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Influence of elicitors application to Monastrell and Tempranillo vineyards on grape nitrogen composition over two vintages

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ABSTRACT

Currently, elicitors have been used to induce the defence mechanisms of vines, i.e., against both abiotic and biotic stresses. Besides, increases in phenolic synthesis in grapevines have been reported after elicitors application. However, its effects on the grape nitrogen composition are not entirely known. Thus, this work aimed to study the effect of methyl jasmonate (MeJ) and a yeast extract (YE) applied in Monastrell and Tempranillo grapevines on grape amino acids during two consecutive seasons. Amino acids in grapes were analysed by HPLC. In the first season, the total amino acid content in grapes decreased in Monastrell after MeJ and YE applications from 2236 to 1580 and 1620 mg L^{-1}, respectively, while in Tempranillo, the total amino acid concentration decreased after YE applications from 2355 to 1811 mg L^{-1}. However, during the second season, elicitors did not affect total amino acid concentration. The most important components of variability in amino acid concentration were the grape variety and the season. These results provide new knowledge of the effect of two elicitors in two important red varieties on grape amino acids content, relevant for wine quality.

KEYWORDS: LAmino acids, assimilable nitrogen, methyl jasmonate, yeast extracts, *Vitis vinifera*

INTRODUCTION

Elicitors are a specific class of molecules originating from different organisms, including carbohydrate polymers, lipids, glycopeptides, and glycoproteins, which can trigger plant defence responses contributing to plant resistance against pathogen attacks (Delaunois *et al.*, 2014). It has been shown that elicitor compounds lead to the accumulation of pathogenesis-related proteins and key enzymes of the phenylpropanoid pathway, some of which possess antimicrobial properties (Agrios, 1988; Repka, 2001). Due to this induced plant pathogenic resistance, several studies have focused on finding more sustainable alternatives for grapevine production to reduce or prevent pathogen diseases in the vineyard (Romanazzi *et al.*, 2009; Jacometti *et al.*, 2010; Romanazzi *et al.*, 2013). Currently, fungicide treatments represent more than half of pesticides applied in viticulture and their use is not without risks to human health (Delaunois *et al.*, 2014). These properties of elicitors to induce a plant's defence mechanism could circumvent the use of synthetic chemical sprays in the vineyards rendering them more sustainable (Romanazzi *et al.*, 2002; Romanazzi *et al.*, 2013; Delaunois *et al.*, 2014; Garde-Cerdán *et al.*, 2017; Gil-Muñoz *et al.*, 2020). Additionally, elicitors improved secondary metabolites synthesis and applied to the grapevines increase the concentration of phenolic compounds, mainly of anthocyanins and stilbenes in grapes and wines (Ruiz-García *et al.*, 2012; Portu *et al.*, 2016; Gil-Muñoz *et al.*, 2017) probably because these elicitor compounds may activate the phenylalanine ammonia-lyase (PAL), the enzyme which catalyses the first step in the phenolic biosynthesis.

Methyl jasmonate (MeJ) is a phytohormone that acts as a signal molecule for plant resistance to various stress types and modulates chlorophyll degradation and anthocyanin biosynthesis (Ruiz-García and Gómez-Plaza, 2013; Portu *et al.*, 2015; Portu *et al.*, 2016; Portu *et al.*, 2017). MeJ is mainly involved in plant responses to wounding and insect feeding and in resistance to pathogens (Gozzo, 2003). Besides, it has been suggested that foliar application of MeJ in the vineyard modified the amino acid concentrations of grapes (Garde-Cerdán *et al.*, 2016; Gutiérrez-Gamboa *et al.*, 2017; Gutiérrez-Gamboa *et al.*, 2018). MeJ is one of the elicitors most used in plants (Ruiz-García and Gómez-Plaza, 2013). Among the biotic elicitors, yeast extracts (YE) are also considered. As several authors have reported (Ferrari, 2010; Abraham *et al.*, 2011; Rahimi *et al.*, 2014; Cai *et al.*, 2014b; Loc *et al.*, 2014; Gutiérrez-Gamboa *et al.*, 2018; Gutiérrez-Gamboa *et al.*, 2019), the application of YE in plants allows triggering defence mechanisms, leading to an accumulation of secondary metabolites, such as phenols, sesquiterpenoids, and other compounds. However, its effect on the grape nitrogen compounds are little studied (Gutiérrez-Gamboa *et al.*, 2017; Gutiérrez-Gamboa *et al.*, 2018).

In grapes, amino acids (which represents around 25–30 % of total nitrogen) and ammonium are the main nitrogen form (Garde-Cerdán and Ancín-Azpilicueta, 2008). Must nitrogen composition plays a key role in wine quality because certain amino acids are precursors of important fermentative volatile compounds, mainly of higher alcohols and ethyl esters, which contribute to the pleasant aroma of wine (Bell and Henschke, 2005; Garde-Cerdán and Ancín-Azpilicueta, 2008). In addition, must nitrogen content can affect the dynamics and progression of alcoholic fermentation (Garde-Cerdán *et al.*, 2011). Low nitrogen content in must could be one of the causes of stuck and sluggish fermentations, which are one of the major oenological problems resulting in increased vinification time and spoilage of wine (Bisson and Butzke, 2000). A high nitrogen concentration in grapes could lead to the synthesis of biogenic amines in musts and wines, which can cause adverse health troubles to the consumers (Smit *et al.*, 2014).

There are few studies focused on the effect of these two elicitors, MeJ and YE, application on the grape nitrogen composition, and the results are variable as it has been observed that there are no effects, positive and even negative effects, depending on the season and the grape variety (Gutiérrez-Gamboa *et al.*, 2017, Gutiérrez-Gamboa *et al.*, 2018), but in none of these works, the Monastrell variety has been studied. Monastrell and Tempranillo (*Vitis vinifera* L.) are two prestigious red varieties of Spain that provide wines of recognised quality, are the most widely planted and are perfectly adapted to the two wine-growing areas of this study.

Based on the aforementioned, this research aimed to study the effect of foliar applications of two different elicitors, methyl jasmonate (MeJ) and a yeast extract (YE), on Monastrell and Tempranillo grapevines on must amino acid composition during two consecutive seasons.

MATERIALS AND METHODS

1. Study site and grapevine treatments

The field study was conducted on a commercial vineyard in Jumilla (Lat: 38°22´58´´N; Long: 1°26´30.8´´W, Murcia, Spain) for Monastrell (*Vitis vinifera* L.) grapevines, and in another plot in Alfaro (Lat: 42º 10´47´´ N; Long: 1º49´53´´ W; La Rioja, Spain) for Tempranillo (*Vitis vinifera* L.) grapevines. Information about vineyard age, clone, rootstock, vine training system, vineyard orientation, yield, among others, is presented in the manuscript reported by Gil-Muñoz *et al.* (2017). Meteorological data were recorded by two weather stations near the plots. Thus, in Jumilla, the annual precipitation (mm), mean annual temperature (ºC) and evapotranspiration (mm) recorded in the 2014 and 2015 seasons were, respectively: 133.20 and 354.20 mm, 15.9 and 15.5 ºC and 1308.9 and 1217.9 mm. In this area, 2015 was the rainiest; however, the highest rainfall occurred during September, the month in which Monastrell is harvested. In Alfaro, the annual precipitation (mm), mean annual temperature (ºC) and evapotranspiration (mm) recorded in the 2014 and 2015 seasons were, respectively: 462.5 and 301.2 mm; 14.3 and 14.1 ºC; 1159.5 and 1173.5 mm. In this area, 2014 was rainier; however, the highest rainfall accumulation occurred during the month of November (141 mm), so this precipitation increased the water reserves available for the development of vines during 2015.

The treatments applied to the grapevines of both varieties were methyl jasmonate (MeJ) (aqueous solution 10 mM + Tween 80 as wetting agent), yeast extract (YE) (1.69 g L^{-1} according to the manufacturer's instructions + Tween 80 as wetting agent) and control (aqueous solution with Tween 80) during two consecutive seasons (2014 and 2015). Plants were sprayed at the beginning of veraison and 12 days after the first application for Monastrell grapes and a week later for Tempranillo grapes. For each plot, the experimental field treatments were applied in triplicate and were arranged in a complete randomised block design.

Grapes were harvested at their optimum technological maturity, i.e., when the weight of 100 berries was constant and the probable alcohol was around 13 % (v/v), then they were destemmed and crushed. In the must obtained, oenological parameters were determined and aliquots of 100 mL of each must sample were frozen at –20 ºC until its free amino acids content could be analysed in the laboratory (approximately 3 months after harvest).

2. Must oenological parameters

Musts were physico-chemically characterised by determining probable alcohol, pH, titratable acidity and malic acid according to the OIV (2003) methodology. As treatments were performed in triplicate, the results of these parameters are the average of three analyses (n = 3).

3. Analysis of amino acids by HPLC

The amino acid analysis of the must was performed by the method described by Garde-Cerdán *et al.* (2014). Free amino acids were analysed by reverse-phase HPLC using an Agilent 1100 Series (Palo Alto, USA), equipped with an automatic liquid sampler (ALS), a fluorescence detector (FD) and a diode array detector (DAD). Each sample was centrifuged at 3320 g for 10 minutes at 20 °C and then, 5 mL of the supernatant was mixed with 100 µL of norvaline and 100 µL of sarcosine (internal standards). This mixture was filtered through a 0.45 µm OlimPeak pore filter (Teknokroma, Barcelona, Spain) and submitted to an automatic precolumn derivatisation with o-phthaldiadehyde (OPA Reagent, Agilent) and with 9-fluorenylmethylchloroformate (FMOC Reagent, Agilent). The injected amount from the derivatised sample was 10 µL. All separations were performed on a Hypersil ODS (250 × 4.0 mm, I.D. 5 µm) column (Agilent) at 40 °C.

Two eluents were used as mobile phases: eluent A: 75 mM sodium acetate, 0.018 % triethylamine (pH 6.9) and 0.3 % tetrahydrofuran; eluent B: water, methanol and acetonitrile (10:45:45, v/v/v). Identification of the compounds was performed by comparing their retention times with their pure reference standards. The pure reference compounds and internal standards were obtained from Sigma-Aldrich (Madrid, Spain). The treatments were carried out in triplicate, so the results for free amino acids are expressed as the average of three analyses (n = 3).

The amino acids analysed were aspartic acid (Asp), glutamic acid (Glu), asparagine (Asn), serine (Ser), glutamine (Gln), histidine (His), glycine (Gly), threonine (Thr), arginine (Arg), alanine (Ala), γ-aminobutyric acid (Gaba), tyrosine (Tyr), cysteine (Cys), valine (Val), methionine (Met), tryptophan (Trp), phenylalanine (Phe), isoleucine (Ile), leucine (Leu), lysine (Lys) and proline (Pro). Total amino acid content (TA) corresponded to the sum of all amino acids, while total amino acids without proline content (TA-Pro) corresponded to the sum of all amino acids excluding proline, an amino acid that is not metabolised by yeast to carry out the alcoholic fermentation.

4. Statistical analysis

The statistical analysis of amino acid concentration was performed using variance analysis (one-way ANOVA). The multivariate factorial analysis accounted for all amino acids, total amino acids and total amino acids without proline concentration using Statgraphics Centurion XVII. Differences between samples were compared using the Duncan test at a 95 % probability level. A linear discriminant analysis (LDA) was performed with all amino acids (InfoStat, www.infostat.com.ar) of each grape variety and season to identify and group the samples.

RESULTS AND DISCUSSION

Oenological parameters in Monastrell and Tempranillo musts, from control and treatments with methyl jasmonate (MeJ) and the yeast extract (YE), during the two seasons (2014 and 2015), are shown in Table 1.

As can be seen, no significant differences were obtained between the control and treated samples in the different parameters measured for either of the two varieties. However, Monastrell musts had a lower acidity and consequently a lower concentration of malic acid, as well as a higher pH than the Tempranillo musts, during the two years of study. This can be explained by the difference in location and the climatological conditions between the two study sites.

The effect of the application of methyl jasmonate (MeJ) and yeast extract (YE) to the Monastrell and Tempranillo grapevines on grape amino acids concentration during the 2014 and 2015 seasons is shown in Tables 2 and 3. It was observed that the elicitor's application to the Tempranillo grapevines had a less important effect than in Monastrell in both study seasons. During the 2014 season, in Monastrell variety, of the 21 amino acids analysed, seven of them were not affected by the elicitor treatments, while in Tempranillo, of the all amino acids analysed, thirteen of them were not affected by the elicitor applications. Thus, MeJ applications to Monastrell grapevines decreased the must concentration of Asp, Glu, Asn, Ser, Gln, Thr, Arg, Ala, Cys, Met, Phe and Ile, decreasing its total amino acids and total amino acids without Pro content, compared to control musts (Table 2). YE treatment applied to Monastrell grapevines decreased the must concentration of Glu, Asn, Ser, Thr, Arg, Tyr and Phe, negatively affecting its total amino acids and total

TABLE 1. Oenological parameters in Monastrell and Tempranillo musts from grapevines untreated (control) and treated with methyl jasmonate (MeJ) and a yeast extract (YE) during two consecutive vintages (2014 and 2015).

	Monastrell			Tempranillo		
2014	Control	MeJ	YE	Control	MeJ	YE
Probable alcohol (% v v^{-1})	14.70 ± 0.41 a	15.11 ± 0.14 a	15.03 ± 0.06 a	14.66 ± 0.28 a	14.45 ± 0.09 a	14.38 ± 0.11 a
pH	4.08 ± 0.03 a	4.06 ± 0.01 a	4.04 ± 0.01 a	3.44 ± 0.04 a	3.43 ± 0.02 a	3.48 ± 0.07 a
Titratable acidity (g L^{-1})*	3.27 ± 0.16 a	3.26 ± 0.04 a	3.42 ± 0.04 a	5.25 ± 0.07 a	5.28 ± 0.16 a	5.25 ± 0.17 a
Malic acid (g L^{-1})	1.84 ± 0.21	1.67 ± 0.06 a	1.62 ± 0.07 a	2.26 ± 0.39 a	1.93 ± 0.14 a	2.13 ± 0.16 a
2015	Control	MeJ	YE	Control	MeJ	YE
Probable alcohol (%v v^{-1})	12.61 ± 0.62 a	12.49 ± 0.98 a	12.83 ± 0.48 a	12.34 ± 1.22 a	13.19 ± 1.06 a	12.40 ± 0.92 a
pH	4.01 ± 0.01 a	4.02 ± 0.02 a	4.09 ± 0.03 a	3.46 ± 0.05 a	3.43 ± 0.06 a	3.47 ± 0.05 a
Titratable acidity (g L^{-1})*	2.95 ± 0.04 a	3.00 ± 0.17 a	2.81 ± 0.15 a	4.63 ± 0.11 a	4.78 ± 0.18 a	4.54 ± 0.45 a
Malic acid (g L^{-1})	1.19 ± 0.01 a	1.20 ± 0.12 a	1.38 ± 0.14 a	1.33 ± 0.25 a	1.29 ± 0.17 a	1.15 ± 0.22 a

All the parameters are given with their standard deviation (n = 3). For each year, grape variety and parameter, different letters in the same row indicate significant differences between treatments ($p \leq 0.05$). * As g L-1 of tartaric acid.

TABLE 2. Amino acids concentration (mg L-1) in musts from untreated (control) and treated Monastrell and Tempranillo grapevines with methyl jasmonate (MeJ) and a yeast extract (YE) during the 2014 vintage.

	Monastrell			Tempranillo		
	Control	MeJ	YE	Control	MeJ	YE
Asp	8.87 ± 1.91 b	4.65 ± 0.91 a	7.67 ± 0.63 b	22.63 ± 2.65 a	19.54 ± 0.96 a	18.80 ± 2.05 a
Glu	24.11 ± 3.41 b	16.11 ± 2.65 a	18.30 ± 0.91 a	162.50 ± 16.29 b	171.21 ± 9.95 b	136.12 ± 11.88 a
Asn	9.61 ± 1.35 b	4.27 ± 0.60 a	6.17 ± 1.53 a	5.50 ± 0.61 a	5.37 ± 0.81 a	4.91 ± 1.82 a
Ser	52.76 ± 9.18 b	34.69 ± 2.35 a	37.02 ± 6.46 a	53.01 ± 0.89 b	53.62 ± 3.65 b	43.82 ± 2.93 a
Gln	93.79 ± 22.73 b	44.73 ± 6.32 a	65.48 ± 12.92 ab	262.57 ± 10.15 b	241.53 ± 19.48 b	200.26 ± 17.03 a
His	42.55 ± 10.14 ab	30.37 ± 2.83 a	44.66 ± 2.86 b	40.92 ± 3.21 a	39.89 ± 1.92 a	33.11 ± 6.82 a
Gly	8.98 ± 1.58 a	6.84 ± 0.63 a	8.65 ± 2.83 a	7.17 ± 0.48 a	8.31 ± 0.36 a	5.86 ± 2.09 a
Thr	44.13 ± 7.29 b	33.25 ± 2.23 a	33.92 ± 4.45 a	45.54 ± 3.27 a	44.18 ± 2.20 a	39.51 ± 4.02 a
Arg	472.88 ± 108.47 b	229.34 ± 19.32 a	281.36 ± 89.13 a	705.21 ± 54.10 b	601.50 ± 50.69 ab	501.65 ± 80.50 a
Ala	109.86 ± 18.45 b	70.43 ± 3.99 a	69.79 ± 11.62 a	93.62 ± 2.27 b	104.26 ± 7.88 b	75.61 ± 6.20 a
Gaba	273.53 ± 41.54 a	223.99 ± 26.27 a	224.16 ± 25.39 a	161.77 ± 17.43 a	150.68 ± 22.41 a	120.14 ± 22.31 a
Tyr	6.43 ± 0.71 b	5.54 ± 0.66 ab	5.01 ± 0.51 a	11.83 ± 1.14 a	12.12 ± 0.94 a	10.91 ± 2.24 a
Cys	10.90 ± 1.93 b	8.32 ± 0.65 a	9.09 ± 0.57 ab	8.73 ± 0.42 a	10.07 ± 1.54 a	7.56 ± 1.53 a
Val	21.90 ± 2.50 a	17.91 ± 1.52 a	20.08 ± 2.91 a	14.81 ± 1.02 a	15.66 ± 0.81 a	14.75 ± 2.61 a
Met	2.18 ± 0.32 b	1.15 ± 0.08 a	1.54 ± 0.58 ab	8.95 ± 0.74 a	12.13 ± 1.35 b	8.69 ± 1.81 a
Trp	32.42 ± 6.20 a	23.73 ± 1.03 a	24.70 ± 4.52 a	23.38 ± 4.98 a	24.19 ± 3.31 a	22.99 ± 6.69 a
Phe	12.53 ± 1.45 b	8.72 ± 0.87 a	9.98 ± 0.84 a	7.96 ± 0.54 a	9.20 ± 0.28 b	8.51 ± 0.58 ab
Ile	8.85 ± 1.15 b	6.42 ± 0.62 a	7.81 ± 1.37 ab	4.59 ± 0.93 a	4.95 ± 0.45 a	5.03 ± 1.60 a
Leu	21.44 ± 3.06 a	17.35 ± 1.74 a	17.85 ± 2.39 a	12.02 ± 1.44 a	12.19 ± 1.10 a	11.47 ± 2.68 a
Lys	5.06 ± 0.89 a	4.01 ± 0.26 a	4.08 ± 0.72 a	2.87 ± 0.58 a	2.64 ± 0.62 a	2.57 ± 1.07 a
Pro	972.73 ± 182.87 a	788.50 ± 62.35 a	723.03 ± 89.68 a	699.69 ± 68.73 ab	793.37 ± 132.16 b	538.22 ± 79.82 a
TA	2235.50 ± 410.31 b	1580.32 ± 108.06 a	1620.35 ± 20.20 a	2355.29 ± 53.81 b	2336.61 ± 238.85 b	1810.51 ± 244.94 a
TA-Pro	1262.77 ± 233.15 b	791.82 ± 45.96 a	897.32 ± 104.48 a	1655.61 ± 81.36 b	1543.23 ± 107.00 b	1272.29 ± 166.59 a

All the parameters are given with their standard deviation (n = 3). For each parameter and grape variety, different letters in the same row indicate significant differences between treatments ($p \leq 0.05$). TA: Total amino acids concentration. TA-Pro: Total amino acids concentration without proline.

TABLE 3. Amino acids concentration (mg L-1) in musts from untreated (control) and treated Monastrell and Tempranillo grapevines with methyl jasmonate (MeJ) and a yeast extract (YE) during the 2015 vintage.

	Monastrell			Tempranillo		
	Control	MeJ	YE	Control	MeJ	YE
Asp	9.79 ± 1.77 b	6.32 ± 0.84 a	6.86 ± 0.99 a	22.71 ± 0.69 a	19.43 ± 1.14 a	21.79 ± 3.10 a
Glu	18.95 ± 4.86 a	22.25 ± 2.72 ab	26.32 ± 1.94 b	105.20 ± 4.02 a	93.38 ± 5.58 a	98.06 ± 11.15 a
Asn	17.34 ± 3.72 a	12.49 ± 2.41 a	12.41 ± 1.59 a	7.94 ± 1.59 a	6.81 ± 1.11 a	7.77 ± 0.45 a
Ser	39.73 ± 5.90 a	31.28 ± 7.91 a	39.61 ± 3.11 a	41.79 ± 1.92 a	40.02 ± 4.42 a	42.67 ± 6.82 a
Gln	83.45 ± 15.76 b	59.74 ± 11.44 a	74.06 ± 2.50 ab	172.17 ± 7.71 a	155.63 ± 15.57 a	179.22 ± 11.19 a
His	37.47 ± 8.82 a	31.23 ± 6.19 a	31.39 ± 4.51 a	22.17 ± 0.32 a	42.48 ± 11.52 b	64.14 ± 9.90 c
Gly	9.63 ± 0.74 a	10.34 ± 2.31 a	9.95 ± 1.76 a	5.54 ± 0.58 a	5.86 ± 0.71 ab	7.27 ± 1.09 b
Thr	27.67 ± 3.16 a	22.80 ± 6.49 a	25.20 ± 4.06 a	25.07 ± 3.62 a	22.17 ± 1.01 a	27.35 ± 3.19 a
Arg	345.92 ± 108.57 a	269.03 ± 79.99 a	285.93 ± 57.24 a	408.04 ± 120.11 a	318.18 ± 54.30 a	426.27 ± 67.14 a
Ala	44.56 ± 7.20 a	30.66 ± 6.82 a	38.07 ± 7.08 a	77.39 ± 10.58 a	68.40 ± 6.73 a	80.14 ± 7.35 a
Gaba	197.30 ± 13.42 a	174.32 ± 39.25 a	185.23 ± 16.17 a	80.05 ± 15.40 a	64.41 ± 6.33 a	92.90 ± 17.11 a
Tyr	21.42 ± 1.68 a	17.31 ± 1.53 a	18.83 ± 4.35 a	11.87 ± 2.69 a	11.42 ± 1.33 a	14.95 ± 0.69 a
Cys	6.91 ± 1.54 a	11.45 ± 1.04 b	13.20 ± 1.10 b	7.05 ± 1.78 a	7.10 ± 1.40 a	9.11 ± 0.47 a
Val	18.75 ± 2.62 a	18.45 ± 2.65 a	21.51 ± 1.85 a	12.44 ± 0.76 a	12.23 ± 1.05 a	13.26 ± 1.15 a
Met	1.47 ± 0.32 a	1.58 ± 0.29 a	2.99 ± 0.53 b	7.15 ± 0.54 a	6.11 ± 0.85 a	6.04 ± 0.58 a
Trp	22.35 ± 1.05 a	26.50 ± 1.49 b	28.09 ± 1.50 b	18.66 ± 16.12 a	27.05 ± 1.31 a	31.11 ± 2.44 a
Phe	6.28 ± 0.53 a	6.11 ± 0.91 a	7.55 ± 0.28 b	7.55 ± 0.54 a	7.56 ± 0.99 a	7.86 ± 1.14 a
Ile	7.52 ± 0.89 a	7.13 ± 0.74 a	9.04 ± 1.37 a	3.22 ± 0.45 a	3.10 ± 0.04 a	3.28 ± 0.25 a
Leu	16.78 ± 3.42 a	16.31 ± 3.63 a	21.28 ± 0.99 a	8.42 ± 1.51 a	8.00 ± 0.47 a	8.77 ± 1.10 a
Lys	1.27 ± 0.29 a	1.58 ± 0.40 a	1.64 ± 0.33 a	2.69 ± 0.16 a	2.57 ± 0.20 a	2.70 ± 0.21 a
Pro	470.10 ± 60.54 a	395.12 ± 70.65 a	512.74 ± 166.52 a	426.48 ± 53.82 a	472.33 ± 87.92 a	451.37 ± 41.48 a
TA	1404.65 ± 147.96 a	1172.01 ± 191.16 a	1371.91 ± 102.81 a	1473.60 ± 124.28 a	1394.26 ± 110.41 a	1596.00 ± 117.69 a
TA-Pro	934.55 ± 162.46 a	776.89 ± 164.97 a	859.16 ± 92.63 a	1047.12 ± 164.12 a	921.92 ± 85.52 a	1144.64 ± 102.23 a

All the parameters are given with their standard deviation (n = 3). For each parameter and grape variety, different letters in the same row indicate significant differences between treatments ($p \leq 0.05$). TA: Total amino acids concentration. TA-Pro: Total amino acids concentration without proline.

amino acids without Pro content, compared to control musts (Table 2). In addition, the musts from grapes treated with YE showed higher content of His than MeJ samples.

In the 2014 season, MeJ applications to Tempranillo grapevines increased Met, and Phe concentrations in must, without affecting its total amino acids and total amino acids without Pro content, compared to control samples (Table 2). YE treatment applied to Tempranillo grapevines decreased Glu, Ser, Gln, Arg, Ala, Met and Pro concentrations in must negatively affecting its total amino acids and total amino acids without Pro content, compared to control samples (Table 2). In addition, the musts from grapes treated with YE showed lower content of Glu, Ser, Gln, Ala, Met, Pro, total amino acids and total amino acids without proline than MeJ samples.

During the 2015 wine-growing season, in Monastrell variety, of the 21 amino acids analysed, seven were affected by the elicitor treatments, while in Tempranillo, of the all amino acids analysed, two of them were affected by the elicitor applications. Thus, MeJ applications to Monastrell grapevines decreased the must concentration of Asp and Gln while increasing Cys and Trp content without affecting its total amino acids and total amino acids without Pro content, compared to control musts (Table 3). YE treatment applied to Monastrell grapevines decreased the must concentration of Asp while increasing the content of Glu, Cys, Met, Trp, and Phe in musts, without affecting its total amino acids and total amino acids without Pro content, compared to control musts (Table 3). In addition, the musts from grapes treated with YE showed higher content of Met and Phe than MeJ samples.

In the 2014 season, MeJ applications to Tempranillo grapevines increased only His concentration in must without affecting its total amino acid content and total amino acids without Pro content, compared to control samples (Table 3). Compared to the control samples, YE treatment applied to Tempranillo grapevines increased His and Gly concentrations in must without affecting its total amino acid content and total amino acids without Pro content (Table 3). Thus, the musts from grapes treated with YE showed lower content of Gly than MeJ samples.

With respect to the effect of elicitor application to the grapevines, Garde-Cerdán *et al.* (2016) showed that MeJ application to the Tempranillo vineyard increased the content of His, Ser, Trp, Phe, Tyr, Asn, Met and Lys. However, this application did not affect the content of total amino acids. In another study, Gutiérrez-Gamboa *et al.* (2017) reported that in Tempranillo grapevines, the application of certain elicitors such as chitosan and the same yeast extract used in our study decreased the concentration of several amino acids together with a reduction of the content of total amino acids. However, they observed that the application of methyl jasmonate to the grapevines slightly affected the must amino acid composition.

On the other hand, in a recent study, Gil-Muñoz *et al.* (2021) showed how MeJ increased the total amino acid content in Monastrell musts although these differences were more noticeable in 2019 compared to 2020; however, Pérez-Álvarez *et al.* (2022), in the same grape variety and seasons, observed that foliar treatments with MeJ supported in nanoparticles had little impact on grape amino acids concentration. Different studies reported that methyl jasmonate, the methyl ester of jasmonic acid (JA), may activate the phenylalanine ammonia-lyase (PAL), which is the key enzyme that catalyses the first step in the phenolic biosynthesis (Belhadj *et al.*, 2006), increasing phenolic composition in grapevines (Portu *et al.*, 2016). Moreover, MeJ induces changes in amino acid concentrations and profiles in plants, e.g., tea (Shi *et al.*, 2014; Shi *et al.*, 2015) and grapes (Garde-Cerdán *et al.*, 2016). Amino acids not only affect the taste but also serve as precursors for some volatile compounds such as higher alcohols and esters (Garde-Cerdán and Ancín-Azpilicueta, 2008). Thereby, nitrogen availability in plant tissues can be affected by enzymatic activity resulting in an important accumulation of secondary metabolites (phenolic and volatile compounds) associated with resource allocation or physiological costs. In this way, it has been shown that nitrogen deficiency increased volicitin-induced volatile emission, jasmonic acid accumulation, and ethylene sensitivity in *Zea mays* seedlings (Schmelz *et al.*, 2003). In addition, in *Lolium perenne,* the concentration of nitrate and several of the studied amino acids decreased after the infection with endophytes (Rasmussen *et al.*, 2008). Redman *et al.* (2001) exposed that there were physiological costs by the application of jasmonic

TABLE 4. Percentage of variance attributable to treatment, season, variety and the interaction of each of them (treatment × season), (treatment × variety), (season × variety) and (treatment × season × variety) of each amino acid compound, total amino acids (TA) and total amino acids without proline (TA-Pro).

	Treatment (T) (%)	Season (S) (%)	Variety (V) (%)	T x S (%)	T x V (%)	S x V (%)	T x S x V (%)	Residual (%)
Asp	4.22***	0.31 NS	90.86***	0.03 NS	0.08 NS	0.02 NS	0.75 NS	3.71
Glu	0.34*	5.55***	84.44***	0.59**	0.53*	6.83***	0.51*	1.20
Asn	9.53**	36.20***	24.91***	0.07 NS	6.03**	10.36***	0.75 NS	12.14
Ser	14.17**	16.48***	16.49***	10.96*	10.57*	1.53 NS	2.25 NS	27.56
Gln	2.76***	4.42***	81.44***	1.58*	0.56 NS	5.79***	1.05*	2.41
His	9.44*	0.04 NS	3.34 NS	14.26*	15.30*	5.57*	28.79***	23.28
Gly	0.05 NS	1.34 NS	35.39***	3.49 NS	2.50 NS	11.27*	9.54 NS	36.42
Thr	5.76*	68.78***	2.40 NS	3.47 NS	1.84 NS	3.05*	1.04 NS	13.66
Arg	13.56**	16.05***	34.09***	5.51*	0.71 NS	9.67**	2.66 NS	17.75
Ala	7.51***	38.69***	21.08***	6.07**	5.19**	9.06***	3.60*	8.69
Gaba	3.04*	21.79***	62.24***	2.32 NS	0.56 NS	0.16 NS	0.60 NS	9.30
Tyr	1.06 NS	49.93***	0.05 NS	1.80 NS	1.64 NS	35.80***	1.04 NS	8.68
Cys	6.61*	0.00 NS	15.79***	28.91***	3.32 NS	6.02*	16.07**	23.27
Val	2.34 NS	3.77*	65.77***	2.35 NS	2.65 NS	1.96 NS	1.96 NS	19.21
Met	0.25 NS	4.63***	78.22***	1.62**	2.29**	7.26***	2.33**	3.40
Trp	2.91 NS	0.10 NS	2.07 NS	21.74*	7.34 NS	1.96 NS	0.94 NS	62.93
Phe	2.85 NS	42.97***	1.38 NS	4.39*	9.15**	16.17***	8.95**	14.12
Ile	2.86 NS	2.68*	72.12***	1.08 NS	2.63 NS	4.41**	2.14 NS	12.08
Leu	1.54 NS	4.64**	71.48***	3.57 NS	1.40 NS	1.92 NS	2.23 NS	13.22
Lys	1.06 NS	40.08***	1.34 NS	2.58 NS	0.13 NS	37.96***	1.39 NS	15.46
Pro	3.33 NS	58.21***	4.21*	7.42*	4.95 NS	3.29*	0.70 NS	17.88
TA	8.61**	50.37***	10.13***	10.05**	4.02*	1.23 NS	2.28 NS	13.31
TA-Pro	10.11**	23.55***	33.13***	7.06**	1.81 NS	7.42**	3.19 NS	13.72

Statistically significant at $*p \le 0.05$, $**p \le 0.01$ and $***p \le 0.001$, respectively. NS: not significant.

acid (JA) in tomato plants (*Solanum lycopersicum* L.) related to a negatively impacts associated with productive factors such as fruit number, fruit weight, number of seeds per fruits, among others. Moreover, it has been reported that the resistant induction through MeJ stimulated the export of resources out of affected tissues and increased allocation to roots in *S. lycopersicum* plants (Gómez *et al.*, 2010). Therefore, it is probable that the decrease in the content of several amino acids in Monastrell and Tempranillo grapevines may be related to some physiological costs or export of nitrogen resources to other tissues after inducing resistance through the use of these aforementioned elicitors.

Table 4 shows the percentage of variance attributable to treatment, season, variety and the interaction of each of them of the individually amino acid, total amino acids concentration, and total amino acids without Pro content. The variety and the season individually were the most dominant factor of variation in amino acid content, followed by interaction between season × variety. Thus, the season was the most dominant factor for the content of Asn, Thr, Ala, Tyr, Phe, Lys, Pro and total amino acids in the musts through elicitor applications. In both grape varieties, the amino acid content in the musts was higher in 2014 than in 2015, which could be mainly due to differences in rainfall, as observed by Garde-Cerdán *et al.* (2009). The variety was the most dominant factor for the concentration of Asp, Glu, Ser, Gln, Gly, Arg, Gaba, Val, Met, Ile, Leu and total amino acids without Pro. This result is quite consistent, since the amino acids content is a function of the grape variety (Garde-Cerdán *et al.*, 2009; Gutiérrez-Gamboa *et al.*, 2018).

The interaction treatment x season was the most dominant factor for the Cys concentration. The interaction among treatment, season and variety was the most dominant factor for the content of His (Table 4).

A linear discriminant analysis (LDA) was performed to identify and evaluate the amino acid concentration in the musts and that could differentiate the treatments, methyl jasmonate (MeJ) and yeast extract (YE), according to the season (2014 and 2015) and variety, Monastrell (Mon) and Tempranillo (Tem). According to all amino acids found in Mon and Tem in the 2014 and 2015 seasons, the initial LDA was worked out. Figure 1 shows the graphical representation of the treatments projections for each group on a plane defined by two main canonical axes (Functions 1 and 2). It shows a wide distance between C-2014-Mon (1), MeJ-2014-Mon (2) and YE-2014-Mon (3), with respect to C-2014-Tem (4), MeJ-2014-Tem (5) and YE-2014-Tem (6) samples. Moreover, Figure 1 shows a close distance between C-2015-Mon (7), MeJ-2015-Mon (8) and YE-2015-Mon (9), with respect to C-2015-Tem (10), MeJ-2015-Tem (11) and YE-2015-Tem (12) samples. Function 1, which accounted for the highest weight of the variance, could separate Monastrell samples with respect to the Tempranillo samples in the 2014 season. The classification matrix of the model indicated a correct global classification of 94.50 %, the eigenvalues of 2001.61 and 206.05, canonical correlation of 1.000 and 0.998, respectively. Function 1 explained the 85.70 % of the total variance and was strongly positively correlated with Ala, Leu and Lys and was negatively correlated with Gln and Arg.

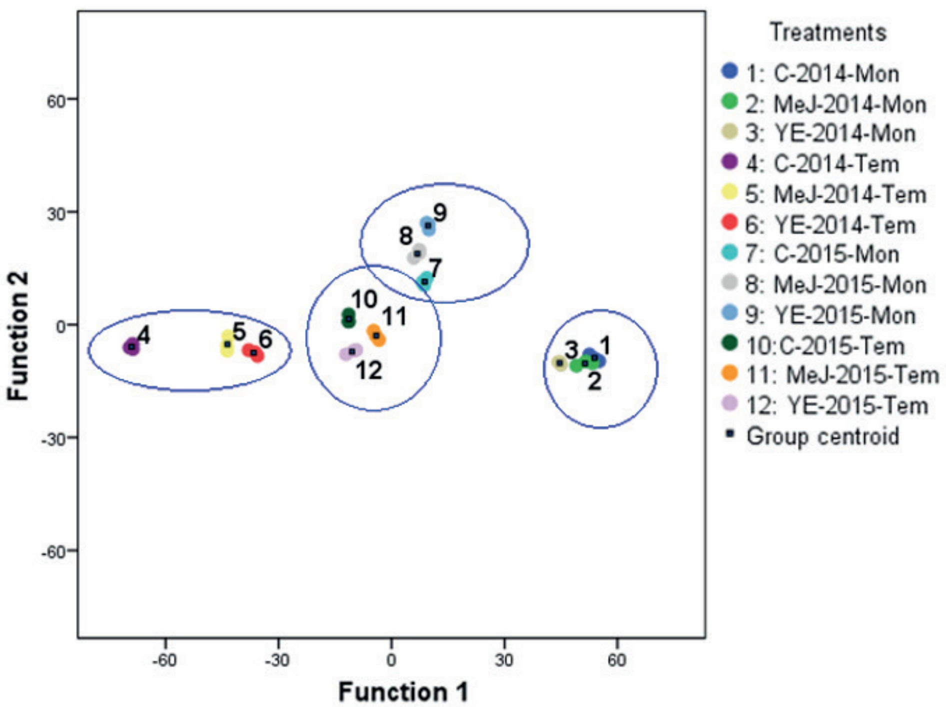

FIGURE 1. Linear discriminant analysis (LDA) performed with all amino acids concentration (mg L-1) in Monastrell (Mon) and Tempranillo (Tm) musts from untreated (C) and treated grapevines with two elicitors: methyl jasmonate (MeJ) and a yeast extract (YE) in 2014 and 2015 vintages.

Function 2 of LDA explained 8.80 % of the total variance and was strongly positively correlated with Cys and Leu but negatively correlated with Thr. According to the information provided by the variables related to Function 1 and Function 2, it has been concluded that Monastrell samples obtained in the 2014 season keep a higher level of Ala, Leu and Lys; however, Tempranillo samples obtained in the 2014 vintage were described by Gln. In addition, Monastrell samples obtained in the 2015 season kept a higher level of Cys and Leu; however, Tempranillo samples obtained in the 2015 season were described by Thr.

In conclusion, the elicitor treatments decreased grape amino acids content depending on the grape variety. The most important variability related to the concentration of the amino acids was the variety and the season individually. These results are important to understand the conditions which affect the effectiveness of elicitor applications on grape amino acids content according to the variety and the vineyard management.

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