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ORIGINAL PAPER

Influence of methyl jasmonate and methyl jasmonate plus urea foliar applications on amino acids composition throughout 'Tempranillo' grape ripening over two seasons

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Abstract

This work studies the impact of foliar application of methyl jasmonate (MeJA) and methyl jasmonate plus urea (MeJA + Ur) on the evolution of amino acid content in 'Tempranillo' grapes during ripening, across two vintages. To achieve this goal, sample grapes were harvested at five different timing. Fol1: 1 day before first foliar application; Fol2: 1 day before second foliar application; Preharvest: 15 days after second foliar application; Harvest: the day of harvest; and Postharvest: 15 days after harvest. The effect of foliar treatments was season dependent, being effective to improve the amino acids content of grapes only in the first vintage. Among the treatments studied, foliar application of MeJA-Ur showed better results. The evolution of amino acids during ripening also was different among seasons. Overall, in the 2019, amino acids reached their highest content at Preharvest or Harvest samples, whereas in the 2020 season, these highest concentrations were reached at Postharvest. Asparagine might serve as a suitable amino acid for controlling grape ripening, as its content decreased from Fol1 to Postharvest in the two vintages. Moreover, differences on the total amino acids content at Harvest date between vintages were observed, probably due to different climatological conditions. Therefore, this study pioneers the examination of the impact of foliar applications of MeJA and MeJA + Ur on the amino acids evolution in 'Tempranillo' grapes during ripening. The need for further research is clear to comprehend the complex interaction between foliar treatments and grape amino acids dynamics for optimizing nitrogen quality of grapes.

Keywords Nitrogen compounds · Urea · Methyl jasmonate · Grapes · Ripening

Introduction

The amino acids of must are key compounds for the growth and development of yeast during the alcoholic fermentation and for bacteria in the course of malolactic fermentation [1]. Their content can affect the kinetic of the fermentation [2]. Furthermore, some of amino acids are precursors of volatile compounds such as higher alcohols, aldehydes, ketones and esters [3]. The amino acid content of grapes changes significantly during ripening and these changes can influence grape and wine quality. Likewise, the initial nitrogen (N) pool in grapes can affect a large number of metabolites that contribute to wine's quality. At berry set commence grape N accumulation [4]. The total amino acid grape content increases from veraison to harvest. However, sometimes the total amino acid content achieves a peak before harvest, after which it stabilized and/or decrease slowly until harvest [5]. Furthermore, the content of amino acids and its profile in grapes can be influenced by different factors such as viticultural practices, environmental conditions, and grapes variety [6]. The amino acid profile of grapes is generally similar from year to year for each variety, whereas the amino acid concentration can vary broadly [7]. However, climatic change is modifying the develop of grapes and therefore, grape composition and flavour. Berry ripening is accelerated under high temperatures, achieving a high content of sugars versus a faster breakdown of acids

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in the grape, which leads to higher alcohol and lower acidity in the resulting wine. These effects may appear by modification of secondary metabolites such as flavonoids, amino acids and carotenoids, affecting aroma and wine color [8]. For this reason, it is interesting to study how the evolution of amino acids in grapes during ripening is developing, in the current climatic change scenario. To mitigate the climatic change effects several approaches have been studied in last years, foliar application of biostimulants to grapevines is one of them [9–12]. Among them, stand out the use of elicitors and nitrogen compounds as foliar treatments to grapevines.

Methyl jasmonate (MeJA) is one of the elicitors more used, is a phytohormone present in several plant tissues and, acts as inductor of secondary metabolites in plants [13]. Its foliar application to vineyard increases the phenolic content in grapes, mainly anthocyanins and stilbenes [14, 15], presumably because MeJA can activate the phenylalanine ammonia-lyase (PAL), enzyme which catalyses the first step in the phenolic biosynthesis pathway [16]. However, its effect can be influenced by grape variety, season, or climate conditions [17–19]. The effect of MeJA foliar application in other fruits also has been studied. In sweet cherry fruits, MeJA treatments were effective in maintaining of fruit firmness, although a decrease on total phenolics, antioxidant capacity and total monomeric anthocyanin values was observed [20]. Another study focused on kiwi concluded that treatments with MeJA can be used as an efficient postharvest tool to reduce weight loss and minimize losses in vitamin C, total phenolics, and total flavonoids [21]. Regarding the effect on amino acids content in grapes, MeJA foliar application to grapevines presents an unclear effect. In this way Garde-Cerdán et al. [22], observed an increase in the content of some amino acids in the must from 'Tempranillo' grapes, whereas Gutiérrez-Gamboa et al. [23] showed a decrease on the must amino acids content. Recently, Garde-Cerdán et al. [24], in their study about the effect of MeJA and MeJA-dopped nanoparticles on nitrogen composition of 'Tempranillo' grapes, have observed a different effect of these foliar treatments depending on the vintage.

Foliar fertilization is another practice that researchers carry out to improve grapes quality. Hannam et al. [25] showed that nitrogen foliar application at veraison-time to vineyard is an effective method of improve YAN (yeast assimilable nitrogen) content in must and it produces changes in amino acid profiles of must. Among the different nitrogen sources, foliar application of urea (Ur) is widespread due to its small molecular size, higher water solubility and low cost [11, 26]. Previous studies reported an increase on the concentration of several amino acids in must of grapes coming from grapevines foliar treated with urea [25, 27]. Nevertheless, Gutiérrez-Gamboa et al. [28] showed a decrease on the concentration of some amino acids and, an increase on the proline content in must from 'Cabernet Sauvignon' grapes foliar treated with urea.

There are previous works [29, 30] which analyze the effect of foliar application of MeJA and MeJA combined with Ur on the phenolic, aromatic and nitrogen composition of 'Tempranillo' wines and phenolic grape composition. Authors concluded that foliar treatments were season dependent and the effect of MeJA + Ur foliar treatment was greater than the effect of MeJA improving the wine chemical composition. However, there is only a recent publication on the effect of MeJA + Ur foliar treatment on the amino acids composition of grapes at the harvest date. This study concluded that, in the first vintage examined, the foliar application of MeJA and MeJA + Ur increased ammonium nitrogen, amino nitrogen, and yeast assimilable nitrogen in 'Tempranillo' grapes compared to the control grapes, with the combined treatment exhibiting a more pronounced effect. However, in the second year of the study, these treatments did not significantly impact nitrogen parameters, suggesting a season-dependent influence, possibly attributed to environmental conditions and variations in grapevine nitrogen content [31].Taking into account the aforementioned, we wonder about the impact of foliar application of MeJA and MeJA + Ur on the amino acids content of grapes during ripening. Based on the hypothesis that both treatments will increase amino acids content in grapes, although, MeJA + Ur will probably have a greater effect on amino acid content compared to the application of MeJA alone. Hence, the aim of this work was to study, for the first time, the evolution (from 1 day before the first foliar application to 15 days after harvest) of the content of the different amino acids on grapes coming from 'Tempranillo' grapevines foliar treated with MeJA and MeJA + Ur over two vintages.

Materials and methods

Vineyard site and experimental layout

This work was conducted in the 2019 and 2020 vintages with grapes from 'Tempranillo' (*Vitis vinifera* L.) variety grown in the experimental vineyard of Finca La Grajera. This vineyard was located in Logroño, La Rioja (Spain) (Lat: 42º26′25.36″ North; Long: 2º30′56.41″ West; 456 m above sea level). Vines were planted in 1997, were trained to a vertical shoot positioned (VSP) trellis system with a grapevine spacing of 2.80 m × 1.25 m and grafted onto a R-110 rootstock. For this trial, three foliar applications were carried out to vineyard: (i) control (sprayed with aqueous solution of Tween 80 alone), (ii) methyl jasmonate (MeJA, 10 mM of methyl jasmonate) and (iii) methyl jasmonate plus urea (MeJA + Ur, 10 mM of methyl jasmonate and a dose of 6 kg N/ha of urea).

The products employed to foliar applications were dissolved in water (the concentration of treatments was decided following previous works [11, 22, 32], and Tween 80 (1 mL/L) were used as wetting agent. Treatments were carried out twice, at veraison and 1 week later. For each treatment, 200 mL of solution was sprayed over leaves. The treatments were performed in triplicate and the experimental layout was arranged in a complete randomized block design along the vineyard. 10 vines were sprayed for each replication and treatment.

Grapes were hand-harvested at five different timing. Fol1: 1 day before the first foliar application; Fol2: 1 day before second foliar application; Preharvest: 15 days after the second foliar application; Harvest: the day of harvest [when grapes reached their optimum technological maturity, i.e., the weight of 100 berries remained constant and the probable alcohol reached 13 (% v/v)]; and Postharvest: 15 days after harvest. For each time of sampling, 150 berries per replicate and treatment was collected haphazard and frozen at – 20 °C until the analyses of amino acids were carried out.

Analysis of amino acids in the musts by HPLC–DAD

The amino acids analysis was carried out following the method described by Garde-Cerdán et al. [33]. In brief, a derivatization of amino acids was performed by reaction of 1.75 mL of borate buffer 1 M (pH 9), 750 µL of methanol (Merck, Darmstadt, Germany), 1 mL of sample (previously filtered), 20 µL of internal standard (2-aminoadipic acid, 1 g/L) (Sigma–Aldrich, Madrid, Spain) and 30 µL of derivatization reagent diethyl ethoxymethylenemalonate (DEEMM) (Sigma–Aldrich). In a screw-cap test tube was done the reaction of derivatization over 30 min in an ultrasound bath (DU-100 ARGO Lab, Modena, Italy). Then, the samples were heated at 70–80 °C in an incubator (INC 120 plus ARGO Lab) for 2 h to complete the degradation of excess DEEMM and reagent by-products.

The analyses were carried out on an Agilent 1260 Infinity II chromatograph (Palo Alto, USA), with a diode array detector (DAD). An ACE HPLC column (C18-HL) (Aberdeen, Scotland) particle size 5 µm (250 mm × 4.6 mm) was employed for the chromatographic separation. Amino acids were eluted following the conditions described by Garde-Cerdán et al. [2]. 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 was used for the detection, and was monitored at 280, 269 and 300 nm. The volume of injection was 50 µL. The identification of the target compounds was performed according to the retention times and the UV–Vis spectral characteristics of corresponding standards (Sigma-Aldrich) derivatizated. Quantification was performed using the calibration graphs of each standard in 0.1 N HCl ($R^2 \geq 0.97$), which underwent the same process of derivatization that the samples.

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

Statistical analysis

The SPSS Version 21.0 statistical package for Windows (SPSS, Chicago, USA) was employed to perform the statistical analysis of the data. The differences among the means of nitrogen compounds data were processed using the variance analysis (ANOVA) ($p \leq 0.05$) and a post hoc Duncan´s multiple range test was carried out. The effect of foliar treatments, time of sampling, seasons and their interaction were analyzed using a multifactor analysis (MANOVA).

Results and discussion

Influence of the foliar MeJA and MeJA + Ur treatments on amino acids content in each time of sampling in grape musts

Table 1 shows the results of must amino acids content from control and treated vines with methyl jasmonate (MeJA) and with methyl jasmonate plus urea (MeJA + Ur), in 2019 season for each time of sampling (Fol1, Fol2, Preharvest, Harvest, and Postharvest). The amino acid present in a higher content in all samples was arginine, except for MeJA + Ur treatment from Preharvest samples, in which glutamine was the predominant. This result is consistent with observations made by Hernández-Orte et al. [7] on the 'Tempranillo' grape variety. Arginine contains four nitrogen atoms in its molecule, making it the most effective nitrogen source for yeasts. Glutamine, Ɣ-aminobutyric acid (GABA), and the sum of threonine and citrulline were found in greater proportion in grapes across all samples. In both Harvest and Postharvest samples, histidine reached similar levels as GABA and the sum of threonine and citrulline. Hernández-Orte et al. [7] studied the amino acid profile of grapes from four varieties over a 3-year period and showed that arginine, proline, histidine, and glutamine were the most prevalent amino acids across all four varieties. In addition, it is noteworthy that arginine, along with ammonium, serves as the main nitrogen sources for yeast through alcoholic fermentation [2]. Valine, isoleucine, leucine and phenylalanine are amino acids that acts as precursors of higher alcohols in alcoholic fermentation [2]. Their representation in grapes, as shown in Fol1, was less than 5% of the total amino acids content. In Fol2, this group of amino acids accounted for approximately 5% in both control and MeJA + Ur grapes, whereas in MeJA grapes, it represented about 8%. In Preharvest samples, it

Table 1 Amino acids concentration (mg/L) in grapes from control, methyl jasmonate (MeJA) and MeJA + Urea (MeJA + Ur) treatments in 2019

	Fol1			Fol2			Preharvest		
	Control	MeJA	MeJA + Ur	Control	MeJA	MeJA + Ur	Control	MeJA	MeJA + Ur
Asp	33.06 ± 1.52B	29.70 ± 0.23A	33.75 ± 3.70A	53.21 ± 3.07C	51.72 ± 1.41B	51.03 ± 1.55B	55.08 ± 4.14aC	61.06 ± 6.46aBC	85.01 ± 15.63bD
Glu	32.24 ± 3.03A	28.25 ± 2.05A	33.47 ± 5.46A	71.26 ± 5.95B	73.51 ± 11.15B	79.97 ± 9.15B	83.82 ± 0.29aCD	117.24 ± 21.55bCD	125.94 ± 6.84bD
Asn	38.07 ± 0.37aD	38.42 ± 3.46aB	52.74 ± 4.46bC	38.94 ± 6.97D	33.18 ± 6.19B	31.76 ± 0.81B	25.58 ± 1.88abC	21.96 ± 2.96aA	28.26 ± 2.01bB
Ser	49.62 ± 3.06A	45.10 ± 4.21A	47.26 ± 8.72A	50.66 ± 0.67A	54.77 ± 4.84A	52.16 ± 7.73A	61.31 ± 3.25C	86.95 ± 16.23B	85.40 ± 15.69B
Gln	156.33 ± 17.36A	155.73 ± 16.89A	144.89 ± 26.47A	180.98 ± 7.37aA	462.60 ± 25.30bC	482.57 ± 68.15bB	391.36 ± 47.58aB	485.44 ± 71.32aC	1138.75 ± 198.66bC
His	63.66 ± 5.40aA	57.49 ± 5.86aA	56.64 ± 9.26aA	77.11 ± 6.66A	93.02 ± 17.23B	97.06 ± 12.65B	132.20 ± 15.65aB	160.77 ± 14.54abC	202.82 ± 33.97bCD
Gly	4.50 ± 0.06aA	4.43 ± 0.30aA	6.34 ± 0.59bB	4.60 ± 0.44abA	5.31 ± 0.35bA	4.09 ± 0.68aA	7.73 ± 0.58B	8.66 ± 1.02B	8.99 ± 0.69C
Thr + Cit	90.71 ± 2.14A	84.09 ± 12.65A	93.53 ± 4.04A	112.24 ± 20.03aAB	152.20 ± 0.96bB	131.45 ± 13.37abB	145.74 ± 22.18aC	156.77 ± 17.46aB	205.41 ± 13.90bC
Arg	222.48 ± 3.02A	201.46 ± 25.71A	230.94 ± 39.23A	331.79 ± 29.54B	331.94 ± 54.55B	364.82 ± 53.99A	576.53 ± 35.75aC	822.77 ± 30.88bC	835.14 ± 85.29bB
Ala	53.48 ± 5.18	52.03 ± 4.64A	52.33 ± 5.69A	65.19 ± 11.68a	88.19 ± 9.80bB	83.77 ± 6.66abB	69.28 ± 6.59a	122.50 ± 0.84bC	121.88 ± 23.28bC
GABA	124.58 ± 17.72AB	114.20 ± 17.52A	118.00 ± 13.73A	105.89 ± 1.42bA	107.41 ± 11.39bA	86.40 ± 7.49aA	155.80 ± 10.70aC	193.68 ± 15.25bB	194.33 ± 21.79bB
Pro	45.71 ± 3.91A	42.14 ± 5.19A	38.99 ± 5.92A	53.14 ± 2.83A	62.56 ± 3.65B	58.06 ± 8.11A	75.07 ± 6.61B	83.58 ± 6.59C	95.27 ± 14.40B
Tyr	7.55 ± 0.78A	7.03 ± 0.98A	7.25 ± 1.29A	8.20 ± 0.79aAB	10.63 ± 1.22bB	8.88 ± 0.89abA	9.97 ± 1.28aBC	18.42 ± 0.94cCD	15.27 ± 1.42bB
Val	14.55 ± 0.45bA	9.75 ± 0.75aA	10.57 ± 1.14aA	19.49 ± 3.53aA	40.73 ± 4.80bB	26.29 ± 4.06aB	41.61 ± 4.37aC	113.39 ± 12.16cE	84.59 ± 7.02bD
Met	4.55 ± 0.42bA	3.65 ± 0.56aA	4.63 ± 0.27bA	7.77 ± 1.11aB	15.08 ± 0.64bB	7.05 ± 0.94aA	13.69 ± 1.34aD	35.98 ± 6.15bD	32.34 ± 1.57bD
Ile + Trp	30.39 ± 2.91bA	21.95 ± 2.14aA	26.78 ± 3.79abA	40.07 ± 0.97aB	65.27 ± 10.96bB	42.85 ± 3.31aB	61.73 ± 4.10aB	142.36 ± 13.31cE	102.48 ± 4.46bD
Leu	15.52 ± 0.72bA	10.72 ± 0.46aA	12.89 ± 1.74aA	23.09 ± 1.61aA	42.63 ± 7.57bB	23.65 ± 0.50aA	48.17 ± 4.37aB	146.08 ± 16.72cE	96.80 ± 6.64bC
Phe	9.88 ± 0.91b	7.63 ± 0.49aA	9.12 ± 1.04abA	10.44 ± 0.91a	30.88 ± 0.56cB	23.05 ± 4.17bB	21.62 ± 2.06a	40.84 ± 3.75bC	36.78 ± 3.11bC
Orn	4.72 ± 0.04b	3.18 ± 0.47aA	3.07 ± 0.40aA	6.18 ± 0.98a	8.27 ± 1.14bB	10.14 ± 0.71bB	5.89 ± 1.02a	15.01 ± 1.88bD	17.68 ± 1.11bD
Lys	7.05 ± 0.64	6.11 ± 0.86A	7.16 ± 0.97A	7.02 ± 0.90	8.38 ± 0.27B	7.86 ± 1.09AB	8.48 ± 0.60a	14.90 ± 0.83bD	15.99 ± 1.90bC

	Harvest			Postharvest		
	Control	MeJA	MeJA + Ur	Control	MeJA	MeJA + Ur
Asp	50.12 ± 2.71aC	66.62 ± 6.88bC	71.64 ± 1.35bC	26.52 ± 2.93aA	37.03 ± 6.85bA	38.87 ± 2.25bAB
Glu	78.22 ± 4.65aC	105.98 ± 17.54bC	106.06 ± 7.25bC	87.26 ± 1.20aD	133.81 ± 1.96cD	107.66 ± 10.96bC
Asn	17.06 ± 1.41aB	23.11 ± 0.65bA	28.03 ± 2.21cB	9.82 ± 0.85aA	17.46 ± 3.10bA	21.07 ± 0.78bA
Ser	58.79 ± 1.68aBC	86.99 ± 13.80bB	86.94 ± 15.23bB	56.58 ± 1.70aB	85.68 ± 7.97bB	84.76 ± 6.74bB
Gln	395.37 ± 42.80aB	527.76 ± 40.78bC	591.26 ± 74.92bB	209.96 ± 37.37aA	356.76 ± 30.53bB	269.62 ± 35.44aA
His	137.38 ± 7.41aB	168.16 ± 7.09bC	183.46 ± 4.97cC	121.47 ± 7.84aB	183.83 ± 27.80bC	233.12 ± 20.82cD
Gly	9.88 ± 0.50bC	7.46 ± 1.29aB	6.85 ± 0.53aB	4.47 ± 0.58aA	8.09 ± 0.18cB	6.69 ± 0.79bB
Thr + Cit	160.43 ± 1.19aC	210.86 ± 18.25cC	188.83 ± 1.09bC	133.70 ± 16.63aBC	189.05 ± 11.84bC	212.94 ± 28.83bC
Arg	677.19 ± 2.65aD	814.95 ± 113.93abC	921.10 ± 155.20bB	612.31 ± 102.05aCD	942.75 ± 47.20bD	986.65 ± 136.24bB
Ala	54.72 ± 9.01a	94.90 ± 5.91bB	84.71 ± 13.86bB	69.44 ± 9.49a	122.24 ± 1.27bC	121.14 ± 15.53bC
GABA	145.02 ± 12.80BC	187.99 ± 30.94B	193.60 ± 28.19B	154.65 ± 15.23C	195.36 ± 35.87B	175.02 ± 1.45B
Pro	82.23 ± 4.44B	82.75 ± 9.68C	104.34 ± 15.44B	105.34 ± 15.63C	109.37 ± 5.59D	116.60 ± 15.06B
Tyr	11.30 ± 1.04aC	15.97 ± 2.49bC	16.07 ± 2.31bB	15.15 ± 1.56aD	20.45 ± 1.08bD	19.43 ± 0.24bC
Val	34.87 ± 2.14aB	67.99 ± 5.95cC	53.87 ± 2.99bC	41.71 ± 4.96aC	88.85 ± 9.88bD	80.88 ± 8.63bD
Met	13.95 ± 1.36aC	25.31 ± 0.81cC	19.65 ± 1.13bB	13.23 ± 1.15aD	32.78 ± 2.54cD	25.91 ± 3.11bC

Table 1 (continued)

	Harvest			Postharvest		
	Control	MeJA	MeJA + Ur	Control	MeJA	MeJA + Ur
Ile + Trp	59.11 ± 1.27aB	102.54 ± 7.33cC	82.92 ± 4.17bC	64.69 ± 2.06aB	120.80 ± 11.53bD	104.54 ± 10.72bD
Leu	48.89 ± 8.25aB	91.05 ± 5.75cC	67.68 ± 1.14bB	53.33 ± 4.26aB	122.69 ± 12.53bD	105.47 ± 11.78bC
Phe	24.98 ± 2.52a	38.83 ± 6.57bC	34.15 ± 1.20bC	23.09 ± 2.66a	34.00 ± 2.94bBC	35.15 ± 3.41bC
Orn	5.21 ± 0.55a	13.37 ± 1.28bCD	15.68 ± 1.42cC	6.07 ± 0.92a	11.80 ± 2.02bBC	10.61 ± 0.34bB
Lys	7.93 ± 1.35a	13.89 ± 1.54bCD	15.73 ± 2.75bC	7.07 ± 0.71a	13.15 ± 0.49cC	10.69 ± 1.24bB

Time of sampling: Fol1, 1 day before first foliar application; Fol2, 1 day before second foliar application; Preharvest, 15 days after second application; Harvest, the day of harvest; Postharvest, 15 days after harvest

All parameters are listed with their standard deviation ($n = 3$). For each compound and time of sampling (Fol1, Fol2, Preharvest, Harvest, or Postharvest), different lowercase letters indicate significant differences between treatments ($p \leq 0.05$). For each compound and treatment (Control, MeJA, or MeJA + Ur), uppercase letters indicate differences among the time of sampling ($p \leq 0.05$). Absence of letters indicate no significant differences ($p > 0.05$)

constituted roughly 7% in both control and MeJA + Ur samples, and 13.5% in MeJA grapes. At Harvest, these amino acids accounted for 6% in both control and MeJA + Ur grapes, and around 9% in MeJA grapes. Finally, in Postharvest samples, the content in control grapes was around 8%, in MeJA grapes it was 11%, and in MeJA + Ur samples, it was 10%. Therefore, foliar application of MeJA increased the content of amino acid precursors of higher alcohols with respect to control and MeJA + Ur grapes during grape ripening. Excluding the aforementioned, the amino acids present in grapes in a lower concentration were ornithine, methionine, glycine, lysine and tyrosine. In all samples collected a different times, their content was lower than 3% of the total amino acids content, except for MeJA grapes from Preharvest (3.3%) and Postharvest (3.1%). Lysine, glycine, and methionine were characterized as minor amino acids in grapes, specifically in certain grape varieties such as 'Monastrell', 'Merlot', and 'Petit Verdot'. A previous work highlighted that glycine and lysine are not a good nitrogen sources for *Saccharomyces cerevisiae* yeast, but they are suitable for non-*Saccharomyces* [2]. Amino acids can be categorized based on the trends observed during the ripening period. Hernández-Orte et al. [7] described that most amino acids exhibited varying development patterns during the ripening stages in different years of their study, with amino acids reaching their highest content before the harvest. Aspartic acid, phenylalanine, ornithine, and lysine demonstrated an increase in their content in grapes until the Preharvest stage, followed by a decrease until Postharvest (Table 1). On the other hand, glutamic acid, histidine, glycine, alanine, valine, methionine, leucine, isoleucine, and tryptophan showed an increase in their content up to the Preharvest stage, followed by a decrease until Harvest and subsequently an increase again until the Postharvest stage. Asparagine was the only amino acid which presented a decrease in its content from Fol1 to Postharvest (Table 1). Proline, for its part, underwent a general increase until Postharvest sample. Given the observed trends in these two amino acids, they could be considered suitable parameters for monitoring grape ripening. The trends observed for glutamine, tyrosine, and arginine depended on the sample (control, MeJA and MeJA + Ur) being studied. Serine and GABA exhibited an increase in their content in grapes up to Preharvest stage, after which their content remained relatively constant (Table 1). The increase in the concentration of free amino acids as the fruit ripens could be due to a decrease in the demand for these metabolites as the growth process progresses through ripening [7]. The range of concentrations measured for all samples of the amino acids at Harvest was consistent with those described by Beel &Henschke [5] except for tryptophan, which was found in higher concentration (38–54 mg/L). MeJA and MeJA + Ur treatments increased the content of several amino acids in Harvest and

Postharvest samples. These amino acids included aspartic acid, glutamic acid, asparagine, serine, histidine, the sum of threonine and citrulline, alanine (treatments also increased its content at Preharvest, and in the case of MeJA, in Fol1), and tyrosine (MeJA treatment also increased its content in Fol1). MeJA treatment increased the glutamine content at Harvest and Postharvest, whereas MeJA + Ur only increased glutamine concentration at Harvest, in comparison to the content of control grapes (Table 1). Grapes from grapevines treated only with MeJA showed a higher content of glycine than MeJA + Ur grapes, which also had higher content than the control grapes in Postharvest samples. On the other hand, MeJA and MeJA + Ur foliar treatments increased the arginine content at Preharvest and Postharvest samples, with MeJA + Ur additionally raising the arginine content at Harvest time compared to control samples. Furthermore, treatments rose GABA concentration at Preharvest when compared with control samples (Table 1). However, foliar treatments did not affect the content of proline in any of the samples studied. Valine, methionine, isoleucine, and leucine underwent a similar pattern; treatments jumped their concentration from Preharvest to Postharvest, and MeJA also increased their content in Fol2, compared to their content in control grapes (Table 1). Grapes from grapevines treated with MeJA showed a high content of tryptophan from Fol2 to Postharvest, whereas MeJA + Ur treatment increased its concentration in Preharvest and Postharvest samples. The content of phenylalanine and ornithine was increased from Fol2 to Postharvest by both treatments studied. Finally, the lysine content rose for treatments from Preharvest to Postharvest samples (Table 1).

In summary, all amino acids underwent an increase in their concentrations at any sampling time, except for proline, due to the effect of foliar treatments compared to control grapes. Therefore, both MeJA and MeJA + Ur treatments affected the biosynthesis of amino acids in grapes during the 2019 season. Garde-Cerdán et al. [24] also observed an enhance of the synthesis of most amino acids during the first season of their study, attributed to MeJA foliar application.

Table 2 presents the results of must amino acids content from control and treated vines with methyl jasmonate (MeJA) and with methyl jasmonate plus urea (MeJA + Ur), in 2020 season for each time of sampling (Fol1, Fol2, Preharvest, Harvest, and Postharvest). The amino acids present in a higher content across all samples were glutamine or arginine, following by alanine, GABA, glutamic acid and histidine. The amino acids content that act as precursors for higher alcohols was: in Fol1, it accounted for 9% in the control sample, 7% in MeJA, and 5% in MeJA + Ur of the total amino acids content; in Fol2, it represented 7% in the control sample, 9.7% in MeJA, and 6% in MeJA + Ur of the total amino acids content; in the Preharvest samples these amino acids accounted for 6.6% in control, 8.4% in MeJA, and 5.8% in MeJA + Ur samples of the total amino acids content; at Harvest, this group of amino acids constituted 10% in control samples, 11% in MeJA, and 9% in MeJA + Ur samples of the total amino acids content; and finally, at Postharvest, these amino acids accounted for 12% in the control, 9.6% in MeJA, and, 9.5% in MeJA + Ur samples. It was again observed that foliar application of MeJA increased the content of amino acid precursors of higher alcohols, this time from Fol1 to Harvest. The amino acids with lower content in all samples were glycine, the sum of threonine and citrulline, ornithine, and lysine. Their content represented less than 3% of the total amino acids content in all samples across various treatments and sampling times. Aspartic and glutamic acids, GABA, histidine, glycine, methionine, and tyrosine showed a more or less pronounced increase in their concentration from Fol1 to Postharvest (Table 2). A similar trend was observed for the following amino acids: serine, glutamine, citrulline + threonine, leucine, valine, isoleucine + tryptophan, phenylalanine and lysine, which presented a minimal or no increase in their concentration from Fol1 to Preharvest, followed by an increase in their content until Postharvest (Table 2). In the 2020 season, asparagine was the only amino acid which displayed a decrease in its content in grapes from Fol1 to Post-harvest, consistent with the evolution observed in 2019. Therefore, as mentioned above, it seems that asparagine could be a suitable amino acid for monitoring grape ripening, since its content decreased from Fol1 to Postharvest in both vintages studied. Arginine and proline exhibited an increase in their content from Fol1 to Harvest, followed by a decrease until Postharvest. Alanine increased its content in grapes from Fol1 to Fol2, underwent a decrease until Preharvest and then slightly increased its content until Postharvest (Table 2). Ornithine demonstrated a decrease from Fol1 to Fol2 and then, an increase until Postharvest. Overall, the foliar treatments did not significantly affect the content of amino acids in grapes during the ripening process in this second season, with some cases indicating a slight decrease. All amino acids presented a concentration range at Harvest that aligned with those previously described by Bell &Henschke [5], except for tyrosine, which showed in control grapes a content higher than 33 mg/L.

Figure 1 shows the total amino acids content, with and without proline, throughout grape ripening for control, MeJA and MeJA + Ur samples in both vintages (2019 and 2020). In Fig. 1a, b, it can be observed that in 2019 season, MeJA and MeJA + Ur treatments increased the total amino acids and the total amino acids without proline content from Fol2 to Postharvest stages. Stand out the notable effect of MeJA + Ur foliar treatment at Preharvest moment; however, the MeJA treatment also led to an increase in both total amino acids and total amino acids without proline, in comparison to the control grapes. However, during the 2020 season, the effect of foliar treatments was totally different. Both

Table 2 Amino acids concentration (mg/L) in grapes from control, methyl jasmonate (MeJA) and MeJA + Urea (MeJA + Ur) treatments in 2020

	Fol1			Fol2			Preharvest		
	Control	MeJA	MeJA + Ur	Control	MeJA	MeJA + Ur	Control	MeJA	MeJA + Ur
Asp	2.15 ± 0.12A	2.14 ± 0.17A	2.49 ± 0.32A	5.80 ± 1.11bB	4.52 ± 0.43abB	4.13 ± 0.60aB	6.96 ± 1.03bBC	5.00 ± 0.28aB	4.50 ± 0.10aB
Glu	63.58 ± 3.30A	54.24 ± 7.54A	61.37 ± 9.92A	95.41 ± 10.56bB	77.27 ± 6.91aB	78.98 ± 0.66aB	104.18 ± 2.37bB	88.39 ± 4.49aBC	97.15 ± 4.94bC
Asn	54.70 ± 7.04bB	49.15 ± 6.75abB	39.86 ± 6.09aC	49.84 ± 6.84B	41.10 ± 4.30B	46.90 ± 4.18C	26.98 ± 5.10A	26.95 ± 5.02A	31.57 ± 5.63B
Ser	65.40 ± 5.35bA	53.05 ± 3.73aA	51.22 ± 7.23aA	66.65 ± 5.68A	56.95 ± 4.44A	60.86 ± 9.15A	63.48 ± 3.72A	61.98 ± 3.28A	68.47 ± 11.00AB
Gln	610.08 ± 76.71bAB	422.83 ± 64.29aAB	371.13 ± 35.82aA	573.63 ± 43.49A	501.88 ± 30.13B	517.90 ± 63.48B	547.82 ± 53.52bA	388.32 ± 25.87aA	475.68 ± 61.93abAB
His	88.15 ± 7.15cA	71.63 ± 8.08bA	55.05 ± 1.55aA	93.50 ± 4.18A	90.30 ± 15.95A	76.88 ± 2.12AB	101.91 ± 6.83A	96.97 ± 6.37A	96.15 ± 16.47B
Gly	8.11 ± 0.72cA	6.79 ± 0.57bA	4.55 ± 0.34aA	8.38 ± 0.32bA	6.63 ± 0.79aA	6.44 ± 0.65aAB	8.41 ± 0.81A	7.53 ± 0.73A	8.17 ± 0.11B
Thr + Cit	12.56 ± 0.69bA	10.90 ± 0.78abA	8.88 ± 1.55aA	12.83 ± 0.94bA	12.78 ± 0.86bAB	10.32 ± 1.20aAB	12.17 ± 0.22A	12.87 ± 1.52AB	12.99 ± 2.32BC
Arg	517.83 ± 34.06bA	413.89 ± 16.52aA	388.50 ± 44.39aA	614.74 ± 23.19bB	525.27 ± 46.81aA	471.57 ± 16.65aA	783.82 ± 14.64C	703.76 ± 106.26B	775.97 ± 87.15B
Ala	148.95 ± 14.64AB	117.87 ± 12.54A	118.58 ± 20.39A	170.74 ± 12.92bB	132.24 ± 11.02aA	150.16 ± 26.11abA	142.85 ± 1.22A	132.40 ± 12.35A	150.82 ± 17.20AB
GABA	103.50 ± 4.59A	92.00 ± 5.53A	101.40 ± 13.72A	131.96 ± 16.41AB	109.89 ± 9.90A	119.65 ± 8.80AB	149.44 ± 20.83B	134.90 ± 13.65A	152.46 ± 18.45B
Pro	28.89 ± 2.01aA	35.76 ± 4.49bA	36.66 ± 2.87bA	93.93 ± 7.80B	77.52 ± 8.86B	77.18 ± 10.04B	99.34 ± 6.03B	103.88 ± 9.71C	111.91 ± 9.78C
Tyr	22.17 ± 1.15bA	18.36 ± 1.42aA	16.92 ± 1.59aA	27.88 ± 2.74A	25.79 ± 2.28B	26.65 ± 4.48B	27.17 ± 3.81A	24.67 ± 2.89B	27.37 ± 4.01B
Val	40.03 ± 5.65bA	22.47 ± 3.81aA	15.92 ± 1.81aA	33.64 ± 5.06bA	45.84 ± 2.52cB	23.22 ± 1.93aAB	33.18 ± 3.21bA	37.74 ± 3.01bB	26.22 ± 3.75aB
Met	11.77 ± 1.14bA	6.13 ± 0.85aA	6.15 ± 1.11aA	17.73 ± 1.55bAB	14.47 ± 1.94abB	13.39 ± 2.22aB	20.24 ± 1.20aB	26.65 ± 2.52bC	24.73 ± 4.06abC
Ile + Trp	66.81 ± 4.79cA	47.64 ± 8.38bA	32.69 ± 3.63aA	60.05 ± 9.43abA	73.68 ± 9.74bBC	48.47 ± 8.26aAB	63.13 ± 4.13A	65.12 ± 6.67AB	54.95 ± 6.54B
Leu	37.40 ± 6.44bA	17.04 ± 2.74aA	11.25 ± 1.31aA	27.88 ± 1.01bA	34.21 ± 1.84cB	17.02 ± 1.61aAB	30.30 ± 5.09aA	40.04 ± 4.76bB	23.30 ± 4.19aB
Phe	31.76 ± 2.01cB	20.88 ± 2.77bA	14.70 ± 0.31aA	24.99 ± 2.53abA	30.13 ± 0.43bAB	22.65 ± 3.64aB	23.15 ± 0.41A	26.63 ± 2.45B	21.91 ± 3.55B
Orn	14.40 ± 2.65bA	9.68 ± 0.67aA	7.16 ± 0.92aA	12.28 ± 0.24abA	12.61 ± 0.05bB	10.81 ± 1.25aA	13.65 ± 1.19A	12.26 ± 1.87AB	11.50 ± 0.20A
Lys	10.15 ± 0.41bA	9.13 ± 0.86bA	7.42 ± 0.69aA	11.67 ± 0.58A	9.74 ± 0.91A	10.23 ± 1.40B	11.68 ± 0.23A	10.97 ± 0.29A	12.76 ± 1.46C

	Harvest			Postharvest		
	Control	MeJA	MeJA + Ur	Control	MeJA	MeJA + Ur
Asp	7.94 ± 0.39CD	6.31 ± 0.70C	6.96 ± 1.24C	8.90 ± 1.13D	7.50 ± 1.21C	6.63 ± 1.28C
Glu	134.46 ± 21.16bD	100.97 ± 9.53aCD	118.42 ± 10.21abD	148.50 ± 14.18bD	112.94 ± 18.31aD	115.98 ± 14.57aD
Asn	21.74 ± 2.54C	24.86 ± 1.84A	21.58 ± 2.68A	26.42 ± 1.61C	25.21 ± 4.44A	28.25 ± 2.22AB
Ser	80.44 ± 12.67B	81.68 ± 15.48B	85.02 ± 15.21B	87.91 ± 5.79B	84.53 ± 11.35B	84.72 ± 7.53B
Gln	727.61 ± 116.69bB	424.42 ± 56.80aAB	473.39 ± 22.28aAB	1148.98 ± 21.60cC	382.75 ± 57.96aA	518.63 ± 78.25bB
His	166.81 ± 12.60B	145.56 ± 14.32B	141.42 ± 15.88C	197.69 ± 21.15bC	136.12 ± 25.12aB	150.30 ± 24.14aC
Gly	15.13 ± 2.63B	11.21 ± 1.14B	13.15 ± 2.22C	17.02 ± 1.31bB	15.18 ± 1.47abC	12.48 ± 1.51aC
Thr + Cit	16.77 ± 1.35B	15.97 ± 3.11B	15.72 ± 1.94C	19.09 ± 1.61C	15.68 ± 2.33B	16.27 ± 1.94C
Arg	1080.53 ± 41.98D	1007.32 ± 107.81C	1061.32 ± 94.21C	1030.70 ± 36.19D	922.19 ± 41.80C	980.60 ± 97.79C
Ala	156.91 ± 18.57AB	143.93 ± 26.20A	156.11 ± 19.71A	158.45 ± 11.53AB	132.34 ± 22.22A	130.68 ± 12.77A
GABA	229.05 ± 37.20C	196.00 ± 29.85B	221.24 ± 36.62C	249.57 ± 20.45C	232.50 ± 49.12B	247.08 ± 38.73C
Pro	140.69 ± 8.79C	149.38 ± 21.23D	144.87 ± 14.06D	127.53 ± 22.66C	135.65 ± 19.91D	131.06 ± 19.88CD
Tyr	44.07 ± 5.16bB	33.31 ± 4.87aC	35.00 ± 4.33abC	44.55 ± 1.48bB	39.80 ± 2.91abD	36.46 ± 3.36aC
Val	85.27 ± 15.79B	71.87 ± 12.23C	61.91 ± 7.21C	122.29 ± 2.66bC	50.64 ± 9.33aB	60.96 ± 5.88aC
Met	33.98 ± 4.71C	32.02 ± 5.15D	29.13 ± 5.03C	37.94 ± 7.14bC	25.15 ± 0.50aC	26.75 ± 2.60aC

Table 2 (continued)

	Harvest			Postharvest		
	Control	MeJA	MeJA + Ur	Control	MeJA	MeJA + Ur
Ile + Trp	114.78 ± 18.99B	119.33 ± 12.86D	95.17 ± 11.77C	142.90 ± 27.01bB	92.95 ± 17.09aC	96.07 ± 12.46aC
Leu	80.25 ± 11.49B	80.72 ± 10.98C	65.14 ± 8.02C	131.13 ± 9.49bC	71.24 ± 2.84aC	73.05 ± 10.12aC
Phe	51.48 ± 2.26cC	30.53 ± 1.51aB	39.11 ± 1.51bC	60.75 ± 0.56bD	29.77 ± 3.84aB	34.20 ± 5.83aC
Orn	14.35 ± 1.27A	12.14 ± 2.39AB	11.40 ± 1.16A	14.69 ± 2.17bA	9.92 ± 0.43aAB	11.97 ± 0.54aA
Lys	15.33 ± 1.63B	15.82 ± 1.42C	14.25 ± 1.83C	15.19 ± 1.96B	13.13 ± 1.79B	12.85 ± 1.19C

Time of sampling: Fol1, 1 day before first foliar application; Fol2, 1 day before second foliar application; Preharvest, 15 days after second application; Harvest, the day of harvest; Postharvest, 15 days after harvest

All parameters are listed with their standard deviation ($n = 3$). For each compound and time of sampling (Fol1, Fol2, Preharvest, Harvest, or Postharvest), different lowercase letters indicate significant differences between treatments ($p \leq 0.05$)

For each compound and treatment (Control, MeJA, or MeJA + Ur), uppercase letters indicate differences among the time of sampling ($p \leq 0.05$). Absence of letters indicate no significant differences ($p > 0.05$)

MeJA and MeJA + Ur produced a decrease in total amino acids content and total amino acids content without proline (Fig. 1c, d) regarding to the amino acids content in control grapes, observed at Fol1, Fol2 and Postharvest stages. Furthermore, no significant differences were observed between the treated grapes and the control grapes at the Preharvest and Harvest stages.

The effect of foliar treatments was different between the two study seasons, suggesting that foliar applications show a dependence on the season in which they are applied. This dependence has already been observed by other authors previously [24]. Mainly, the different effect of foliar treatments observed could be explained by differences on the pre harvest rainfall recorded among seasons. In 2020 season, the preharvest rainfalls were higher (32.9 l/m^2) than in 2019 vintage (11.5 l/m^2). In addition, a previous study reported data on nitrogen compound content in grapes at harvest [24]. In the 2020 season, the nitrogen content in the control grapes was approximately twice as high as that in 2019. Thus, the impact of foliar treatments was less pronounced when the grapes had a higher content of nitrogen compounds.

It should be noted the differences on the total content (with and without Pro, Fig. 1) of amino acids in musts between the two years of the study. In 2019 at harvest moment, total amino acids content of control must was around 2070 mg/L, whereas in 2020 this content was around 3215 mg/L, which can be explained by climatological conditions, since they play a key role in the amino acid content of the must [7].

Overall, the variation in amino acids evolution during ripening differed between seasons. In 2019, amino acids attained their peak content in preharvest or harvest samples, aligning with findings by Hernández-Orte et al. [7]. In contrast, during the 2020 season, the highest concentrations were observed at post-harvest, a notable deviation from the previous vintage, potentially attributed to climatic change.

Multifactor analysis of variance of amino acids in musts

Tables 3 and 4 show the results of the multifactor analysis of variance of amino acids content, during the 2019 and 2020 seasons, considering the two factors under investigation: treatment and sampling time. In 2019 (Table 3), the treatments influenced the content of all individual amino acids, except for asparagine and proline in MeJA treatment. Additionally, the total amino acids content, both with and without proline, was affected by the treatments, with the MeJA + Ur treatment showing a more substantial impact compared to the MeJA treatment (Table 3). The "sampling time" factor also significantly affected the content of all individual amino acids at various sampling points, as well as the total amino acid content with and without proline,

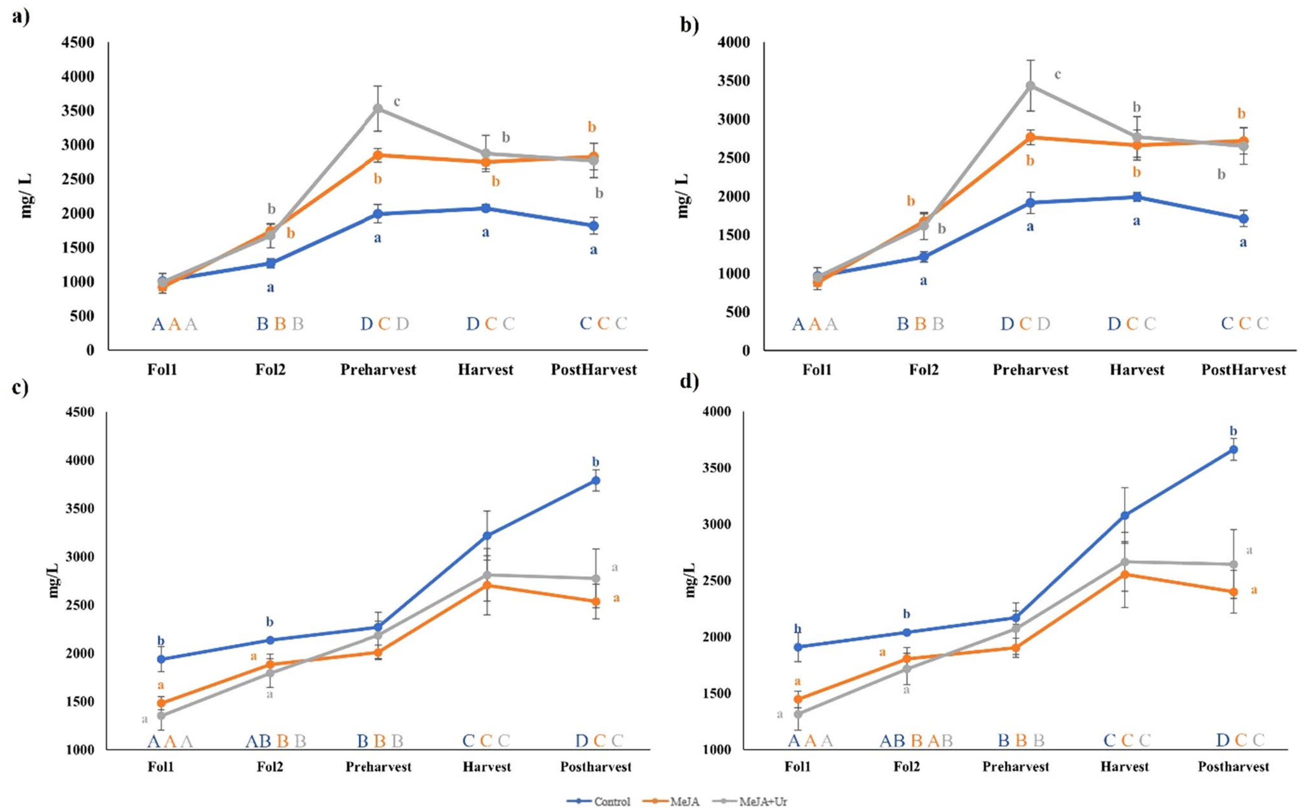

Fig. 1 Amino acids concentration (mg/L) in grapes from control and treated vineyards with foliar application, methyl jasmonate (MeJA) and methyl jasmonate plus urea (MeJA + Ur): **a** total amino acids concentration from 2019 season, **b** total amino acids concentration without proline from 2019 season, **c** total amino acids concentration from 2020 season, **d** total amino acids concentration without proline from 2020 season. Different lowercase letters indicate significant differences between treatments at each time of maturation ($p \leq 0.05$). Uppercase letters indicate differences among time of ripening for each treatment ($p \leq 0.05$). Absence of letters indicates no significant differences

reaching the highest content at Preharvest for several amino acids (Table 3). The interaction between the two factors was statistically significant for all individual amino acids, except for proline, and also affected the total amino acids content (Table 3). In 2020 season (Table 4), the studied foliar treatments impacted the individual content of several amino acids, leading to a decrease in their content in grapes when compared with control grapes. The sampling time also influenced the grape's content of both individual and total amino acids. However, in this vintage, the maximum concentration values were observed either at Harvest or Postharvest stage (Table 4). In this vintage, the interaction between both factors was significant for all amino acids, except for aspartic and glutamic acids, threonine + citrulline, arginine, alanine, GABA, proline, tyrosine and lysine. These findings further confirm the dependence of the effect of foliar application's effect on the vineyard in relation to the specific season.

Table 5 shows the percentage of variance attributed to each factor (season, sampling time, and treatment), and their interactions. The main source of variability was the sampling time, which is logical considering the changes in amino acid content during grape ripening. The season also showed a significant influence on specific amino acids, such as aspartic acid, the sum of threonine and citrulline, alanine and tyrosine (Table 5). However, the effect of the treatments was minor, producing an effect lower than 5% in all amino acids. Overall, the interaction effect among factors influenced the concentration of amino acids in grapes, with the most substantial impact observed only for glutamine.

Table 3 Multifactor analysis of variance of amino acids content in 2019 season, with the two factors studied: treatment (Control, MeJA, MeJA + Ur) and time of sampling (Fol1, Fol2, Preharvest, Harvest, Postharvest) and their interaction (treatment × time of sampling)

	Treatment (T)		Time of sampling (S)						Interaction
	Control	MeJA	MeJA + Ur	Fol1	Fol2	Preharvest	Harvest	Postharvest	T*S
Asp	43.60a	49.23b	56.06c	32.17a	51.99b	67.05c	62.79c	34.14a	***
Glu	70.56a	91.76b	90.62b	31.32a	74.91b	109.00d	96.75c	109.57d	***
Asn	25.89a	26.83a	32.37b	43.08d	34.63c	25.27b	22.73b	16.11a	***
Ser	55.39a	71.89b	71.30b	47.32a	52.53a	77.89b	77.57b	75.67b	*
Gln	266.80a	397.65b	525.42c	152.31a	375.38c	671.85e	50480d	278.78b	***
His	106.36a	132.65b	154.62c	59.26a	89.06b	165.26cd	163.00c	179.47d	***
Gly	6.24a	8.79b	6.59ab	5.09a	4.67a	8.46c	8.06c	6.42b	***
Thr + Cit	128.57a	158.59b	166.43b	89.44a	131.96b	169.31c	186.70d	178.56cd	***
Arg	484.06a	622.78b	667.73b	218.29a	342.85b	744.81c	804.42cd	847.24d	**
Ala	62.42a	95.97b	92.77b	52.61a	79.05b	104.56c	78.11b	104.27c	***
GABA	137.19a	159.72b	153.47b	118.93b	99.90a	181.27c	175.54c	175.01c	*
Pro	72.30a	76.08ab	682.65b	42.28a	57.92b	84.64c	89.77c	110.43d	N.S.
Tyr	10.43a	14.50c	13.38b	7.28a	9.24b	14.55c	14.45c	18.35d	***
Val	30.45a	64.14c	51.24b	11.62a	28.83b	79.86e	52.24c	70.48d	***
Met	10.64a	22.56c	17.91b	4.28a	9.67b	27.33e	19.64c	23.97d	***
Ile	18.24a	47.13c	34.19b	5.96a	18.41b	55.17d	35.57c	50.81d	***
Trp	32.96a	43.45c	37.72b	20.41a	30.98b	47.01c	45.95c	45.86c	***
Ile + Trp	51.20a	90.58c	71.91b	26.37a	49.39b	102.19d	81.52c	96.67d	***
Leu	37.80a	82.63c	61.30b	13.04a	29.79b	97.02d	69.21c	93.83d	***
Phe	18.00a	30.44c	27.65b	8.87a	21.46b	33.08c	32.65c	30.74c	***
Orn	5.62a	10.69b	11.44b	3.66a	8.20b	13.46d	11.42c	9.50b	***
Lys	7.51a	11.29b	11.49b	6.77a	7.75a	13.12c	12.52c	10.30b	***
Total aas [a]	1631.01a	2216.41b	2366.35c	974.01a	1559.48b	2789.37d	2563.89c	2469.54c	***
Total aas[a] without Pro	1558.71a	2140.34b	2283.70c	931.73a	1501.56b	2704.73d	2474.12c	2359.11c	***

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

[a]Total aas: concentration of total amino acids

Table 4 Multifactor analysis of variance of amino acids content in 2020 season, with the two factors studied: treatment (Control, MeJA, MeJA + Ur) and time of sampling (Fol1, Fol2, Preharvest, Harvest, Postharvest) and their interaction (treatment × time of sampling)

	Treatment (T)			Time of sampling (S)					Interaction
	Control	MeJA	MeJA + Ur	Fol1	Fol2	Preharvest	Harvest	Postharvest	T*S
Asp	6.35b	5.09a	4.94a	2.26a	4.82b	5.48b	7.07c	7.68c	N.S.
Glu	109.23b	86.76a	94.38a	59.73a	83.89b	96.57c	117.95d	125.81d	N.S.
Asn	35.94	33.45	33.63	47.90c	45.95c	28.50b	22.73a	26.63ab	*
Ser	72.78	67.64	70.06	56.56a	61.48a	64.64a	82.38b	85.72b	*
Gln	721.62c	424.04a	471.35b	468.01a	531.14b	470.61a	541.81b	683.45c	***
His	129.61b	108.12a	103.96a	71.61a	86.89b	98.34b	151.27c	161.37c	*
Gly	11.41b	9.47a	8.96a	6.48a	7.15ab	8.04b	13.16c	14.89d	*
Thr + Cit	14.68b	13.64ab	12.84a	10.78a	11.98ab	12.68b	16.15c	17.01c	N.S.
Arg	805.52b	714.49a	735.59a	440.07a	537.19b	754.51c	1049.72e	977.83d	N.S.
Ala	155.58b	131.76a	141.27a	128.47a	151.05b	142.02ab	152.32b	140.49ab	N.S.
GABA	172.71	153.06	168.37	98.97a	120.50a	145.60b	215.43c	243.05d	N.S.
Pro	98.07	100.44	100.33	33.77a	82.87b	105.04c	144.98e	131.42d	N.S.
Tyr	33.17b	28.38a	28.48a	19.15a	26.77b	26.40b	37.46c	40.27c	N.S.
Val	62.88c	45.71b	37.64a	26.14a	34.23b	32.38ab	73.02c	77.96c	***
Met	24.33b	20.88a	20.03a	8.01a	15.20b	23.87c	31.71d	29.94d	**
Ile + Trp	89.53c	79.74b	65.47a	49.05a	60.73a	61.06a	109.76b	110.64b	**
Leu	61.39c	48.65b	37.95a	21.90a	26.37ab	31.21b	75.37c	91.80d	***
Phe	38.43b	27.59a	26.52a	22.45a	25.92b	23.90ab	40.37c	41.57c	***
Orn	13.87b	11.32a	10.57a	10.41a	11.90b	12.47b	12.63b	12.19b	**
Lys	12.80b	11.76a	11.50a	8.90a	10.55b	11.80c	15.13e	13.72d	N.S.
Total aas[a]	2669.92b	2121.99a	2183.08a	1590.63a	1936.59b	2153.88	2910.42d	3033.46d	***
Total aas[a] without Pro	2571.84b	2021.55a	2082.74a	1556.86a	1853.71b	2048.84c	2765.44d	2902.04d	***

For each amino acid and factor, different letters indicate significant differences between samples ($p \leq 0.05$)

Interaction: N.S., not significant ($p > 0.05$); ***$p \leq 0.001$; **$p \leq 0.01$; *$p \leq 0.05$

[a]Total aas: concentration of total amino acids

Table 5 Percentage of variance attributable to season, time of sampling, treatment and their interactions

	Season (%)	Sampling (%)	Treatment (%)	Season × Sampling (%)	Season × Treatment (%)	Sampling × Treatment	Season × Sampling × Treatment (%)	Residual (%)
Asp	**77.26******	8.94***	0.82***	7.62***	1.27***	1.15***	1.33***	1.62
Glu	4.03***	**68.78******	0.20 N.S.	5.06***	9.21***	2.01*	3.64***	7.07
Asn	6.58***	**69.75******	1.08*	3.45***	2.40***	3.14**	5.48***	8.11
Ser	1.43*	**50.62******	3.10*	7.05***	8.35***	7.63**	1.78 N.S.	20.03
Gln	9.15***	14.09***	2.94***	20.60***	**22.52******	16.86***	9.07***	4.77
His	3.05***	**65.46******	0.96*	7.34***	9.54***	3.10***	4.48***	6.06
Gly	24.24***	**38.31******	1.59***	20.10***	3.29***	3.45***	3.65***	5.37
Thr + Cit	**82.84******	6.40***	1.07***	5.09***	1.27***	1.03***	0.99***	1.32
Arg	8.25***	**77.12******	0.70**	2.29***	4.20***	2.11***	1.12 N.S.	4.20
Ala	**59.67******	9.65***	0.74 N.S.	5.69***	10.32***	3.50**	1.34 N.S.	9.10
GABA	2.00**	**65.83******	0.24 N.S.	13.68***	2.80**	1.54 N.S.	1.46 N.S.	12.44
Pro	9.76***	**73.31******	0.51 N.S.	7.80***	0.24 N.S.	0.89 N.S.	0.98 N.S.	6.52
Tyr	**60.30******	26.40***	0.10 N.S.	4.59***	3.11***	0.85 N.S.	1.13*	3.52
Val	0.00 N.S.	**47.40******	2.31***	16.96***	14.94***	6.95***	8.37***	3.07
Met	4.89***	**62.97******	2.72***	5.46***	9.42***	6.24***	3.65***	4.65
Ile + Trp	1.07**	**53.39******	4.75***	13.35***	10.70***	5.38***	5.58***	5.79
Leu	2.20***	**55.00******	3.98***	13.35***	10.90***	5.68***	6.62***	2.27
Phe	5.24***	**43.29******	0.44*	10.99***	19.58***	11.24***	5.56***	3.66
Orn	11.00***	**25.48******	2.17***	10.25***	24.55***	12.21***	6.62***	7.72
Lys	8.57***	**45.25******	3.75***	6.45***	13.15***	8.48***	4.91***	9.45
Total aas[a]	2.91***	**61.88******	0.54*	9.41***	13.98***	3.75***	3.76***	3.77
Total aas without Pro	2.60***	**59.81******	0.53*	10.28***	14.91***	3.91***	4.06***	3.89

Statistically significant at ***$p \leq 0.001$; **$p \leq 0.01$; *$p \leq 0.05$. N.S.: not significant ($p > 0.05$)

[a]Total aas: total amino acids

Bold indicates which factor was most influential for each amino acid

Conclusions

The influence of foliar treatments with MeJA and MeJA + Ur on 'Tempranillo' grapes, applied at veraison and 1 week later, during ripening was studied in this research. The evolution of the different amino acids varied between vintages. Overall, during the 2019 season, amino acids reached their highest concentration in grapes at Preharvest moment, whereas in 2020, this maximum was achieved at Postharvest stage. Moreover, the season dependence of the treatments is evident, as the effect of both foliar treatments differed significantly depending on the vintage. In the first season, foliar treatments increased the content of several amino acids in grapes, while no such improvement was observed in 2020 season. The asparagine content in grapes could be used to follow the ripening of grapes, as it decreased from Fol1 to the Postharvest stage in the two vintages studied. As well as the MeJA foliar application increased the content of amino acid precursors of higher alcohols in both seasons. In conclusion, further in-depth research is needed to comprehend the impact of foliar treatments on the amino acid content of grapes, to develop an effective tool for enhancing the nitrogen quality of grapes.

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Data availability The authors declare that the data supporting the findings of this study are available within the paper.

Declarations

Conflict of interest The authors declare there are no conflicts of interest.

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

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