (g/L)	1.6 ± 0.3a	1.5 ± 0.3a	1.3 ± 0.2a	1.6 ± 0.09a	2.1 ± 0.2a	2.0 ± 0.3a	1.3 ± 0.1a	1.5 ± 0.1a	1.9 ± 0.07a	2.4 ± 0.2b
Glu (g/L)	103.3 ± 6.9a	95.1 ± 12.9a	95.2 ± 16.1a	99 ± 15.3a	97.1 ± 8.7a	91.6 ± 7.8a	114.3 ± 9.0a	112.6 ± 6.2a	115.9 ± 7.5a	113.3 ± 3.3a
Fru (g/L)	121.6 ± 8.5a	113.2 ± 12.3a	111.9 ± 12.3a	112.6 ± 15.4a	110.8 ± 11.1a	103.7 ± 5.3a	119.9 ± 9.1a	121.9 ± 17.1a	121.0 ± 7.3a	119.4 ± 2.5a
Glu+Fru (g/L)	224.9 ± 15.1a	208.3 ± 25.1a	202.8 ± 28.1a	211.6 ± 30a	208 ± 19.6a	195.3 ± 13.2a	234.2 ± 18a	234.5 ± 21a	237 ± 14.6a	232.6 ± 5.0a
Amino N (mg N/L)	68.6 ± 11.4a	84.8 ± 13.9a	64.5 ± 8.8a	69.5 ± 13.7a	73 ± 12.7a	68.6 ± 12.7a	73.3 ± 5.1a	71.7 ± 9.2a	68.6 ± 19a	62.3 ± 6.4a
Ammonium N (mg N/L)	33.5 ± 6.6a	41 ± 5.7a	41.9 ± 8.9a	32.5 ± 5.8a	32 ± 4.8a	27 ± 9.3a	41.9 ± 7.2a	42.9 ± 9.8a	32.8 ± 5.6a	27.8 ± 8.7a
YAN (mg N/L)	109.8 ± 21.4a	119.5 ± 21.7a	114.5 ± 19.2a	103 ± 19.5a	103.3 ± 14.7a	96.3 ± 26.5a	116 ± 5.3a	111.5 ± 19.8a	91.3 ± 27a	87.5 ± 14.4a

All the parameters are given with their standard deviation (n = 4).
*As g/L of tartaric acid; Glu: glucose; Fru: fructose; YAN: yeast assimilable nitrogen. For each parameter, water status regime and season, different letters indicate significant differences between treatments ($p \leq 0.05$).

Table 2
Multifactorial analysis (mean values ± standard deviation) of the enological parameters and nitrogen fractions across elicitors treatments (T), water status (W) and seasons (S) factors and their interactions (T × W, T x S, W x S, T x W x S).

	Treatments (T)		Water status (W)		Season (S)		Multifactorial analysis[1]			
	Control	Ap-MeJ	Rainfed	RDI	2019	2020	T x W	T x S	W x S	T x W x S
Weight of 100 berries (g)	175.8 ± 22.7a	171.0 ± 25.0a	160.7 ± 21.0a	186.1 ± 19.3b	164.9 ± 21.3a	181.9 ± 23.4b	ns	ns	ns	ns
ºBrix	22.2 ± 1.9a	21.3 ± 1.9a	22.3 ± 1.7a	21.1 ± 2.0a	20.7 ± 2.0a	22.7 ± 1.2b	ns	ns	ns	ns
Probable alcohol (%, v/v)	12.9 ± 1.3a	12.3 ± 1.3a	13.0 ± 1.2a	12.2 ±1.4a	11.9 ± 1.4a	13.3 ± 0.8b	ns	ns	ns	ns
pH	3.6 ± 0.1b	3.5 ± 0.1a	3.6 ± 0.1a	3.6 ± 0.1a	3.5 ± 0.1a	3.7 ± 0.1b	ns	ns	ns	ns
Total acidity* (g /L)	4.9 ± 0.6a	5.1 ± 0.6a	5.0 ± 0.7a	5.1 ± 0.6a	5.5 ± 0.4a	4.6 ± 0.4b	ns	ns	ns	ns
Tartaric acid (g/L)	4.2 ± 1.5a	4.7 ± 1.5a	4.6 ± 1.7a	4.3 ± 1.4a	3.0 ± 0.6a	5.9 ± 0.6b	ns	ns	ns	ns
Malic acid (g/L)	1.6 ± 0.2a	1.8 ± 0.5a	1.4 ± 0.2a	2.0 ± 0.3b	1.6 ± 0.4a	1.8 ± 0.4a	**	ns	ns	ns
Glu (g/L)	108.1 ± 11.8a	103.2 ± 13.3a	106.3 ± 12.2a	104.9 ± 13.4a	97.3 ± 11.9a	114.0 ± 6.2b	ns	ns	ns	ns
Fru (g/L)	118.8 ± 10.2a	114.4 ± 12.2a	119.3 ± 11.6a	114.2 ± 10.7a	112.5 ± 11.8a	120.5 ± 9.4b	ns	ns	ns	ns
Glu+Fru (g/L)	226.9 ± 21.1a	217.2 ± 24a	225.5 ± 22.0a	219.1 ± 23.9a	209.0 ± 23.2a	234.6 ± 14.4b	ns	ns	ns	ns
Amino N (mg N/L)	70.3 ± 11.3a	66.8 ± 9.9a	69.9 ± 8.3a	66.8 ± 12.7a	67.8 ± 11.2a	69.0 ± 10.4a	ns	ns	ns	ns
Ammonium N (mg N/L)	35.5 ± 7.1a	35.5 ± 11.1a	39.9 ± 8.2b	30.0 ± 7.0a	34.3 ± 8.8a	36.8 ± 9.4a	ns	ns	ns	ns
YAN (mg N/L)	105.1 ± 20.4a	102.9 ± 21a	112.9 ± 16.0b	93.8 ± 20.6a	106.8 ± 20.2a	101.6 ± 21.0a	ns	ns	ns	ns

*As g/L of tartaric acid; Glu: glucose; Fru: fructose; YAN: yeast assimilable nitrogen. All the parameters are given with their standard deviation (n = 4). Different letters indicate significant differences between treatments, water status and seasons ($p \leq 0.05$). [1]Statistical significance: **$p \leq 0.01$ and ns, not significant ($p > 0.05$).

nanoparticles and water regime on must nitrogen compounds of Monastrell grapevines. The slight differences observed on enological parameters between musts from these nano-elicitors respect to the control treatments agree with the results obtained by other authors when applied different elicitors in conventional way, i.e.,Romanazzi et al. (2013) using chitosan (CHT) on Bois noir cv. grapevines, Portu et al. (2016) when applied MeJ, CHT and yeast extract (YE) on Tempranillo cv. grapevines and Garde-Cerdán et al. (2016) and Gutiérrez-Gamboa et al. (2018) who applied MeJ foliar on Tempranillo grapevines. Portu et al. (2016) found increases of tartaric acid content in grapes from MeJ treatment. Previous studies in Monastrell grapes showed that the application of MeJ had no effect on berry weight (Ruiz-García et al., 2013; Gil-Muñoz et al., 2017). However, the effect of applied MeJ to Monastrell grapes on parameters such as ºBrix, total acidity and pH, presented different responses depending on the type of clone (Ruiz--García et al., 2013) and the season (Gil Muñoz at el., 2017).

When different irrigation managements are studied, authors as Girona et al. (2009), Intrigliolo and Castel, (2010), and Pérez-Álvarez et al. (2021b), reported that deficit irrigation regime generally favours greater berry weights, as well as can be observed in this work respect to the rainfed samples (Table 2). However, others such as Niculcea et al. (2013, 2014), Romero et al. (2013), and Molero de Ávila et al. (2020) reported that the effect of water stress usually is slight, and depends on the variety, soil, and vintage. The oenological parameters of grapes can also be influenced by the response of the vineyard to variation of conditions environmental such a high temperature and irradiance (Torres et al., 2017), which are indirectly influenced by the water regime. Thus, although rainfed samples tended to have more ºBrix and tartaric acid than RDI musts, the differences were not significant, possibly conditioned by the rainfall during the cycle that minimized the water stress created in the study, since these parameters are generally strongly affected by water stress as observed Garrido et al. (2016) in their Tempranillo assay. Romero et al. (2013) studied the effect to applied RDI at 30% ETc in Monastrell grapes during three consecutive seasons and found different results between seasons in total acidity, pH and malic acid parameters. However, they observed that the content of ºBrix

and tartaric acid did not change in the different seasons.

3.2. Influence of the foliar treatments on amino acids content

Table 3 shows the musts amino acids concentration from control Monastrell grapevines and treated with Ap and Ap-MeJ under different water deficit conditions (rainfed and RDI) in 2019 and 2020 seasons.

Regarding the effect of the foliar application on the amino acids content, the response of the plants was different for each combination water deficit regime-season studied (Table 3). Thus, in 2019, where all the three foliar treatments were applied, the Ap treatment had the greatest influence on the musts, increasing the content of certain amino acids (Gly, GABA, Cys, Ile+Trp, total amino acids and total amino acids without Pro), more in the case of the RDI regime than in rainfed, compared to the control plants and those treated with Ap-MeJ (Table 3). The nanoparticle (Ap) has nitrates in its structure (Ramírez-Rodríguez et al., 2020a; Pérez-Álvarez et al., 2021a), that are a source of nitrogen for the plant. It is probable that this nitrogen is released more easily from the nanoparticle (Ap) than when MeJ covers its surface (Ap-MeJ), which could explain the more pronounced increase in the content of certain amino acids when using Ap than when applying Ap-MeJ. In 2020, the content of amino acids such as His, Ala, Met, and Ile+Trp-in rainfed samples, and Cys-and Lys-in RDI regime, increased in the Ap-MeJ treated musts with respect to the control. It is noteworthy that His, the aromatic amino acids (Trp, Phe-and Tyr) and the branched chain amino acids (Val, Ile-and Phe) are among those that increased in 2019 after applying the Ap treatment to the vines under the RDI water regime but not in those under rainfed water status (Table 3). Histidine is the precursor of histaminol and, as well as the aromatic amino acids, are considered as a suitable nitrogen sources for the *Saccharomyces cerevisiae* yeast, being consumed very quickly during the alcoholic fermentation (Martínez-Moreno et al., 2014). However, His-also is decarboxylated to histamine, the biogenic amine causing major problems related to human intoxications and allergies (Ruiz-Capillas and Herrero, 2019). The branched chain amino acids are direct precursors of higher alcohols during the alcoholic fermentation, that contribute to wine aroma, taste and appearance (Bell and Henschke, 2005; Garde-Cerdán et al., 2009; Gómez-Plaza et al., 2012). In addition, Phe-also acts as precursor of phenolic compounds and the 2-phenylethanol compound, responsible for a pleasant rose odor, contributing to wine quality (Garde-Cerdán et al., 2016; Gil-Muñoz et al., 2017).

In general, the few researchers that have evaluated the effect of MeJ applications on the Monastrell grape variety, have focused on the effect on the volatile, phenolic and aromatic composition of the grapes (Gómez-Plaza et al., 2012; Ruiz-García et al., 2013; Gil-Muñoz et al., 2017). However, there are no references that have studied the effects of MeJ application on grape nitrogen compounds in this variety. Other authors observed different results in the effect of the MeJ elicitor depending on the variety studied. Thus, Garde-Cerdán et al. (2016) observed that the application of MeJ in Tempranillo grapevines

Table 3
Individual amino acids content (mg/L) in Monastrell musts from control grapevines and from grapevines treated with nanoparticles (Ap) and nanoparticles doped with MeJ (Ap-MeJ), under non irrigated (rainfed) and regulated deficit irrigation (RDI) conditions, in 2019 and 2020 seasons.

	2019						2020			
	Rainfed			RDI			Rainfed		RDI	
Amino acids	Control	Ap	Ap-MeJ	Control	Ap	Ap-MeJ	Control	Ap-MeJ	Control	Ap-MeJ
Aspartic acid	11.6 ± 3.1a	13.4 ± 1.3a	8.6 ± 1.7a	11 ± 2.1a	13.5 ± 0.5a	12.9 ± 1.8a	8.2 ± 0.3a	7.5 ± 1.0a	9.4 ± 2.5a	7.9 ± 0.4a
Glutamic acid	30.3 ± 7.5a	39.2 ± 4.1a	30.8 ± 4.0a	32.6 ± 3.0a	33.4 ± 1.0a	30.8 ± 5.6a	27.7 ± 3.8a	24.3 ± 1.3a	22.1 ± 3.2a	27.4 ± 2.3a
Asparagine	16.3 ± 2.1a	16.3 ± 1.5a	15.4 ± 4.4a	9.5 ± 1.1a	11.5 ± 0.3a	11.1 ± 1.3a	5.5 ± 2.3a	6.1 ± 0.5a	3.5 ± 0.7a	4.2 ± 0.8a
Serine	48.9 ± 6.5a	48.0 ± 9.3a	42.0 ± 5.2a	47.8 ± 8.1a	58.6 ± 11.1a	41.7 ± 8.9a	53.7 ± 5.0a	56.3 ± 4.9a	48.8 ± 8.1a	48.2 ± 2.4a
Glutamine	53.1 ± 12.0a	82.4 ± 19.8a	65.1 ± 22.8a	84.6 ± 12.8a	116.4 ± 30.6a	81.4 ± 46.0a	59.5 ± 7.5a	49.2 ± 3.0a	60.4 ± 28.7a	42.4 ± 3.6a
Histidine	30.3 ± 6.0a	42.3 ± 6.7a	36 ± 13.1a	35.1 ± 3.5ab	41.8 ± 10.4b	20.4 ± 8.2a	17.9 ± 0.9a	20.5 ± 0.4b	17.8 ± 7.9a	14.8 ± 2.5a
Glycine	7.0 ± 0.6a	8.3 ± 0.9b	6.7 ± 0.5a	5.8 ± 1.7a	6.9 ± 1.7a	4.8 ± 0.6a	4.6 ± 0.1a	4.9 ± 0.7a	3.0 ± 0.6a	3.4 ± 0.2a
Threonine	60.1 ± 9.8a	72.0 ± 16.4a	49.1 ± 3.5a	50.0 ± 11.6a	67.1 ± 11.7a	47.9 ± 1.4a	64.3 ± 12.3a	73.5 ± 6.5a	57.7 ± 12.1a	53.7 ± 9.4a
Arginine	288.3 ± 103.3a	289.1 ± 54.7a	236.0 ± 62.1a	170.0 ± 60.9a	266.5 ± 46a	192.9 ± 24.2a	161.9 ± 45.6a	135.6 ± 3.8a	112.9 ± 88.9a	127.1 ± 9.7a
Alanine	66.0 ± 16.2a	75.2 ± 10.8a	56.7 ± 11.9a	61.4 ± 13.2a	72.8 ± 6.7a	57.4 ± 16.4a	63.2 ± 7.4a	88.9 ± 5.5b	54.5 ± 16.2a	73.9 ± 3.0a
GABA	181 ± 13.5a	167.2 ± 22.3a	164.1 ± 15.0a	171.2 ± 32.2a	234.1 ± 22.3b	174 ± 9.0a	153.6 ± 8.1a	156 ± 15.7a	150.5 ± 25.3a	175.2 ± 7.9a
Proline	55.7 ± 13.0a	46.0 ± 7.9a	44.4 ± 2.9a	55.9 ± 18.0a	53.3 ± 14.3a	38.7 ± 6.7a	82.6 ± 16.8a	63.2 ± 5.7a	61.3 ± 7.5a	55.5 ± 5.0a
Tyrosine	5.1 ± 2.1a	4.9 ± 1.4a	3.9 ± 1.0a	3.9 ± 0.2b	3.5 ± 0.4a	2.6 ± 0.3a	9.5 ± 1.0a	9.2 ± 0.2a	7.2 ± 1.7a	7.6 ± 0.4a
Valine	20.5 ± 1.8a	24.7 ± 7.6a	16.5 ± 2.2a	19.1 ± 1.9ab	24.0 ± 5.0b	15.5 ± 2.3a	19.0 ± 2.9a	22.4 ± 3.2a	15.9 ± 3.4a	14.8 ± 2.7a
Methionine	4.1 ± 2.7a	4.2 ± 1.8a	4.7 ± 1.5a	3.9 ± 1.1a	4.6 ± 0.3a	4.0 ± 1.0a	1.8 ± 0.4a	3.5 ± 0.5b	0.8 ± 0.8a	1.5 ± 0.3a
Cystine	0.8 ± 0.1a	1.1 ± 0.3a	0.7 ± 0.3a	0.5 ± 0.1b	0.9 ± 0.2c	0.2 ± 0.0a	1.5 ± 0.3a	1.4 ± 0.2a	0.8 ± 0.3a	1.3 ± 0.0b
Ile+Trp	29.9 ± 2.9a	36.2 ± 6.0a	31.0 ± 2.8a	19.5 ± 0.8a	25.3 ± 2.8b	20.5 ± 1.6a	23.5 ± 1.4a	28.7 ± 1.3b	21.5 ± 3.0a	20.9 ± 2.2a
Leucine	21.6 ± 0.2a	27.3 ± 6.9a	20.7 ± 3.0a	17.9 ± 1.8a	20.4 ± 5.3a	16.0 ± 0.8a	11.6 ± 1.1a	13.4 ± 0.8a	10.3 ± 1.9a	9.8 ± 1.9a
Phenylalanine	16.7 ± 1.2a	22.2 ± 6.7a	18.1 ± 2.0a	20.7 ± 4.1ab	23.8 ± 2.4b	14.8 ± 3.4a	6.8 ± 1.2a	6.2 ± 0.6a	7.0 ± 1.0a	9.1 ± 3.6a
Ornithine	3.1 ± 1.3a	3.1 ± 0.2a	2.8 ± 1.2a	1.7 ± 0.2a	2.2 ± 0.7a	1.7 ± 0.5a	2.6 ± 0.1a	2.9 ± 0.3a	2.2 ± 0.7a	2.3 ± 0.2a
Lysine	6.0 ± 0.4ab	6.6 ± 0.4b	5.2 ± 0.6a	4.2 ± 0.5a	5.2 ± 1.3a	4.8 ± 0.5a	4.7 ± 0.3a	5.1 ± 0.2a	3.7 ± 0.1a	4.1 ± 0.2b
Total amino acids	956.2 ± 165.1a	1029.3 ± 138.8a	858.6 ± 114.3a	826.4 ± 11.4a	1085.7 ± 134.9b	794.1 ± 71.3a	783.6 ± 64.5a	778.9 ± 24.1a	671.2 ± 168.9a	705.2 ± 27.1a
Total amino acids without proline	900.5 ± 153.6a	983.3 ± 133.2a	814.2 ± 113.5a	770.4 ± 101.8a	1032.4 ± 128.3b	755.4 ± 72.2a	701 ± 50.5a	715.7 ± 25.2a	610 ± 162.3a	649.7 ± 28.7a

All the parameters are given with their standard deviation (n = 4).
GABA: γ-aminobutyric acid, Ile+Trp: isoleucine + tryptophan.
For each parameter, water status regime and season, different letters indicate significant differences between treatments ($p \leq 0.05$).

increased the aromatic amino acids, especially phenylalanine, respect to those grapes from the control treatment. Also, Gutiérrez-Gamboa et al. (2017) found increases in Phe-and Met-content when foliarly treated grapevines with MeJ, however, the content of some amino acids decreased when the elicitor applied were chitosan and yeast extract. Gutiérrez-Gamboa et al. (2018) observed that after applying foliarly MeJ in the vineyard, some amino acids decreased in Tempranillo grapes but increased in Graciano, and the total amino acids level decreased in Garnacha but was not affected in Graciano grapes.

On the other hand, according to the multifactorial analysis of the control and Ap-MeJ data (Table 4) can be observed that the water regime to which the Monastrell plants were subjected had a greater influence on the amino acid content of the musts than did the foliar treatment applied, which only modified the proline content (higher in control than in Ap-MeJ grapes). Thus, amino acids such as Asn, Gly, Thr, Arg, Val, Cys, Ile+Trp, Orn, and Lys, as a consequence total amino acids and total amino acids without proline, increased their content in rainfed plants compared to RDI ones (Table 4). The season was the factor that most influenced on the amino acids content of the Monastrell samples with a, generally, higher content in those grapes from 2019 respect to those from 2020 (Table 4). The interactions between the studied factors had little influence on the amino acids concentration of the samples. The treatment (T) x water regime (W) interaction was significant in the case of the amino acids His-and Lys. Meanwhile, the interaction between treatment (T) and season (S) only was significant on Val-and Cys-content (Table 4). The interaction water regime x season was only significant in the case of Asn-and Ile+Trp-concentration. The content of Asp, Cys, Phe, and Lys-was influenced by the triple interaction of treatments with water regime and season (T x W x S). Thus, in general, the interactions between the three factors studied were not significant (Table 4). According to Intrigliolo and Castel (2010), these results support the need of conducting multi-year studies when analyzing the effects of irrigation practices under field conditions.

Previous works that studied the effect of water stress on the biosynthesis of amino acids in grapes reported that the individual differences in amino acid composition can be influenced by the stage of reproductive development (Niculcea et al., 2013), the type of grape variety (Niculcea et al., 2014) and season (Ju et al., 2018). Romero et al. (2013) applied three different water deficit conditions (sustained deficit irrigation, SDI, irrigated at 40% of the ETc throughout the cycle, RDI at 30% and at 20% of the ETc from budburst to fruit set) in Monastrell grapevines, and did not observe differences in the total amino acids content in grapes. Similarly, the regulated deficit irrigation (RDI) and partial root-zone irrigation (PRI) treatments proposed by Romero et al. (2019) did not affect the amino acids content of Monastrell grapes, however, the rootstock on which the variety was grafted was determinant in modifying the content of most of the amino acids in grapes. In the study of Romero et al. (2016b), the irrigation system had higher effect on grape amino acids concentration than the irrigation volume (low or high) applied to their Monastrell grapevines. Thus, PRI treatment enhanced the content of total amino acids, His, Arg, Ala, Cys-and Ile--compared to grapes from RDI system. Besides, these authors found significant effects on total and specific amino acids content between the irrigation system and water volume and the irrigation system and season interactions. Authors such as Dry et al. (2000a,b) and Chaves et al. (2007) among others, reported that the improvement in grape quality and compounds content in PRI grapes is related to the better microclimate and exposure of the clusters to light due to the great reduction in grapevine growth produced by these irrigation systems. Nevertheless, and according to those results reported by Romero et al. (2016a), the differences in yield and amino acids content between irrigation systems cannot be accounted for only by changes in bunch exposure o microclimate, and could be a reflection of intrinsic changes in grapevine physiology and berry metabolism produced by the irrigation system applied. These same authors suggested that a restrictive water treatment could increase the synthesis and accumulation of amino acids with functions - including antioxidant and osmoprotective - that can protect plants subjected to abiotic stress. However, other authors such as Intrigliolo et al. (2016) reported that severe water restriction could improve grape composition but mostly due to its dehydration.

Table 4
Multifactorial analysis (mean values ± standard deviation) of the amino acids content (mg/L) across elicitors treatments (T), water status (W) and seasons (S) factors and their interactions (T × W, T x S, W x S, T x W x S).

	Treatments (T)		Water status (W)		Season (S)		Multifactorial analysis[1]			
Amino acids	Control	Ap-MeJ	Rainfed	RDI	2019	2020	T x W	T x S	W x S	T x W x S
Aspartic acid	10.2 ± 2.5a	9.2 ± 2.5a	9.0 ± 2.3a	10.3 ± 2.5a	11.0 ± 2.5b	8.3 ± 1.5a	ns	ns	ns	*
Glutamic acid	28.3 ± 5.8a	28.5 ± 4.4a	28.4 ± 5.1a	28.5 ± 5.3a	31.1 ± 5.0b	25.6 ± 3.5a	ns	ns	ns	ns
Asparagine	8.6 ± 5.4a	9.7 ± 5.2a	11.2 ± 5.8b	6.8 ± 3.5a	13.5 ± 3.8b	4.8 ± 1.6a	ns	ns	*	ns
Serine	50.1 ± 6.4a	47.0 ± 8.3a	50.3 ± 7.5a	46.3 ± 7.3a	44.6 ± 7.2b	52.2 ± 5.9a	ns	ns	ns	ns
Glutamine	64.4 ± 19.3a	59.5 ± 27.0a	56.7 ± 13.1a	67.2 ± 29.7a	71.0 ± 26.7b	52.9 ± 15.0a	ns	ns	ns	ns
Histidine	25.3 ± 9.1a	23.9 ± 11.4a	26.9 ± 10.5a	22.0 ± 9.7a	30.9 ± 10.1b	17.8 ± 4.1a	*	ns	ns	ns
Glycine	5.1 ± 1.7a	5.0 ± 1.3a	5.7 ± 1.2b	4.4 ± 1.5a	6.0 ± 1.2b	4.1 ± 0.9a	ns	ns	ns	ns
Threonine	58.5 ± 11.4a	56.1 ± 11.9a	62.0 ± 11.8b	52.3 ± 9.1a	51.8 ± 8.4a	62.5 ± 11.9b	ns	ns	ns	ns
Arginine	184.7 ± 98.9a	177.8 ± 57.9a	213.6 ± 87b	149.4 ± 62.3a	223.7 ± 79.1b	132.7 ± 51.9a	ns	ns	ns	ns
Alanine	61.1 ± 13.3a	67.5 ± 16.8a	67.6 ± 15.9a	60.9 ± 14.4a	60.3 ± 13.6a	68.9 ± 16.3a	ns	ns	ns	ns
GABA[1]	162.3 ± 22.7a	166.8 ± 13.9a	162.5 ± 15.8a	167.0 ± 21.3a	172.1 ± 17.3b	157.7 ± 17.3a	ns	ns	ns	ns
Proline	64.4 ± 17.0b	49.2 ± 10.8a	61.4 ± 18.0a	52.5 ± 12.8a	48.2 ± 12.4a	66.6 ± 14.3b	ns	ns	ns	ns
Tyrosine	6.3 ± 2.5a	5.9 ± 2.8a	6.8 ± 2.8a	5.3 ± 2.3a	4.0 ± 1.4a	8.4 ± 1.3b	ns	ns	ns	ns
Valine	18.6 ± 2.8a	17.2 ± 3.8a	19.5 ± 3.1b	16.3 ± 2.8a	17.7 ± 2.7a	18.1 ± 4.0a	ns	*	ns	ns
Methionine	2.9 ± 2.0a	3.5 ± 1.6a	3.7 ± 1.9a	2.7 ± 1.7a	4.2 ± 1.6b	1.9 ± 1.1a	ns	ns	ns	ns
Cysteine	0.9 ± 0.4a	0.9 ± 0.5a	1.1 ± 0.4b	0.7 ± 0.4a	0.6 ± 0.3a	1.2 ± 0.3b	ns	*	ns	*
Ile+Trp	23.6 ± 4.5a	25.3 ± 5.2a	28.3 ± 3.5b	20.6 ± 1.9a	25.2 ± 5.8a	23.6 ± 3.7a	ns	ns	**	ns
Leucine	15.1 ± 4.9a	15.0 ± 4.4a	16.4 ± 4.8a	13.5 ± 3.9a	19.0 ± 2.8b	11.3 ± 1.9a	ns	ns	ns	ns
Phenylalanine	12.8 ± 6.6a	11.8 ± 5.3a	11.9 ± 5.8a	12.6 ± 6.1a	17.6 ± 3.3b	7.4 ± 2.3a	ns	ns	ns	*
Ornithine	2.4 ± 0.9a	2.5 ± 0.8a	2.9 ± 0.9b	2.0 ± 0.5a	2.4 ± 1.1a	2.5 ± 0.5a	ns	ns	ns	ns
Lysine	4.6 ± 0.9a	4.8 ± 0.5a	5.3 ± 0.6b	4.3 ± 0.5a	5.0 ± 0.8a	4.4 ± 0.6a	*	ns	ns	*
Total amino acids	809.4 ± 159.9a	784.2 ± 84.0a	881.3 ± 142.4b	816.5 ± 180.8a	925.0 ± 156.3b	734.7 ± 96.2a	ns	ns	ns	ns
Total amino acids -Proline	745.5 ± 156.8a	733.7 ± 87.9a	822.9 ± 146.0b	763.6 ± 179.7a	876.0 ± 150.8b	669.1 ± 89.2a	ns	ns	ns	ns

Abbreviations: GABA: γ-aminobutyric acid, Ile+Trp: isoleucine + tryptophan. For each parameter, treatments, water status regime and season, different lowercase letters indicate significant differences between treatments ($p \leq 0.05$). [1]Statistical significance: * $p \leq 0.05$, ** $p \leq 0.01$ and ns, not significant ($p > 0.05$).

The total amino acid content also can be modulated by interaction between the water stress and field temperature. Torres et al. (2017) demonstrated a greater accumulation of the total amino acids in grapes from Tempranillo vineyards exposed to higher temperatures (28/18 °C; day/night) and a water deficit (50% ETc) from the evaluation period to maturity. However, in this Monastrell study, the main temperature of both years (throughout total season and during the grapevine cycle from April to 10th October) was the same (15.3 ºC and 20.1 ºC, in 2019 and 2020, respectively), and the evapotranspiration (ETo) was similar in both periods (1270 and 915 mm in 2019 *vs* 1185 and 882 mm in 2020, respectively) but the rainfall profile and soil water reserve, differed; in 2019, rainfall during August and September (veraison and grape ripening stages), equaled soil water reserves between grapevines from rainfed and RDI irrigation treatments. However, in 2020 the spring rains provided all the water available during the cycle for the rainfed grapevines. It is well documented that climatic conditions and soil characteristics before and during berry development, determine plant physiology and grape composition, although these conditions can be managed to some extent by optimizing agronomic practices (Masclaux-Daubresse et al., 2010). Thus, the increase in the content of most of the amino acids, and the total amino acids with and without proline in grapes from 2019 compared to those from 2020, when only the concentrations of Thr, Pro, Tyr, and Cys-were higher than those from the 2019 grapes (Table 4), could be related to the phenological stage at which the plants require and dispose of water. Intrigliolo and Castel (2010) reported that water application after veraison is beneficial for grape maturity, although to achieve an increase in the concentration of phenolic compounds in Tempranillo wines, water stress must be applied during the preveraison period. When deficit irrigation was applied before veraison, Koundouras et al. (2009) also observed an increase of compounds, especially anthocyanins in the skin of Cabernet Sauvignon berries. Girona et al. (2009) showed that in postveraison only a moderate stress (stem water potential < -1.0 MPa) can have positive effects on grape composition. Thus, in ours Monastrell grapevines, the RDI conditions provided in 2020 was not sufficient to improve the nitrogen composition of the grapes with respect to the rainfed ones. Similarly, the elicitor effect of the treatments was not reflected in the biosynthesis of the amino acids in the grapes. Other authors such as Garde-Cerdán et al. (2016) and Pérez-Álvarez et al. (2017), also observed the strong influence of season on the amino acids content of grapevines to which MeJ and urea were foliarly applied, respectively.

3.3. Discriminant analysis

In order to classify the samples, discriminant analysis was performed using the grape amino acids concentration from control and treated grapevines with nanoparticles without (Ap) or with MeJ (Ap-MeJ), under rainfed and RDI water regime, during 2019 and 2020 seasons (Fig. 1). Function 1 explained 99% of the variance and Function 2 explained 1%, representing 100% of all variance. Both Functions were strongly correlated with aspartic acid and cysteine content. Discriminant did not present a clear separation between foliar treatments, although a separation between seasons can be observed. In addition, the 2019 samples showed greater variability due to the effect of the treatments than those from 2020. These obtained results suggest that under the experimental conditions of the present experiment the variations in climatic conditions or other viticultural factor played a more important role than the watering regimes and elicitors treatments applied.

4. Conclusions

In order to improve the efficiency and uptake the elicitor MeJ, Ap and Ap-MeJ nanoparticles have been applied to Monastrell vines, for the first time, under a rainfed and a regulated deficit irrigation (RDI) watering regime. It was observed that the season, following by the water regime, were the most determining factors and, however, the foliar applications had a barely effect on grape enological parameters and musts amino acids content. In the case of the must nitrogen fractions, only the irrigation regime modified the ammonium and YAN content, which was higher in the rainfed samples than in the RDI ones. Although

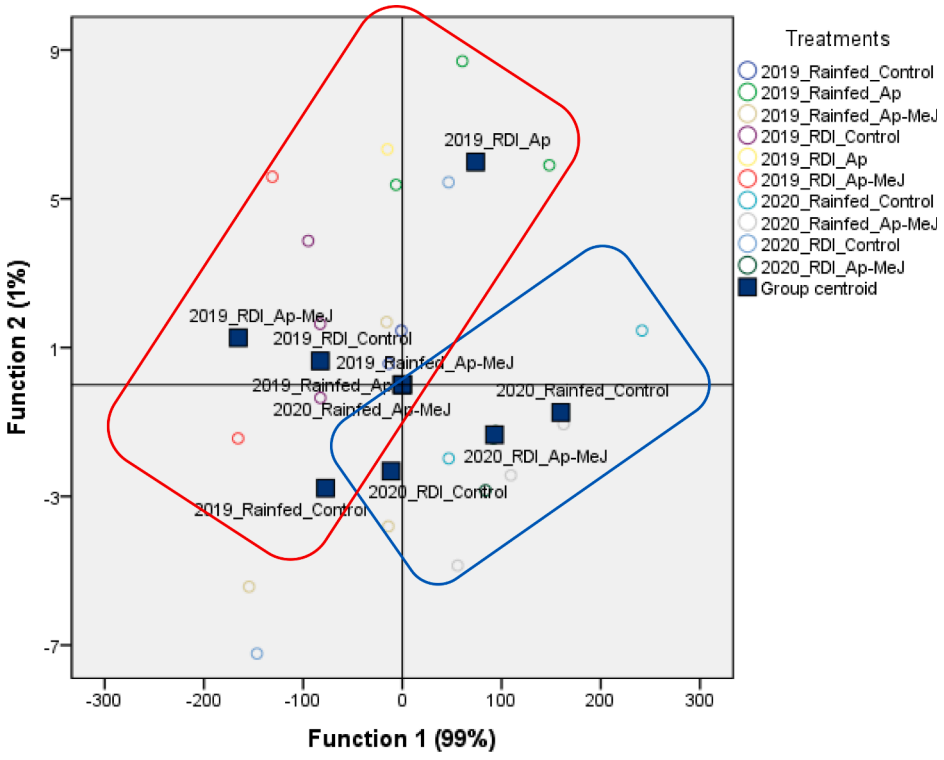

Fig. 1. Discriminant analysis performed with amino acids concentration (mg/L) in musts from control Monastrell grapevines and treated with nanoparticles (Ap) and nanoparticles doped with MeJ (Ap-MeJ), under non irrigated (rainfed) and regulated deficit irrigation (RDI) conditions, in 2019 and 2020 seasons.

the climatic conditions were similar in both seasons, in 2019, rainfall was concentrated during the period of berry ripening, equaling the water availability of plants under rainfed regime and RDI. However, it was the spring rains of 2020 that provided the water available for the entire cycle of the rainfed vines in that campaign. This difference in the water profile and the phenological moment in which the plants require and dispose of water could be one of the factors that most influenced the effect of the water regime and vintage on the enological parameters and amino acids content of the samples. In conclusion, this study could not confirm the effect of MeJ loaded in nanoparticles on the must nitrogen content, therefore more research should be carried out in order to optimize the application dose.

CRediT authorship contribution statement

E.P. Pérez-Álvarez: Conceptualization, Formal analysis, Writing – original draft. **P. Rubio-Bretón:** Investigation, Writing – review & editing. **D.S. Intrigliolo:** Funding acquisition, Conceptualization, Writing – review & editing. **B. Parra-Torrejón:** Methodology, Writing – review & editing. **G.B. Ramírez-Rodríguez:** Methodology, Writing – review & editing. **J.M. Delgado-López:** Funding acquisition, Conceptualization, Methodology, Writing – review & editing. **T. Garde-Cerdán:** Conceptualization, Supervision, Project administration, Funding acquisition, Investigation, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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