



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/m² in 2019 and 217.8 L/m² 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]: °Brix, 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-amino adipic 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 \times 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) derivatized. 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 \pm 11.07a	141.81 \pm 27.18a	116.94 \pm 4.62a	199.57 \pm 7.27a	207.67 \pm 40.39a	194.90 \pm 20.65a
°Brix	24.70 \pm 0.72b	22.23 \pm 1.17a	23.37 \pm 0.49ab	22.30 \pm 0.92a	22.17 \pm 2.31a	22.37 \pm 0.38a
Probable alcohol (% v/v)	14.63 \pm 0.49b	12.92 \pm 0.80a	13.71 \pm 0.35ab	12.97 \pm 0.63a	12.89 \pm 1.58a	13.01 \pm 0.26a
pH	3.83 \pm 0.05a	3.78 \pm 0.10a	3.82 \pm 0.09a	3.76 \pm 0.01a	3.70 \pm 0.07a	3.73 \pm 0.06a
Total acidity (g/L)*	4.61 \pm 0.11a	5.20 \pm 0.36b	5.13 \pm 0.26ab	4.12 \pm 0.33a	4.54 \pm 1.08a	4.03 \pm 0.21a
Glu + Fru (g/L)	249.86 \pm 9.97b	215.50 \pm 12.29a	231.40 \pm 10.82ab	216.42 \pm 10.70a	218.62 \pm 26.56a	223.84 \pm 2.98a
Glu (g/L)	120.18 \pm 5.13b	102.88 \pm 6.89a	110.89 \pm 4.94ab	107.31 \pm 4.54a	106.08 \pm 12.84a	108.61 \pm 2.98a
Fru (g/L)	129.68 \pm 4.84b	112.62 \pm 5.43a	120.51 \pm 6.26ab	109.11 \pm 6.53a	112.54 \pm 13.76a	114.72 \pm 0.98a
Malic acid (g/L)	2.24 \pm 0.24a	2.54 \pm 0.32a	2.51 \pm 0.56a	1.21 \pm 0.08a	1.54 \pm 0.22a	1.39 \pm 0.18a
Ammonium nitrogen (mg N/L)	78.00 \pm 8.22a	106.34 \pm 15.68a	101.40 \pm 20.40a	121.16 \pm 3.52a	101.66 \pm 19.58a	114.66 \pm 6.24a
Amino nitrogen (mg N/L)	118.51 \pm 14.33a	202.11 \pm 50.59b	175.71 \pm 24.66ab	152.53 \pm 14.33a	139.63 \pm 35.64a	152.24 \pm 5.50a
YAN (mg N/L)	196.51 \pm 21.18a	308.45 \pm 64.76b	277.11 \pm 44.31ab	273.69 \pm 17.69a	241.29 \pm 55.05a	266.90 \pm 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 °Brix and probable degree, than the musts from the MeJ foliar application (Table 1). Wang et al. [19] found a reduction in °Brix, 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/m², compared to 11.5 L/m² 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.

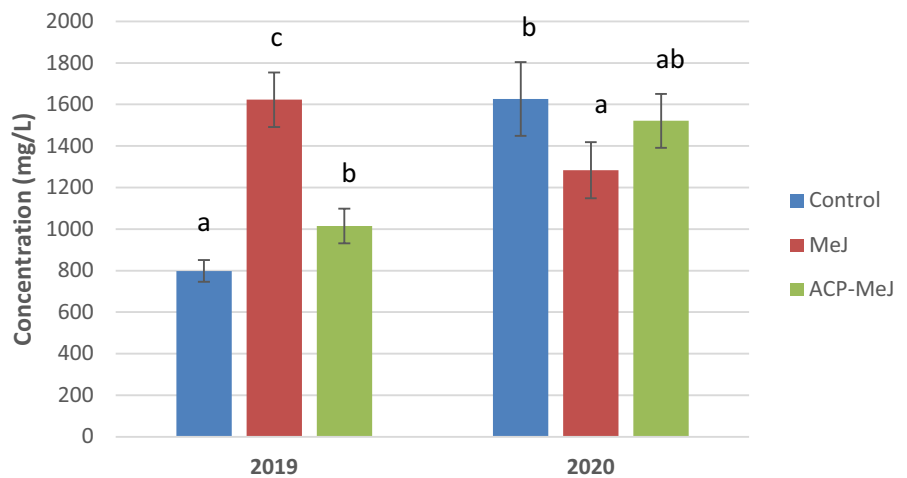
Table 2 Amino acids content (mg/L) in musts from control, methyl jasmonate (MeJ), and ACP-MeJ treatments, in 2019 and 2020 vintages

	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

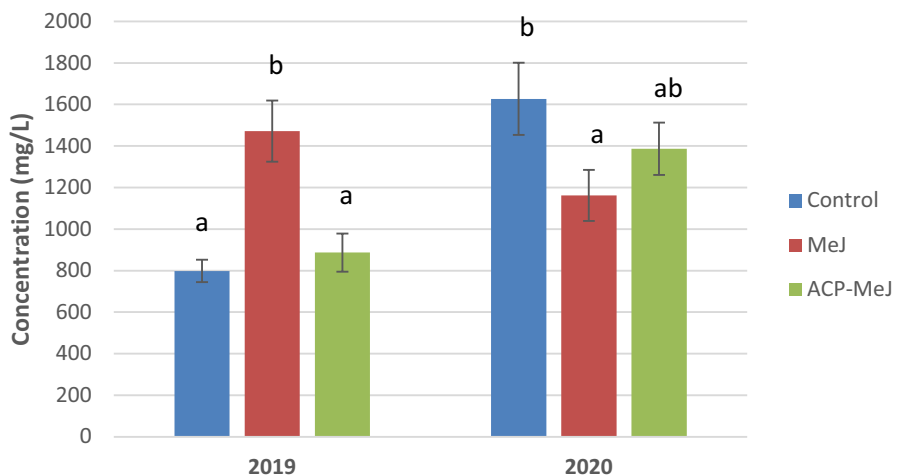
All parameters are listed with their standard deviation ($n=3$). For each vintage and compound, different letters indicate significant differences between the samples ($p \leq 0.05$)

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 ($p \leq 0.05$)

(a) Total amino acids



(b) Total amino acids without Pro



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)
<i>Treatment (T)</i>												
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
<i>Vintage (V)</i>												
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
<i>Interaction</i>												
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 elicitor able to trigger plant defense responses against abiotic stress (i.e., drought).

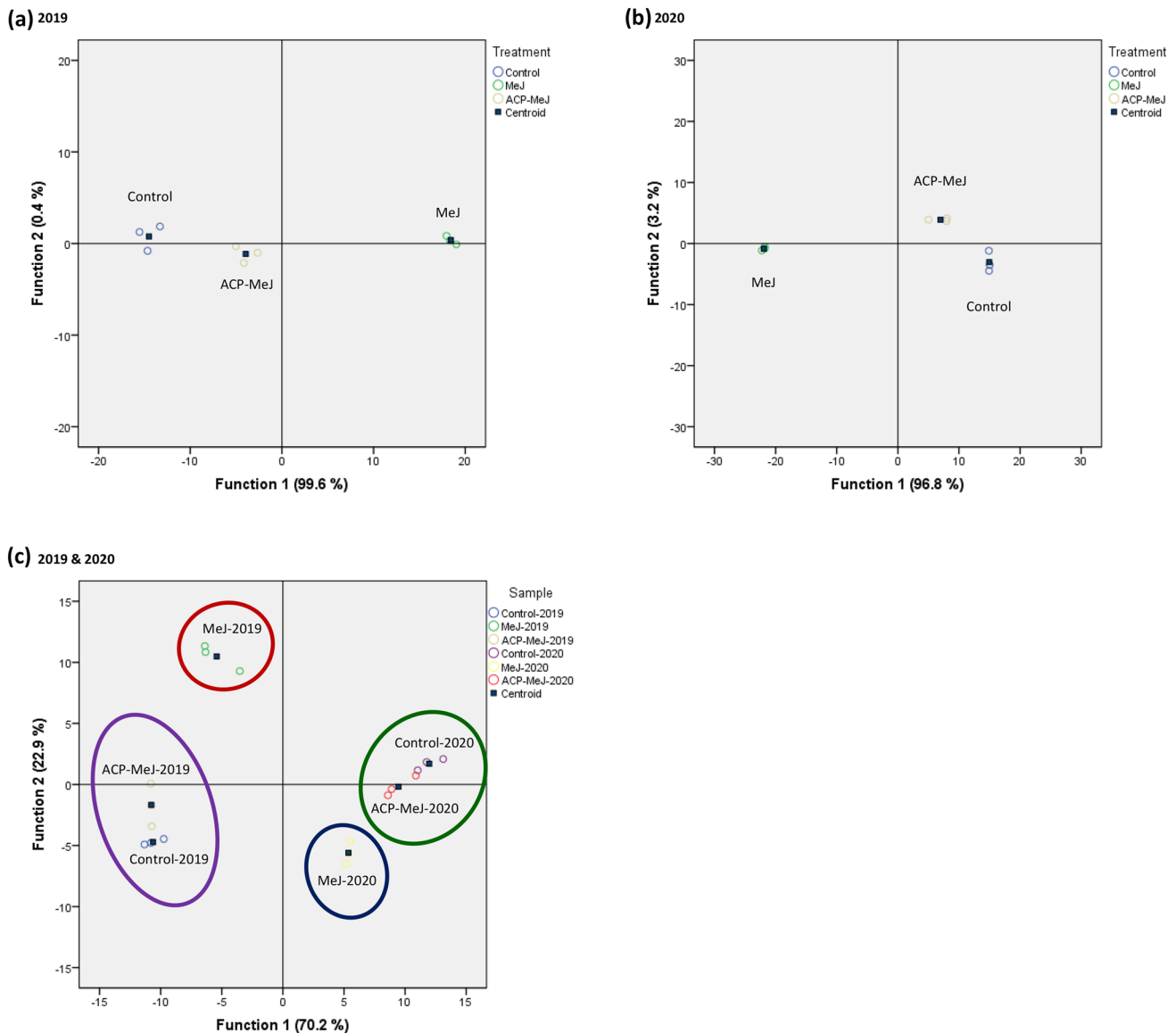


Fig. 2 Discriminant analysis of amino acids' content (mg/L) in musts from control, methyl jasmonate (MeJ), and nanoparticles doped with this elicitor (ACP-MeJ) treatments, in **a** 2019, **b** 2020, and **c** 2019 and 2020 vintages

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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 requirements This article does not contain any studies with human or animal subjects.

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