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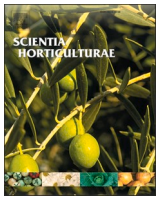

Research Paper

Methyl jasmonate and/or urea, conventionally and on nanoparticles, foliar applications: Influence on grape amino acids composition

M. González-Lázaro [a], E.P. Pérez-Álvarez [a,*], B. Parra-Torrejón [b], S. Marín-San Román [a], I. Sáenz de Urturi [a], R. Murillo-Peña [a], J.M. Delgado-López [b], T. Garde-Cerdán [a,*]

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

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ABSTRACT

Nanotechnology in agriculture provides a strategic and sustainable solution to climate challenges. In addition, foliar biostimulants serve to mitigate the effects of climate change in viticulture. This work aims to evaluate the impact of foliar applications of methyl jasmonate (MeJ), urea (Ur), and their combination (MeJ+Ur), applied either conventionally (free) or supported on amorphous calcium phosphate nanoparticles (ACP), on the amino acids composition of Tempranillo grapes. These nitrogen compounds were analyzed by high pressure liquid chromatography (HPLC). Among the conventional applications, MeJ+Ur proved to be the most effective in increasing the amino acids content in grape must. Moreover, the combined application of MeJ and Ur showed a synergistic effect, enhancing their effect on the grape amino acids content. The ACP-MeJ+Ur foliar treatment resulted in the highest increase in total amino acids content among all applications studied, using significantly less MeJ and Ur compared to the conventional treatment. Discriminant analysis highlighted the distinctiveness of MeJ+Ur and ACP-MeJ+Ur, emphasizing their influence on the amino acids composition of grapes. Nanotechnology in viticulture offers a promising approach for sustainable practices, reducing environmental impact while maintaining grape quality.

1. Introduction

The emergence of nanotechnology has positioned its application in agriculture as a crucial tool for realizing the objective of global sustainable food production. Active nanoparticles can act as a direct source of micronutrients or serve as delivery platform for bioactive agrochemicals, thereby promoting improved crop growth, enhanced yield, and superior crop quality. The physico-chemical properties of nanoparticles, including size, shape, surface chemistry, and coatings, exert significant influences on crops, soil health, and the surrounding ecosystem. Hence, the judicious selection of nanoparticles with appropriate physico-chemical characteristics, along with their application through suitable agricultural methods, represents an intelligent strategy approach to achieving sustainable agriculture and elevate overall plant performance (Balusamy et al., 2023; Dilnawaz et al., 2023; Garde-Cerdán et al., 2021, 2023c). Among nanomaterials, biomimetic calcium phosphate nanoparticles (ACP NPs) stand out due to their intrinsic ability to host foreign molecules, such as methyl jasmonate (MeJ) and urea, making them valuable nanocarriers for precise delivery (Delgado-López et al., 2014; Ramírez-Rodríguez et al., 2020; Pérez-Álvarez et al., 2021; Parra-Torrejón et al., 2021). Their composition, mainly Ca and P, provides plants with essential macronutrients. Additionally, the tunable solubility of ACP NPs enables the gradual and controlled release of the active compounds, thereby reducing the required dosages and, consequently, minimizing associated negative environmental impacts (Ramírez-Rodríguez et al., 2020; Pérez-Álvarez et al., 2021; Parra-Torrejón et al., 2021).

One of the current challenges in agriculture is mitigating the adverse effects of climate change on crop productivity and vulnerability, which is essential for ensuring food security and sustained agricultural development. Innovations in agro-nanotechnology, focusing on "low input but maximum output" farming practices, are well-aligned with the goal of achieving desired crop production (Rani Sarkar et al., 2022). Under these circumstances, winegrowers are actively investigating and adopting various approaches, including the foliar application of biostimulants, to enhance the quality of both grapes and wines

* Corresponding author.
E-mail addresses: evapilar.perez@icvv.es (E.P. Pérez-Álvarez), teresa.garde.cerdan@csic.es (T. Garde-Cerdán).

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(Garde-Cerdán et al., 2023c; Marín-San Román et al., 2023; Parra-Torrejón et al., 2021; Pérez-Álvarez et al., 2022). Methyl jasmonate (MeJ) is a widely studied elicitor in viticulture, commonly applied as a foliar treatment to enhance the chemical composition of grapes. MeJ is capable of inducing a response in grapevines that stimulates the synthesis of secondary metabolites, including nitrogen, phenolic, and volatile compounds (Garde-Cerdán et al., 2016, 2023a, 2023b, 2024; Gil-Muñoz et al., 2018; Portu et al., 2017). However, due to its high cost, low water solubility and high volatility, alternatives are being sought to use it in a lower dose, and nanotechnology is a huge tool to achieve this goal (Garde-Cerdán et al., 2023c, 2023d; Gil-Muñoz et al., 2021).

On the other hand, foliar fertilization, especially with urea at veraison, is widely implemented in viticulture due to its cost-effectiveness and rapid plant uptake (Gutiérrez-Gamboa et al., 2019a). Over the last few decades, advancements in scientific understanding of plant responses to foliar fertilization have led to increased adoption of this practice in agriculture. Leaves, through their cuticles and stomata, can uptake nutrients non-selectively, in contrast to the more selective uptake by roots (Verdenal et al., 2021). Foliar urea application during veraison has been shown to effectively enhance grape nitrogen content without affecting plant vigor (Lasa et al., 2012). The hydrophilic nature of urea facilitates the efficient transport of nitrogen metabolites from leaves to sink organs. In this context, as with MeJ, urea has also been applied supported on nanoparticles. Pérez-Álvarez et al. (2021) reported that calcium phosphate nanoparticles doped with urea successfully decrease the amount of urea required for fertilization, thereby mitigating the environmental impact while maintaining grape quality. Gaiotti et al. (2021) investigated the use of urea-doped nanoparticles as an alternative to conventional fertilizers and observed a similar nutritional status in plants whether they were nourished with nanoparticles or a conventional fertilizer. Furthermore, the yield and quality parameters of grapes were found comparable among plants treated with different nitrogen sources. More recently, Giménez-Bayón et al. (2024) investigated the impact of foliar applications of urea and urea-doped nanoparticles on stilbene synthesis in Monastrell vineyards during the 2020 ripening period. The study found that both treatments led to an increase in total stilbene content compared to the control. Notably, conventional urea application resulted in more pronounced increases than nano-urea, although nano-urea still outperformed the control. Torres-Díaz et al. (2024) explored the effects of foliar applications ACP nanoparticles doped with methyl jasmonate and urea on the volatile compounds content in Tempranillo grapes. The nanoparticle treatments reduced undesirable C6 and carbonyl compounds, while increasing desirable aroma compounds, even at lower doses of MeJ and Ur. These findings suggest that nanotechnology could enhance grape aromatic quality and promote more sustainable viticulture practices by reducing biostimulant use.

For all these reasons, the study of foliar applications using nanotechnology can open an interesting path towards sustainable viticulture. To our knowledge, this is the first study to jointly examine the foliar application of MeJ, Ur, and their combination (MeJ+Ur), applied conventionally or via nanoparticles, on the amino acids content of Tempranillo grapes. These foliar treatments have been studied individually in several previous studies, but their individual application and the combination of both, particularly using nanotechnology as a tool to mitigate the effects of climate change in viticulture, has not been previously compared. Our initial hypothesis is that foliar treatments will enhance the amino acids content in grapes. Additionally, treatments using nanoparticles, which require a significantly lower doses of the product, may improve the nitrogen composition of the musts, potentially in a similar manner to conventional foliar applications. Therefore, the objective of this work was to investigate the influence of the foliar applications of methyl jasmonate, urea, and their combination, applied both conventionally and via nanoparticles, on the amino acids composition of Tempranillo grapes.

2. Materials and methods

2.1. Vineyard site and experimental design

The Tempranillo grapes (*Vitis vinifera* L.), clone RJ-43, used in this study were cultivated in a commercial vineyard located in Monte Cantabria, Logroño, La Rioja, Spain (42°28'48" N, 2°26'05" W), during the 2021 harvest season. The vineyard was planted in 2015 and is situated at an altitude of 492 meters above sea level, with a planting density of 2,922 vines per hectare. The rows are spaced 3.00 m, with 1.20 m between individual vines. Grapevines were grafted on SO4 rootstock. The vines are trained on a single vertical trellis system and are pruned leaving 4 or 5 thumbs per plant and two buds per thumb. The region experiences a semi-arid continental Mediterranean climate, characterized by warm, dry summers and a concentrated rainfall period in spring.

The study involved the application of various treatments via foliar, including a control group (water), conventional methyl jasmonate solution (MeJ, 10 mM), amorphous calcium phosphate nanoparticles (ACP) loaded with MeJ (ACP-MeJ, 1 mM), urea solution (Ur, 6 kg N/ha), ACP loaded with urea (ACP-Ur, 0.4 kg N/ha), a combination of MeJ and urea (MeJ+Ur, 10 mM + 6 kg N/ha), and ACP loaded with both MeJ and urea (ACP-MeJ+Ur, 1 mM + 0.4 kg N/ha). Urea and MeJ were sourced from Sigma-Aldrich (Madrid, Spain). The preparation of ACP-Ur and ACP-MeJ followed the methodology previously described by Parra-Torrejón et al. (2021) and Pérez-Álvarez et al. (2021), respectively. The ACP-MeJ+Ur nanoparticles were synthesized by mixing equal volumes (2 L) of two solutions: i) an aqueous solution containing 0.2 mol/L calcium chloride and 0.2 mol/L sodium citrate, and ii) an aqueous solution containing 0.12 mol/L dipotassium phosphate and 0.1 mol/L sodium carbonate. After 5 minutes, the ACP precipitate was collected and washed multiple times with ultrapure water by centrifugation (3428 RCF, 15 min). The ACP nanoparticles were then dispersed in 1.5 L of a 4.3 % (m/v) urea solution, followed by the addition of 4 mL of MeJ. The mixture was stirred for 24 hours, after which the ACP--MeJ+Ur nanoparticles were collected by centrifugation as previously described and stored at room temperature. MeJ and urea loading were quantified using UV-Vis spectroscopy and elemental analysis, respectively, following the protocols outlined in previous studies (Parra-Torrejón et al., 2021; Ramírez-Rodríguez et al., 2020). All solutions were prepared using ultrapure water (0.22 μS, 25 °C, Milli-Q©, Millipore, Merck, Darmstadt, Germany).

Foliar applications were carried out using an electric sprayer (Pulmic Pegasus 15 Advance, Grupo Sanz, Spain). The treatments were applied to plants arranged in rows within an edaphologically homogeneous section of the vineyard, avoiding the edges. The experimental design employed randomized block layout with 7 treatments, each conducted in triplicate ($7 \times 3 = 21$ trials), with 5 vines per replicate (105 plants in total). Each plant received 200 mL of the solution twice, at veraison and one week later. Untreated rows were left between the treated ones to prevent cross-contamination. Treatments were conducted when the ambient temperature was below 20°C, typically between 8:00 a.m. and 10:00 a.m. For the treatments, aqueous solutions were prepared using Tween 80 (Sigma-Aldrich) as a wetting agent at a concentration of 1 mL/L. Grapes were harvested at their optimal point of technological maturity, defined as the stage when the weight of 100 berries remained constant and the probable alcohol content was around 13 % (v/v). A sample of 150 berries per replicate and treatment was collected haphazard, frozen and stored at -20°C until the amino acids analyses were performed. Another set of 100 berries was separated and weighed to calculate the average berry weight. The must obtained from crushing the grape berries was analyzed for classical parameters, which data were shown in Torres-Díaz et al. (2024).

2.2. Analysis of amino acids in the musts by HPLC-DAD-FLD

The separation, identification, and quantification of amino acids

were conducted using High-Performance Liquid Chromatography (HPLC) on an Agilent 1260 Infinity Series system, equipped with a Diode Array Detector (DAD) and a Fluorescence Detector (FLD). The amino acids analysis followed the method described by Garde-Cerdán et al. (2016).

Sample preparation involved homogenizing 40 berries using a Masticator homogenizer (IUL Basic, Barcelona, Spain). The homogenized samples were then centrifuged at 4,000 rpm for 10 minutes at 20°C. For each must sample (5 mL), 100 μL of sarcosine (used as an internal standard for proline quantification) and 100 μL of norvaline (used as an internal standard for primary amino acids quantification) were added. The mixture was filtered through a 0.22 μm filter and underwent automatic derivatization. Primary amino acids were derivatized using o-phthaldialdehyde (OPA Reagent, Agilent), while proline, a secondary amino acid, was derivatized using 9-fluorenylmethylchloroformate (FMOC Reagent, Agilent). A 10 μL volume of the derivatized sample was injected, and the column temperature was maintained at a constant 40°C. Separations were performed on a Hypersil ODS column (250 × 4.0 mm, I.D. 5 μm, Agilent).

The mobile phases consisted of:

A: 75 mM sodium acetate and 0.018 % triethylamine (pH 6.9) + 0.3 % tetrahydrofuran
B: a mixture of water, methanol, and acetonitrile (10:45:45, v/v/v).

Amino acids were identified by comparing retention times with standards (Sigma-Aldrich) and UV–Vis spectral characteristics. Quantification was based on calibration graphs for each standard ($R^2 > 0.96$). Detection was carried out using the DAD at two wavelengths (λ = 338 nm for primary amino acids; λ = 262 nm for the secondary amino acid, proline); and the FLD (λ excitation = 340 nm, λ emission = 450 nm, for primary amino acids; λ excitation = 266 nm, λ emission = 305 nm, for the secondary amino acid, proline).

Since the treatments were conducted in triplicate, the results for must nitrogen compounds are expressed as the mean of the three replicates (n = 3).

2.3. Statistical analyses

The data were analyzed using SPSS statistical software, Version 21.0 for Windows (SPSS, Chicago, USA). Amino acids data were subjected to variance analysis (ANOVA) with a significance level set at $p \leq 0.05$. Post-hoc comparisons between samples were conducted using Duncan test at a 95% confidence level. Discriminant analysis was employed to classify the different samples based on their amino acids composition.

3. Results and discussion

3.1. Effect of foliar treatments on must amino acids composition

Table 1 shows the amino acids content in musts from the seven treatments under study. Nitrogen (N) plays a crucial role in various biological processes in both grapevines and fermentative microorganisms. Additionally, amino acids are precursors of some volatile compounds (Garde-Cerdán and Ancín-Azpilicueta, 2008). Therefore, grapes with an adequate amino acids content are essential to elaborate quality wines. All amino acids concentrations measured in the musts were within the ranges described in the literature (Bell and Henschke, 2005), except for the arginine and alanine values in ACP-MeJ+Ur sample, which exceeded the specified range. Additionally, the cysteine content in the Ur sample and the tryptophan content across all samples studied were higher than those described in the literature (Bell and Henschke, 2005). In all the wines elaborated in this work, *Saccharomyces cerevisiae* was the yeast employed. *S. cerevisiae* exhibits a selective approach in nitrogen consumption, employing distinct molecular mechanisms to prioritize specific nitrogen sources. Ammonium ions and free amino

Table 1
Amino acids content (mg/L) in must from control, methyl jasmonate (MeJ), methyl jasmonate on nanoparticles (ACP-MeJ), urea (Ur), urea on nanoparticles (ACP-Ur), methyl jasmonate + urea (MeJ+Ur), and methyl jasmonate + urea on nanoparticles (ACP-MeJ+Ur) foliar treatments.

	Control	MeJ	ACP-MeJ	Ur	ACP-Ur	MeJ+Ur	ACP-MeJ+Ur
Aspartic acid	26.13 ± 1.98 c	22.59 ± 2.12 ab	20.65 ± 0.05 a	22.79 ± 1.56 ab	24.48 ± 0.03 bc	20.87 ± 2.66 a	20.00 ± 0.71 a
Glutamic Acid	52.17 ± 1.34 d	44.75 ± 3.52 c	28.22 ± 0.52 a	36.94 ± 5.84 b	45.05 ± 2.85 c	26.21 ± 3.56 a	26.73 ± 4.96 a
Asparagine	13.03 ± 1.49 a	23.58 ± 2.17 b	13.42 ± 0.91 a	14.02 ± 2.23 a	10.73 ± 0.17 a	37.05 ± 2.40 c	12.75 ± 2.08 a
Serine	64.58 ± 1.68 bc	71.93 ± 1.51 c	44.30 ± 2.42 a	58.69 ± 10.34 bc	66.68 ± 8.42 c	69.65 ± 10.27 c	52.75 ± 7.22 ab
Glutamine	686.20 ± 58.63 b	834.02 ± 3.43 c	514.03 ± 52.49 a	836.52 ± 110.88 c	736.30 ± 22.95 bc	745.72 ± 86.88 bc	540.17 ± 84.04 a
Histidine	107.59 ± 0.94 bc	124.02 ± 7.75 c	61.38 ± 5.36 a	94.29 ± 17.64 b	108.70 ± 17.68 bc	106.58 ± 10.73 bc	67.68 ± 5.91 a
Glycine	6.97 ± 0.17 bc	9.31 ± 0.70 d	5.39 ± 0.39 a	6.18 ± 0.50 ab	7.72 ± 1.06 c	9.24 ± 0.38 d	5.95 ± 0.49 ab
Threonine	89.07 ± 1.03 b	90.01 ± 1.24 b	62.12 ± 0.17 a	74.30 ± 14.28 ab	91.28 ± 10.68 b	88.65 ± 13.48 b	73.49 ± 9.07 ab
Arginine	1410.49 ± 1.42 b	1407.12 ± 109.73 b	1041.26 ± 48.06 a	975.11 ± 21.29 a	1622.71 ± 165.87 c	1879.19 ± 140.54 d	2596.82 ± 168.44 e
Alanine	148.33 ± 3.34 bc	155.66 ± 17.64 c	111.58 ± 0.12 a	122.20 ± 19.29 ab	143.63 ± 24.65 abc	202.33 ± 27.96 d	267.81 ± 9.68 e
γ-Aminobutyric acid (GABA)	274.07 ± 3.64 c	230.72 ± 3.40 b	192.63 ± 8.15 a	272.84 ± 6.05 c	324.09 ± 28.88 d	355.50 ± 19.65 e	454.99 ± 27.96 f
Tyrosine	13.40 ± 0.46 e	10.12 ± 0.51 cd	7.36 ± 0.26 b	12.06 ± 0.34 de	17.13 ± 2.90 f	8.78 ± 0.43 bc	4.20 ± 0.09 a
Cysteine	5.60 ± 0.29 ab	6.70 ± 0.08 b	4.34 ± 0.10 a	9.94 ± 2.05 c	5.59 ± 0.90 ab	8.38 ± 0.53 c	4.98 ± 0.76 ab
Valine	31.51 ± 2.02 b	45.38 ± 7.94 c	35.78 ± 3.01 bc	15.63 ± 3.00 a	20.23 ± 2.51 a	41.37 ± 8.54 bc	31.47 ± 6.06 b
Methionine	17.62 ± 1.04 a	26.78 ± 2.46 b	15.74 ± 1.85 a	17.28 ± 2.80 a	16.84 ± 2.86 a	24.69 ± 3.60 b	24.70 ± 4.22 b
Tryptophan	76.53 ± 0.83 c	73.51 ± 0.94 c	48.57 ± 1.63 a	48.54 ± 0.47 a	77.28 ± 12.01 c	68.84 ± 10.18 bc	60.25 ± 7.51 ab
Phenylalanine	28.38 ± 3.45 c	23.63 ± 2.73 b	18.67 ± 2.37 a	22.40 ± 1.78 ab	22.77 ± 1.44 ab	20.00 ± 2.05 ab	19.06 ± 0.88 a
Isoleucine	21.08 ± 2.36 b	36.56 ± 7.54 c	19.27 ± 3.29 b	11.60 ± 1.78 a	18.33 ± 3.24 ab	43.63 ± 4.85 d	20.73 ± 1.24 b
Leucine	39.70 ± 5.27 b	70.61 ± 13.21 c	31.57 ± 5.84 ab	24.21 ± 2.89 a	40.59 ± 4.09 b	82.08 ± 9.44 c	33.13 ± 0.83 ab
Lysine	10.42 ± 0.98 d	11.95 ± 0.44 e	7.84 ± 0.21 b	3.80 ± 0.28 a	7.65 ± 0.58 b	8.94 ± 0.58 c	4.71 ± 0.51 a
Proline	992.11 ± 41.36 c	836.03 ± 7.73 ab	784.63 ± 2.52 a	969.82 ± 100.21 c	959.12 ± 34.52 c	915.35 ± 40.85 bc	809.22 ± 57.33 a
Total amino acids	4114.98 ± 97.95 c	4154.98 ± 157.30 c	3068.74 ± 122.71 a	3649.16 ± 185.42 b	4366.91 ± 268.46 c	4763.04 ± 148.23 d	5131.59 ± 225.51 e
Total amino acids whitout proline	3122.87 ± 56.59 c	3318.95 ± 165.03 c	2284.12 ± 120.19 a	2679.34 ± 175.99 b	3407.79 ± 241.25 c	3847.69 ± 188.13 d	4322.37 ± 270.21 e

All nitrogen compounds are listed with their standard deviation (n=3). Different letters indicate significant differences among samples ($p \leq 0.05$).

acids are readily assimilated by yeast, contributing to yeast assimilable nitrogen (YAN) (Gil-Muñoz et al., 2021). Overall, the amino acids found in higher concentrations in all must samples were arginine, proline and glutamine, representing collectively around a 75 % of the total amino acids (Table 1). In contrast, the least abundant amino acids were cysteine, glycine, lysine, tyrosine, and asparagine, together accounting for 1.5 % of the total amino acids. This observation aligns with previous findings in Tempranillo musts, considering the influence of vintage on the amino acids content of grapes (Garde-Cerdán et al., 2024; Gutiérrez-Gamboa et al., 2019b). Control grapes showed the highest content of glutamic acid and phenylalanine (Table 1). In contrast, MeJ grapes presented a higher content of asparagine, glutamine, glycine, valine, methionine, isoleucine, leucine, and lysine than control grapes. This indicates that foliar application of MeJ enhanced the content of several amino acids, although the total amino acids content, both with and without proline, did not differ significantly from that of the control grapes (Table 1). These findings are consistent with those of Garde-Cerdán et al. (2016), who also reported an increase in certain amino acids after MeJ foliar application. Nevertheless, Garde-Cerdán et al. (2023c) observed that the effects of MeJ foliar application can vary depending on the vintage under consideration, likely due to climatic conditions. In the first year of their study, characterized by lower rainfall, MeJ foliar application enhanced the synthesis of most amino acids. Conversely, in the second year, control must exhibited a higher total amino acids content than the MeJ must.

ACP-MeJ must showed a lower content of most of amino acids than control grapes, and both the total amino acids content and the total amino acids content without proline were lower (Table 1). This suggest that ACP-MeJ treatment did not enhance amino acids biosynthesis in the grapes. This result contrasts with the findings of Gil-Muñoz et al. (2021) in Monastrell variety, where an increase in amino acids content was observed after ACP-MeJ foliar treatment. Moreover, Garde-Cerdán et al. (2023c) noted that the impact of ACP-MeJ foliar application varied depending on the vintage. In the first vintage, ACP-MeJ foliar treatment led to an increased in six amino acids and total amino acids content compared to the control sample. However, in the second year, amino acids content in ACP-MeJ grapes was similar to the must control sample. This differential response to ACP-MeJ foliar application may be attributed to the plant's nitrogen requirements or varying climatological conditions. Ur must exhibited higher levels of glutamine and cysteine compared to control grapes, while displaying lower content of aspartic acid, glutamic acid, arginine, valine, tryptophan, phenylalanine, isoleucine, leucine, lysine, total amino acids, and total amino acids without proline (Table 1). However, ACP-Ur foliar treatment increased the content of arginine, GABA, and tyrosine, but resulted in lower content of glutamic acid, valine, phenylalanine, and lysine compared to control must. No significant differences were observed in the total amino acids content, either with or without proline, when compared with control must (Table 1). These results differ somewhat from those described by Pérez-Álvarez et al. (2021), who found that nanoparticles doped with urea led to grapes with the highest glutamic acid content, whereas our study showed a lower glutamic acid content than in control must. In addition, they observed that phenylalanine, tryptophan, and threonine levels were comparable to those in grapes treated with conventional urea (6 kg N/ha), which is consistent with our findings for phenylalanine. The MeJ+Ur must displayed higher levels of asparagine, glycine, arginine, alanine, GABA, cysteine, methionine, isoleucine, leucine, total amino acids, and total amino acids without proline compared to control must (Table 1). Additionally, it showed lower concentrations of aspartic acid, glutamic acid, tyrosine, phenylalanine, and lysine compared to the control must. The foliar application of MeJ and MeJ+Ur resulted in an increase in eight and nine amino acids, respectively. Notably, the impact of MeJ+Ur was more pronounced, as it not only elevated specific amino acids but also enhanced the overall total amino acids content (Table 1). This suggests a potential synergistic effect between MeJ and Ur in the biosynthesis of amino acids, as previously described by Garde-Cerdán et al. (2024). The ACP-MeJ+Ur foliar treatment increased the content of arginine, alanine, GABA, total amino acids, and total amino acids without proline compared to the control must. Furthermore, it also led to lower content of aspartic and glutamic acids, glutamine, histidine, tyrosine, methionine, tryptophan, phenylalanine, lysine, and proline relative to the control must (Table 1). It is noteworthy that this treatment was the most effective in significantly augmenting the total amino acids content, both with and without proline, while using 10 times less MeJ and 15 times less Ur than the conventional treatment. It is important to highlight that the increase in total amino acids content was mainly driven by the increase in arginine, alanine and GABA contents.

The Pro/Arg ratio is commonly employed to classify grape varieties based on the relationship between non-assimilable nitrogen (proline) and assimilable nitrogen (arginine). This index serves as a valuable indicator for assessing the nutritional value of grape must for yeast in a specific cultivar (Bell and Henschke, 2005). Proline and arginine are the predominant amino acids in grapes, exerting a significant influence during alcoholic fermentation. Yeasts prioritize the consumption of arginine as the initial amino acid, whereas proline exhibits limited assimilation, particularly under anaerobic fermentation settings (Stines et al., 2000). In our study, the control must showed a Pro/Arg ratio lower than 1 (0.70), indicating that Tempranillo variety does not an accumulating variety of Pro. However, previous works have classified Tempranillo as a proline accumulator (Garde-Cerdán et al., 2014; López et al., 2012). This discrepancy may be attributed to the fact that proline/arginine ratio also is an index influenced by vintage and grape maturity (Bell and Henschke, 2005). In addition, the foliar treatments applied affected this index. The ratios for MeJ (0.59), ACP-Ur (0.59), MeJ+Ur (0.48) and ACP-MeJ+Ur (0.31) showed a reduction in this value. This reduction may result from either a decrease in proline content or an increase in arginine content due to the effect of these foliar treatments. Bell and Henschke (2005) reported that nitrogen application in vineyards decreases the proline/arginine ratio in grapes, regardless of whether the vines are nitrogen-deficient or adequately supplied with nitrogen. The ACP-MeJ+Ur treatment yielded the lowest Pro/Arg ratio, suggesting that the ACP-MeJ+Ur must had a higher amount of arginine available for the yeast, which would create more favorable conditions for fermentation. The observed difference in the Pro/Arg ratio between the MeJ+Ur and ACP-MeJ+Ur musts is noteworthy. Specifically, the application of MeJ+Ur treatment with nanoparticles, indicating a reduction in the dosage of applied treatments, resulted in a more significant increase in arginine content and a more pronounced decrease in proline content. Conversely, ACP-MeJ (0.75) must showed a similar value for this index than control must, while ACP-Ur (0.59) also presented a Pro/Arg ratio lower than that of the control. In contrast, the conventional application of urea (0.99) resulted in a Pro/Arg ratio higher than of the control must.

Additionally, some amino acids serve as precursors of fermentative aromatic compounds, i.e., threonine, tyrosine, valine, methionine, isoleucine, tryptophan, leucine, and phenylalanine. The MeJ foliar application increased the levels of four of these precursors (valine, methionine, isoleucine, and leucine), while ACP-Ur raised only the tyrosine content. The MeJ+Ur treatment also increased the levels of methionine, isoleucine, and leucine, whereas ACP-MeJ+Ur increased only the methionine content. These nitrogen compounds contribute significantly to the fermentative *bouquet* of wine and its organoleptic quality. However, foliar application of ACP-MeJ and Ur did not enhance the levels of any of these key aromatic precursors, suggesting that among the treatments studied, MeJ foliar treatment is more likely to positively influence the wine *bouquet*.

3.2. *Influence of the foliar application methods on must nitrogen compounds*

Table 2 shows the impact of the different foliar application methods

Table 2
Influence of treatment type on the amino acids composition of the must. One-factor ANOVA.

	MeJ/ACP-MeJ		Ur/ACP-Ur		MeJ+Ur/ACP-MeJ+Ur		MeJ/Ur/MeJ+Ur			ACP-MeJ/ACP-Ur/ACP-MeJ+Ur		
Amino acids	F	*p*	F	*p*	F	*p*	F	*p*		F	*p*	
Aspartic acid	**77.08**	**0.00**	**17.85**	**0.01**	6.24	0.07	0.719	0.52	a/a/a	**64.40**	**0.00**	a/b/a
Glutamic Acid	**64.92**	**0.00**	4.68	0.10	0.02	0.89	**13.19**	**0.01**	b/b/a	**28.25**	**0.00**	a/b/a
Asparagine	**55.97**	**0.00**	6.49	0.06	**175.61**	**0.00**	**78.08**	**0.00**	b/a/c	3.40	0.10	a/a/a
Serine	**281.08**	**0.00**	1.08	0.36	5.44	0.08	2.10	0.20	a/a/a	**8.92**	**0.02**	a/b/a
Glutamine	**111.04**	**0.00**	2.35	0.20	**8.67**	**0.04**	1.21	0.36	a/a/a	**12.84**	**0.01**	a/b/a
Histidine	**132.53**	**0.00**	1.00	0.37	**30.27**	**0.01**	4.13	0.07	b/a/ab	**15.80**	**0.00**	a/b/a
Glycine	**71.32**	**0.00**	5.16	0.09	**85.12**	**0.00**	**32.63**	**0.00**	b/a/b	**8.73**	**0.02**	a/b/a
Threonine	**1478.23**	**0.00**	2.72	0.17	2.61	0.18	1.76	0.25	a/a/a	**9.90**	**0.01**	a/b/a
Arginine	**27.98**	**0.01**	**44.99**	**0.00**	**32.10**	**0.00**	**57.07**	**0.00**	b/a/c	**95.54**	**0.00**	a/b/c
Alanine	**18.73**	**0.01**	1.41	0.30	**14.69**	**0.02**	**9.95**	**0.01**	a/a/b	**87.40**	**0.00**	a/b/c
γ-Aminobutyric acid (GABA)	**55.84**	**0.00**	**9.05**	**0.04**	**25.43**	**0.01**	**83.46**	**0.00**	a/b/c	**92.08**	**0.00**	a/b/c
Tyrosine	**69.73**	**0.00**	**9.06**	**0.04**	**325.17**	**0.00**	**43.18**	**0.00**	b/c/a	**48.28**	**0.00**	b/c/a
Cysteine	**1034.97**	**0.00**	**11.37**	**0.03**	**40.37**	**0.00**	**5.28**	**0.05**	a/b/ab	2.53	0.16	a/a/a
Valine	3.83	0.12	4.15	0.11	2.68	0.18	**16.17**	**0.00**	b/a/b	**11.13**	**0.01**	b/a/b
Methionine	**38.73**	**0.00**	0.04	0.86	0.00	1.00	**8.37**	**0.02**	b/a/b	**7.33**	**0.02**	a/a/b
Tryptophan	**526.23**	**0.00**	**17.16**	**0.01**	1.39	0.30	**15.15**	**0.00**	b/a/b	**9.24**	**0.01**	a/b/b
Phenylalanine	5.67	0.08	0.08	0.79	0.53	0.51	2.08	0.21	a/a/a	**5.45**	**0.04**	a/b/a
Isoleucine	**13.26**	**0.02**	**9.92**	**0.03**	**62.79**	**0.00**	**30.50**	**0.00**	b/a/b	0.58	0.59	a/a/a
Leucine	**21.92**	**0.01**	**32.11**	**0.00**	**79.98**	**0.00**	**31.07**	**0.00**	b/a/b	4.06	0.08	ab/b/a
Lysine	**209.66**	**0.00**	**106.51**	**0.00**	**91.22**	**0.00**	**251.86**	**0.00**	c/a/b	**43.29**	**0.00**	b/b/a
Proline	**119.76**	**0.00**	0.03	0.87	6.82	0.06	3.46	0.10	a/b/ab	**17.90**	**0.00**	a/b/a
Total amino acids	**88.93**	**0.00**	**14.52**	**0.02**	5.60	0.08	**34.52**	**0.00**	b/a/c	**70.94**	**0.00**	a/b/c
Total amino acids whitout proline	**77.08**	**0.00**	**17.85**	**0.01**	6.24	0.07	6.24	0.07	b/a/c	**64.40**	**0.00**	a/b/c

For each compound, different letters indicate differences between treatments ($p \leq 0.05$).

on the amino acid content in grape must. The results show that the method of application significantly influenced the levels of nearly all amino acids when comparing MeJ (conventional) with ACP-MeJ (nanoparticles). Notably, only valine and phenylalanine did not exhibit significant differences between the conventional and nanoparticle-based treatments. In this study, MeJ generally resulted in a greater increase in amino acids content compared to ACP-MeJ. This finding is consistent with Garde-Cerdán et al. (2023c), who observed a similar trend in the first vintage of their study. It should be remembered that foliar application of MeJ implies 10 times the amount of the elicitor in comparison with ACP-MeJ. When comparing the effects of urea applied in conventional form (Ur) *versus* urea applied via nanoparticles (ACP-Ur), the results differed from those observed with MeJ. In this case, the ACP-Ur treatment led to a significant increase in the levels of eight amino acids, as well as in levels of the total amino acids content, both with and without proline, when compared to the Ur treatment, which only increased cysteine (Table 2). The enhanced effectiveness of the ACP-Ur treatment is particularly noteworthy, especially considering that it involved a 15-fold lower urea dose than the conventional application. Pérez-Álvarez et al. (2021) also reported that foliar application of ACP-Ur could increase amino acids content similarly to conventional treatment but with a dose 15 times lower. Comparing MeJ+Ur and ACP-MeJ+Ur treatments, MeJ+Ur foliar application increased the content of asparagine, glutamine, histidine, glycine, tyrosine, cysteine, isoleucine, leucine, and lysine in the must, whereas ACP-MeJ+Ur only increased arginine, alanine, and GABA (Table 2). Therefore, the conventional foliar application of MeJ+Ur was more effective than ACP--MeJ+Ur, in terms of the number of amino acids affected. However, when compared to the control, ACP-MeJ+Ur showed a greater increase in total amino acids content, both with and without proline, than MeJ+Ur.

Regarding the comparison of the three treatments applied in their conventional forms, the MeJ+Ur treatment resulted in the highest content of asparagine, arginine, alanine, GABA, total amino acids and total amino acids without proline (Table 2). In addition, the MeJ+Ur must showed similar levels of glutamic acid, histidine, glycine, valine, methionine, tryptophan, isoleucine, and leucine to the MeJ must, and a comparable content of cysteine and proline than Ur must. The MeJ sample presented the highest content of lysine and a similar content of glutamic acid than Ur must. Meanwhile, the Ur sample showed the highest tyrosine content . No significant differences were detected in the levels of aspartic acid, serine, glutamine, threonine, and phenylalanine among the three conventional treatments. These findings indicate that MeJ+Ur was the most effective conventional foliar treatment for increasing the amino acids content of must. As mentioned above, there may be a synergic effect between Ur and MeJ, which, when applied together, intensifies their effect on the amino acids content of grapes. Finally, when comparing the effects of the three treatments applied on nanoparticles (Table 2), ACP-MeJ+Ur showed the highest content of arginine, alanine, GABA, methionine, total amino acids, and total amino acids without proline (Table 2). Although ACP-Ur affected a broader range of amino acids than ACP-MeJ+Ur, its impact on total amino acids was lower than ACP-MeJ+Ur, but higher than that of ACP-MeJ sample. The ACP-Ur must was characterized by the highest content of aspartic acid, glutamic acid, serine, glutamine, histidine, glycine, threonine, tyrosine, phenylalanine, and proline, and a similar content of lysine than ACP-MeJ sample. Furthermore, ACP-Ur presented a similar content of tryptophan than ACP-MeJ+Ur. No significant differences were found among the treatments applied with nanoparticles in terms of cysteine and isoleucine content. Finally, the ACP-MeJ must did not stand out in the content of any amino acid, but it did show a similar valine content to ACP-MeJ+Ur (Table 2). Therefore, among the foliar applications using nanoparticles, ACP-MeJ+Ur was the most effective in increasing the total amino acids content, although ACP-Ur led to the highest content of several amino acids.

In line with the initial hypothesis, all vineyard foliar treatments enhanced the content of certain amino acids, except for ACP-MeJ treatment, which did not result in any improvement. Similarly, the Ur foliar application increased the content of only two amino acids and did not significantly affect the total amino acids content. Notably, ACP-MeJ+Ur treatment, which utilized a significantly lower dose, proved to be the most effective in enhancing the nitrogen fraction of the musts.

3.3. Discriminant analysis

Fig. 1 shows the results of the discriminant analysis conducted on the amino acids of the musts (control, MeJ, ACP-MeJ, Ur, ACP-Ur, MeJ+Ur, and ACP-MeJ+Ur). The analysis was performed with the concentrations

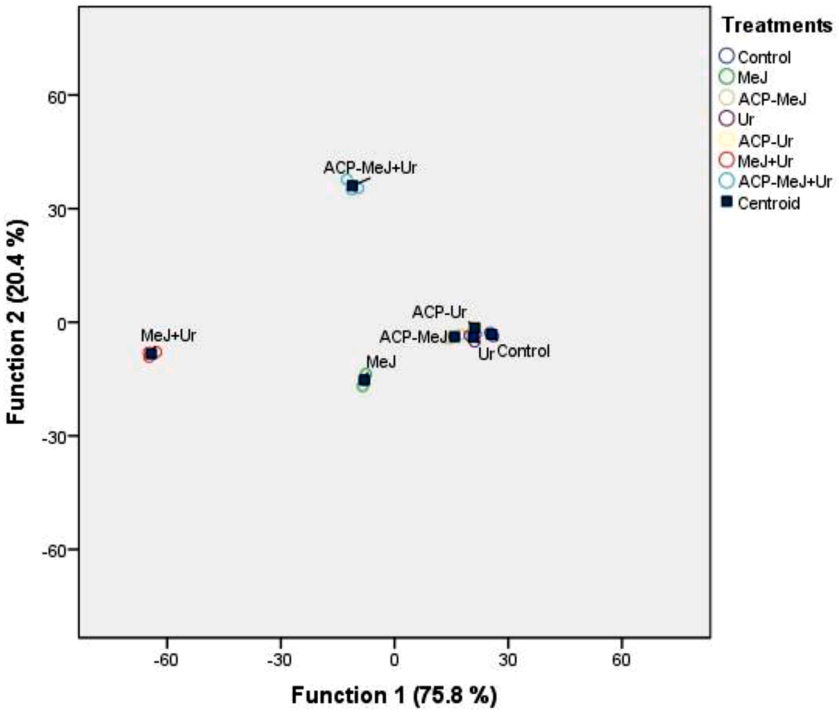

Fig. 1. Discriminant analysis of the amino acids belonging to the must samples obtained in grapes from control, methyl jasmonate (MeJ), methyl jasmonate on nanoparticles (ACP-MeJ), urea (Ur), urea on nanoparticles (ACP-Ur), methyl jasmonate + urea (MeJ+Ur), and methyl jasmonate + urea on nanoparticles (ACP-MeJ+Ur) foliar treatments.

of the identified amino acids, treating each variable as independent. In the discriminant analysis, the samples were grouped as follows: MeJ, ACP-MeJ, Ur, ACP-Ur, and control samples formed one group; MeJ+Ur formed another group; and ACP-MeJ+Ur formed a distinct group on the discriminant plot. Function 1 explained 75.8 % of the variance, and Function 2 explained 20.4 %, together accounting for 96.2 % of the total variance. Considering the coefficients of the canonical functions, the variables contributing most to the discriminant model in Function 1 were valine, asparagine, glutamic acid, arginine, and lysine. Furthermore, the variables that most favoured the discriminant model in Function 2 were: valine, glycine, GABA, arginine, and asparagine. The discriminant plot revealed that samples with similar or lower total amino acids content were clustered near to control must (Fig. 1). In contrast, MeJ+Ur and ACP-MeJ+Ur, which presented higher content of total amino acids, were positioned separately from each other and from the other group. MeJ+Ur stood out on the left side of the discriminant plot due to its higher content of asparagine and arginine. ACP-MeJ was located in the upper center of the discriminant, highlighting its higher content of arginine and GABA.

4. Conclusions

Addressing the challenges posed by climate change on agricultural productivity is essential for ensuring food security and sustained agricultural development. In viticulture, researchers are exploring different approaches, such as the foliar application of biostimulants, to enhance grape and wine quality. Methyl jasmonate (MeJ), a studied elicitor in viticulture, shows promise in improving grape chemical composition. Additionally, foliar fertilization, particularly utilizing urea at veraison, is widely adopted in viticulture due to its cost-effectiveness and rapid plant uptake. Therefore, the combination of MeJ and urea (MeJ+Ur) treatment has been shown to be exceptionally effective in enhancing the amino acids content of must when applied conventionally, suggesting a synergistic relationship between these compounds.

In addition, the integration of nanotechnology in agriculture has become crucial for achieving global sustainable food production goals. Nanotechnology presents an avenue for effective utilization of sources. According to our results, nanoparticle-based foliar applications of urea (ACP-Ur) offer potential benefits and ACP-MeJ+Ur foliar treatment showed the highest increase in total amino acids content among all the studied foliar applications. The results suggest that targeted foliar treatments can be used to modulate the nitrogen composition of grape musts, potentially improving the fermentation process. The ability to enhance arginine content while reducing proline could lead to more efficient fermentations, with fewer risks of stuck or sluggish fermentations, particularly in anaerobic conditions where proline is less accessible to yeast.

Finally, the results from the discriminant analysis underscored the distinctiveness of the MeJ+Ur and ACP-MeJ+Ur samples, indicating their significant impact on the amino acid composition of the grapes. However, it is noteworthy that the nanotreatment used 10 times less MeJ and 15 times less Ur compared to the conventional treatment, efficiently leveraging resources for maximum impact. These approaches, utilizing nanotechnology in viticulture, demonstrate a promising and efficient path toward sustainable viticulture by reducing environmental impact while preserving grape quality.

CRediT authorship contribution statement

M. González-Lázaro: Writing – original draft, Investigation, Data curation. **E.P. Pérez-Álvarez:** Writing – review & editing, Funding acquisition, Conceptualization. **B. Parra-Torrejón:** Writing – review & editing, Methodology. **S. Marín-San Román:** Writing – review & editing, Investigation, Formal analysis. **I. Sáenz de Urturi:** Writing – review & editing, Methodology, Formal analysis. **R. Murillo-Peña:** Writing – review & editing, Methodology, Investigation. **J.M. Delgado-López:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **T. Garde-Cerdán:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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