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

Leticia Martínez-Lapuente[1], Teresa Garde-Cerdán[1,*], Eva P. Pérez-Álvarez[1], Zenaida Guadalupe[1], Belén Parra-Torrejón[2] and Belén Ayestarán[1,*]

[1] Instituto de Ciencias de la Vid y del Vino (Universidad de La Rioja, CSIC, Gobierno de La Rioja), Ctra. de Burgos, Km. 6. 26007 Logroño, Spain
[2] Departamento de Química Inorgánica. Facultad de Ciencias, Universidad de Granada. Av. Fuente Nueva, s/n. 18071 Granada, Spain

## 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

*correspondence:
teresa.garde.cerdan@csic.es
belen.ayestaran@unirioja.es

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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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