

## Influence of foliar application of phenylalanine and urea at two doses to vineyards on grape volatile composition and amino acids content

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### Summary

**The aim was to study the effect of phenylalanine (Phe) and urea (Ur) foliar applications to vines at two dosages on grape volatile and amino acid content. Results showed that the foliar application of both dosages of phenylalanine and the highest dose of Ur favored the synthesis of the aromatic positive compounds, decreasing the presence of C6 compounds in the grapes. Total amino acid content was not modified by the treatments. The treatment that most affected the concentration of amino acids was the lowest dose of Ur, increasing the content of seven amino acids. Phe applications increased the concentration of this amino acid. Therefore, foliar treatments with Phe and Ur were a suitable tool to improve grape volatile composition without affecting grape total nitrogen content.**

**Key words:** nitrogen compounds; aromatic composition; must; grapevine; fertilization; leaves application; *Vitis vinifera*.

### Introduction

Vine requires the input of nitrogen fertilizers to growth and to guarantee an appropriate grape juice nitrogen composition. 140 mg N·L<sup>-1</sup> of yeast assimilable nitrogen (YAN), ensure a correct development of the alcoholic fermentation (BELL and HENSCHKE 2005). Traditionally, vineyard fertilization is carried out by adding fertilizer to the soil, to be absorbed by plant roots. Foliar fertilization allows a quickly and efficient assimilation of applied products by the plants (LASA *et al.* 2012).

Nitrogen addition to the must can be limited or even avoided, if nitrogen fertilization is suitable. A common practice in winemaking is to supplement the must with diammonium phosphate (DAP) to prevent problems related to nitrogen deficiency, but the must continues to be poor in amino acids, and the nitrogen supplementation of grape juice with amino acids improved the wine volatile composition (GARDE-CERDÁN and ANCÍN-AZPILICUETA 2008, TORREA *et al.* 2011). Among the flavors, the compounds that have been more studied belong to the family of terpenes.

C<sub>13</sub> norisoprenoids derived from oxidative degradation of carotenoids. Few esters are present in grapes, whose aroma is usually described as fruity. Benzenoids free forms are derived from phenolic acids. C6 compounds are produced from harvest time until the beginning of fermentation, which are responsible for certain herbaceous and vegetal character of musts and wines (ZALACAIN *et al.* 2007).

It was considered of interest to study the effect of foliar application to the vineyard of different nitrogen sources on grape and wine composition. In a first season, phenylalanine (Phe) and urea (Ur) were the nitrogen sources that provided the best results in order to improve grape and wine quality. These treatments were applied at a dose of 0.9 kg N·ha<sup>-1</sup> and increased the concentration of several amino acids, the varietal aroma and trans-piceid through Phe applications, while Ur improved stilbenes in grape and wines (GARDE-CERDÁN *et al.* 2014, GARDE-CERDÁN *et al.* 2015a, b). Based on this, we considered in the present work to increase the dosage of both nitrogen sources, in a second year of study, using Phe and Ur, at the same dose as the first year (0.9 kg N·ha<sup>-1</sup>) and increasing dosage (1.5 kg N·ha<sup>-1</sup>). The aim was to study the effect of foliar applications to vineyard of two different doses of Phe and Ur on grape volatile and amino acid content.

### Material and Methods

**Samples and grapevine treatments:** The field study was performed on *Vitis vinifera* 'Tempranillo' grapevines located in an experimental vineyard of La Grajera (42°26'27"N, 2°30'54"W, at 640 m above sea level) during the 2013 vintage. Four foliar treatments were tested compared with an untreated control, using urea and phenylalanine at two different doses: Ur1 and Phe1 at 150 mg N·vine<sup>-1</sup> and Ur2 and Phe2 at 250 mg N·vine<sup>-1</sup>. The treatments were prepared as aqueous solution with their corresponding concentration, using Tween 80 as wetting agent at 0.1 % v/v. In the untreated grapevines a water solution containing only Tween 80 was applied. The treatments were repeated to the grapevines at veraison and one week later. 200 mL·vine<sup>-1</sup> of each solution were sprayed over the leaves, applying in each treatment 0.9 kg N·ha<sup>-1</sup> for Ur1 and Phe1 and 1.5 kg N·ha<sup>-1</sup> for Ur2 and Phe2, as-

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suming a total of 3,000 plants·ha<sup>-1</sup>. The treatments were performed in triplicate and were arranged in a complete randomized block design with 3 vines for each replication. Subsequently, the grapes were harvested at their optimum technological maturity, and then were destemmed and crushed. Immediately after, the oenological parameters were determined in the musts. Aliquots of each sample were frozen in order to determine their volatile composition and individual amino acids content.

**Oenological parameters:** Must oenological parameters were analysed according to OIV (2003).

**Analysis of grape volatile compounds:** Volatile compounds were analyzed by the method exposed by GARDE-CERDÁN *et al.* (2015a). These compounds were extracted by a Solid Phase Microextraction (SPME) fiber (DVB/CAR/PDMS, 50/30 mm) (Supelco, Bellefonte, PA, USA). Volatile compounds were separated in a Hewlett-Packard G1800C, GCD series II (Palo Alto, CA, USA) gas chromatograph-electron ionization detector (GC-MS) equipped with a SPB™-20 fused silica capillary column (30 m x 0.25 mm I.D. x 0.25 mm film thickness) (Supelco).

**Analysis of amino acids by HPLC:** Amino acid analysis was performed by the method described by Garde-Cerdán *et al.* (2014). Reversal-phase HPLC Agilent 1100 Series (Palo Alto, USA) was used. 5 mL of the sample was mixed with 100 µL of norvaline, and 100 µL of sarcosine (internal standards). This mixture was submitted to an automatic precolumn derivatization with OPA (Agilent) and with FMOC (Agilent).

**Statistical analysis:** The statistical analysis was performed using SPSS Version 21.0 statistical package for Windows (SPSS, Chicago, USA). The volatile compounds and amino acids statistical analysis was submitted to a variance analysis (ANOVA), and the means were separated by Duncan test, at  $p \leq 0.05$ . For volatile and nitrogen compounds, several comparisons were carried out. On the one hand, each treated sample was compared with the control (appears as ANOVA Control in Tabs 1 and 2, F of Fisher and  $p$ -value). On the other hand, the samples Phe1 and Phe2, and Ur1 and Ur2, were compared in order to study the effect of the dose for each nitrogen source (shown in capital letters in Tabs 1 and 2); and on the other hand, samples Phe1 and Ur1, and Phe2 and Ur2, were compared in order to study the effect of the nitrogen source for each dose (shown in lowercase letters in Tabs 1 and 2).

## Results and discussion

**Effect of treatments on must oenological parameters:** The results on must oenological parameters are discussed by PORTU *et al.* (2015). In brief, the treatments applied to the grapevines did not affect most of the oenological parameters, except for total acidity, tartaric acid and malic acid. These differences were more related to the dose applied rather than the nitrogen source applied.

**Effect of treatments on grape volatile content:** Relative area of benzyl alcohol, total benzenoids and total positive compounds increased, and

total terpenoids, (Z)-3-hexen-1-ol and total C6 compounds decreased through Phe1 applications. In a previous study, the same trend was observed (GARDE-CERDÁN *et al.* 2015a). Phe2 increased relative area of geraniol, total terpenoids, methyl jasmonate, benzyl alcohol, 2-phenylethanol, and total positive compounds compared to control (Tab. 1). Ur1 applications increased relative area of geraniol,  $\beta$ -ionone, and benzyl alcohol, while (Z)- $\beta$ -damascenone, 2-phenylethanal and total benzenoids were decreased compared to control. Terpenoids, C<sub>13</sub> norisoprenoids, esters, benzenoids and C6 compounds decreased their content in the grapes after the treatment with urea (GARDE-CERDÁN *et al.* 2015a). Ur2 applications increased relative area of geraniol, methyl jasmonate, benzyl alcohol and total positive compounds, while 2-phenylethanal, (E)-2-hexenal and C6 compounds decreased with respect to the control grapes. If the dosage for the same nitrogen source was compared, Phe1 increased TDN and (E)-2-hexenal relative area compared to Phe2. Phe2 samples showed higher relative area of  $\alpha$ -terpineol, total terpenoids and methyl jasmonate than Phe1. Ur1 decreased relative area of (Z)- $\beta$ -damascenone, total esters, benzyl alcohol, 2-phenylethanol, 2-phenylethanal and total benzenoids respect to Ur2.

Respect to the lowest dose of the nitrogen sources, Phe1 samples showed higher 2-phenylethanal and total benzenoids relative area than Ur1, while (Z)- $\beta$ -damascenone,  $\beta$ -ionone, total esters and (Z)-3-hexen-1-ol was higher in Ur1 samples. Respect to the highest dose, Phe2 presented higher geranyl acetone and total terpenoids relative area than Ur2, while Ur2 samples showed higher linalool,  $\beta$ -ionone, methyl jasmonate, TDN, hexyl acetate and total esters relative area than Phe2. ANCÍN-AZPILICUETA *et al.* (2013) reported that Ur treatment applied to the grapevines did not affect the concentration of 1-hexanol and benzyl alcohol in wine. In this work, 1-hexanol in grapes was not affected by the treatment, while the relative area of benzyl alcohol was greater in Ur2 samples.

$\alpha$ -Terpineol and geranyl acetone were the major terpenoids in the grapes, while (E)- $\beta$ -damascenone and  $\beta$ -ionone were the major C<sub>13</sub> norisoprenoids found. Concentration of these compounds depends on microclimate factors such as temperature and light, thereby nitrogen supplementation to the grapevines increases vegetative growth and consequently canopy density in comparison to grapevines receiving no supplementary nitrogen (BELL and HENSCHKE 2005). Nitrogen fertilization increased the content of  $\beta$ -damascenone but decreased the concentration of vitispirane, actinidol, and TDN in 'Riesling' wines (LINSSENMEIER and LÖHNERTZ 2007). 2-Hexen-1-ol acetate was the major ester found in grapes, while 2-phenylethanol and 2-phenylethanal were the major benzenoids found. The major C6 compounds in grapes were 1-hexanol and (E)-2-hexen-1-ol. C6 compounds concentration in grapes varied according to the season, vine vigor as well as canopy exposure (YUAN *et al.* 2018). Lower nitrogen supplementation to soil in Pinot noir decreased (E)-2-hexenal and 1-hexenal content in grapes (YUAN *et al.* 2018). These authors suggested that lower nitrogen supply affected either the fatty acid substrates or the enzymes activities, which could result in lower content of C6 compounds.

Table 1

Volatile compound relative area in samples from control vines and from vines treated with different doses (1: 0.9 kg N·ha<sup>-1</sup>; 2: 1.5 kg N·ha<sup>-1</sup>) of phenylalanine (Phe) and urea (Ur)

	Control	ANOVA Control															
		Phe1		Phe2		Ur1		Ur2		Phe1		Phe2		Ur1		Ur2	
		F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
<b>Terpenoids</b>																	
Linalool	0.576 ± 0.009	0.47 ± 0.06A,a	0.54 ± 0.02A,a	0.57 ± 0.06A,a	0.66 ± 0.03A,b	6.359	0.128	7.964	0.106	0.021	0.898	12.812	0.070				
α-Terpinol	1.2 ± 0.2	0.949 ± 0.009A,a	1.5 ± 0.1B,a	1.3 ± 0.1A,a	1.47 ± 0.03A,a	5.055	0.154	3.569	0.199	0.093	0.789	3.401	0.206				
Geraniol	0.21 ± 0.02	0.27 ± 0.02A,a	0.37 ± 0.05A,a	0.31 ± 0.02A,a	0.36 ± 0.04B,a	14.413	0.063	21.462	<b>0.044</b>	48.230	<b>0.020</b>	29.298	<b>0.032</b>				
Geranyl acetone	1.6 ± 0.2	1.36 ± 0.03A,a	1.6 ± 0.1A,b	1.7 ± 0.2A,a	1.15 ± 0.05A,a	2.860	0.233	0.001	0.982	0.518	0.546	12.211	0.073				
Total	3.57 ± 0.02	3.05 ± 0.05A,a	3.98 ± 0.04B,b	3.8 ± 0.4A,a	3.64 ± 0.03A,a	192.637	<b>0.005</b>	185.055	<b>0.005</b>	1.002	0.422	10.100	0.086				
<b>C<sub>13</sub> norisoprenoids</b>																	
β-Cyclocitral	0.9 ± 0.2	1.1 ± 0.2A,a	1.0 ± 0.1A,a	1.4 ± 0.2A,a	1.27 ± 0.03A,a	1.860	0.306	0.826	0.459	8.066	0.105	6.994	0.118				
(Z)-β-Damascenone	1.47 ± 0.09	1.44 ± 0.07A,a	1.5 ± 0.2A,a	0.27 ± 0.01A,b	1.9 ± 0.2B,a	0.071	0.814	0.066	0.821	338.582	<b>0.003</b>	8.661	0.099				
(E)-β-Damascenone	18.4 ± 0.5	17.4 ± 0.6A,a	18 ± 3A,a	20 ± 2A,a	22 ± 2A,a	3.573	0.199	0.037	0.865	3.102	0.220	11.613	0.076				
β-Ionone	3.7 ± 0.7	4.7 ± 0.2A,a	4.9 ± 0.3A,a	6.1 ± 0.3A,b	5.5 ± 0.6A,b	5.106	0.152	5.514	0.143	23.592	<b>0.040</b>	8.053	0.105				
Methyl jasmonate	n.d.	n.d.A,a	0.137 ± 0.006B,a	n.d.A,a	0.291 ± 0.008A,b	n.d.	n.d.	1090.384	<b>0.001</b>	n.d.	n.d.	2509.196	<b>0.000</b>				
TDN	0.24 ± 0.07	0.297 ± 0.008B,a	0.2541 ± 0.0003A,a	0.31 ± 0.07A,a	0.327 ± 0.008A,b	1.476	0.348	0.143	0.742	1.157	0.395	3.290	0.211				
Total	24.7 ± 0.8	24.7 ± 0.9A,a	26 ± 3A,a	28 ± 1A,a	31 ± 2A,a	0.001	0.983	0.152	0.734	10.084	0.086	14.516	0.063				
<b>Esters</b>																	
Hexyl acetate	0.77 ± 0.07	1.0 ± 0.1A,a	0.78 ± 0.08A,a	1.3 ± 0.2A,a	1.2 ± 0.2A,b	5.539	0.143	0.005	0.950	11.014	0.080	9.517	0.091				
2-Hexen-1-ol acetate	1.3 ± 0.3	0.8 ± 0.1A,a	1.1 ± 0.1A,a	0.9 ± 0.1A,a	1.4 ± 0.3A,a	5.503	0.144	0.743	0.480	4.192	0.177	0.302	0.638				
Total	2.0 ± 0.2	1.813 ± 0.003A,a	1.9 ± 0.2A,a	2.15 ± 0.08A,b	2.6 ± 0.1B,b	3.310	0.210	0.764	0.474	0.562	0.532	14.864	0.061				
<b>Benzenoid compounds</b>																	
Benzyl alcohol	n.d.	0.31 ± 0.03A,a	0.40 ± 0.03A,a	0.23 ± 0.03A,a	0.33 ± 0.02B,a	232.211	<b>0.004</b>	292.596	<b>0.003</b>	132.082	<b>0.007</b>	718.801	<b>0.001</b>				
2-Phenylethanol	1.71 ± 0.03	2.7 ± 0.7A,a	2.8 ± 0.2A,a	1.78 ± 0.01A,a	2.5 ± 0.3B,a	4.008	0.183	44.029	<b>0.022</b>	9.985	0.087	17.137	0.054				
2-Phenylethanal	11.8 ± 0.1	19 ± 3A,b	18 ± 5A,a	5.2 ± 0.5A,a	9.3 ± 0.2B,a	11.997	0.074	3.317	0.210	339.052	<b>0.003</b>	217.751	<b>0.005</b>				
Total	13.50 ± 0.08	22 ± 2A,b	21 ± 5A,a	7.2 ± 0.5A,a	12.1 ± 0.5B,a	28.264	<b>0.034</b>	4.702	0.162	268.346	<b>0.004</b>	14.211	0.064				
Total positive compounds	43.6 ± 0.7	51 ± 1A,a	53 ± 2A,a	43 ± 2A,a	50 ± 2A,a	57.245	<b>0.017</b>	42.413	<b>0.023</b>	0.043	0.855	21.066	<b>0.044</b>				
<b>C6 compounds</b>																	
1-Hexanol	28 ± 1	17 ± 4A,a	20 ± 3A,a	26 ± 7A,a	22 ± 3A,a	14.545	0.062	9.108	0.094	0.119	0.763	6.907	0.119				
(Z)-3-Hexen-1-ol	1.1 ± 0.2	0.44 ± 0.03A,a	0.8 ± 0.1A,a	0.86 ± 0.07A,b	1.01 ± 0.05A,a	32.939	<b>0.029</b>	3.888	0.187	3.938	0.186	0.733	0.482				
(E)-2-Hexen-1-ol	12.05 ± 0.02	18 ± 3A,a	13 ± 1A,a	12 ± 1A,a	13.9 ± 0.7A,a	5.321	0.147	3.109	0.220	0.043	0.854	13.067	0.069				
Hexanal	6.1 ± 0.6	7.6 ± 0.4A,a	10 ± 2A,a	11 ± 7A,a	9 ± 1A,a	8.705	0.098	11.937	0.075	1.034	0.416	12.351	0.072				
(E)-2-Hexenal	9 ± 1	6.2 ± 0.4B,a	3.9 ± 0.2A,a	6 ± 3A,a	3.9 ± 0.6A,a	9.822	0.089	30.468	<b>0.031</b>	1.405	0.358	27.560	<b>0.034</b>				
Total	56.4 ± 0.7	49 ± 1A,a	48 ± 4A,a	57 ± 2A,a	50 ± 2A,a	57.245	<b>0.017</b>	8.567	0.100	0.043	0.855	21.066	<b>0.044</b>				

All parameters are listed with their standard deviation (n = 3). For each volatile compound, different capital letters indicate significant differences between Phe1 & Phe2 and between Ur1 & Ur2 (doses effect) treatments and small letters indicate significant differences between Phe1 & Ur1 and between Phe2 & Ur2 (nitrogen source effect) ( $p \leq 0.05$ ). Total positive compounds as expressed as the sum of terpenes + C<sub>13</sub> norisoprenoids + esters + benzenoid compounds. Significant values between control and each of the treatments are in bold ( $p \leq 0.05$ ). TDN: 1,1,6-trimethyl-1,2-dihydronaphthalene.

Table 2

Amino acid concentration (mg·L<sup>-1</sup>) in samples from control vines and from vines treated with different doses (1: 0.9 kg N·ha<sup>-1</sup>; 2: 1.5 kg N·ha<sup>-1</sup>) of phenylalanine (Phe) and urea (Ur)

	Control	ANOVA Control											
		Phe1			Phe2			Ur1			Ur2		
		F	p	F	p	F	p	F	p	F	p	F	p
Arginine (Arg)	490.72 ± 45.63	419.16 ± 42.48A,a	490.87 ± 71.81A,a	550.69 ± 40.59A,a	488.94 ± 52.19A,a	2.635	0.246	0.000	0.998	1.929	0.299	0.001	0.974
Proline (Pro)	429.71 ± 12.85	449.42 ± 60.16A,a	505.40 ± 24.19A,a	481.66 ± 6.65A,a	458.76 ± 20.57A,a	0.205	0.695	15.268	0.060	25.772	<b>0.037</b>	2.870	0.232
Glutamine (Gln)	409.31 ± 129.24	407.17 ± 70.20A,a	465.19 ± 55.64A,a	425.14 ± 17.83A,a	419.36 ± 69.74A,a	0.000	0.985	0.315	0.631	0.029	0.880	0.009	0.932
Glutamic acid (Glu)	97.62 ± 8.55	118.41 ± 19.35A,a	124.11 ± 18.13A,a	129.59 ± 2.03A,a	109.73 ± 15.23A,a	1.933	0.299	3.496	0.202	26.492	<b>0.036</b>	0.962	0.430
g-Aminobutyric acid (GABA)	89.43 ± 22.02	73.60 ± 4.91A,a	93.36 ± 4.30B,b	90.36 ± 1.97B,b	79.09 ± 1.57A,a	0.984	0.426	0.061	0.827	0.004	0.958	0.438	0.576
Histidine (His)	62.10 ± 1.65	57.71 ± 1.93A,a	65.20 ± 5.54A,a	71.04 ± 3.30A,b	70.74 ± 4.76A,a	5.954	0.135	0.575	0.527	11.719	0.076	5.892	0.136
Alanine (Ala)	53.55 ± 5.25	51.91 ± 9.12A,a	59.45 ± 6.29A,a	64.99 ± 3.47A,a	54.17 ± 2.92A,a	0.048	0.847	1.037	0.416	6.612	0.124	0.022	0.896
Serine (Ser)	39.05 ± 0.10	43.96 ± 7.06A,a	48.24 ± 5.30A,a	45.53 ± 1.06B,a	42.13 ± 0.11A,a	0.969	0.429	6.018	0.134	73.559	<b>0.013</b>	816.532	<b>0.001</b>
Tryptophan (Trp)	38.60 ± 0.20	48.33 ± 2.86A,a	49.09 ± 2.24A,a	46.59 ± 0.32A,a	43.99 ± 1.36A,a	22.958	<b>0.041</b>	43.390	<b>0.022</b>	890.088	<b>0.001</b>	30.670	<b>0.031</b>
Threonine (Thr)	33.99 ± 2.50	36.36 ± 2.00A,a	40.70 ± 6.52A,a	44.15 ± 4.81A,a	37.93 ± 1.12A,a	1.093	0.406	1.844	0.307	7.024	0.118	4.143	0.179
Phenylalanine (Phe)	22.25 ± 3.28	37.30 ± 1.72A,b	43.82 ± 3.62A,b	23.61 ± 2.90A,a	24.07 ± 0.89A,a	33.030	<b>0.029</b>	38.913	<b>0.025</b>	0.193	0.703	0.571	0.529
Cysteine (Cys)	18.51 ± 0.23	14.42 ± 0.86A,a	17.20 ± 0.99A,a	20.51 ± 1.44A,b	16.72 ± 0.81A,a	41.910	<b>0.023</b>	3.303	0.211	3.742	0.193	9.145	0.094
Aspartic acid (Asp)	16.43 ± 2.75	20.64 ± 0.69A,a	19.55 ± 1.72A,a	20.70 ± 1.40A,a	19.02 ± 3.25A,a	4.402	0.171	1.849	0.07	3.714	0.190	0.741	0.480
Citrulline (Cit)	16.34 ± 0.64	13.17 ± 0.01A,a	15.42 ± 1.25A,a	17.58 ± 2.21A,a	15.15 ± 1.36A,a	48.801	<b>0.020</b>	0.856	0.452	0.577	0.527	1.258	0.379
Leucine (Leu)	13.76 ± 0.75	13.81 ± 0.92A,a	16.53 ± 2.23A,a	14.54 ± 0.11A,a	13.74 ± 0.88A,a	0.003	0.964	2.768	0.238	2.101	0.284	0.001	0.980
Valine (Val)	12.53 ± 0.19	14.02 ± 1.18A,a	16.82 ± 2.15A,a	15.33 ± 0.14B,a	13.56 ± 0.46A,a	3.086	0.221	7.860	0.107	278.248	<b>0.004</b>	8.525	0.100
Tyrosine (Tyr)	11.60 ± 0.61	13.28 ± 0.66A,a	14.98 ± 0.54A,b	13.82 ± 0.79A,a	13.17 ± 0.18A,a	7.055	0.117	34.653	<b>0.028</b>	9.885	0.088	12.343	0.072
Asparagine (Asn)	9.70 ± 0.44	8.38 ± 1.82A,a	10.98 ± 1.51A,a	10.12 ± 1.09A,a	12.65 ± 1.21A,a	0.994	0.424	1.334	0.367	0.262	0.659	10.508	0.083
Methionine (Met)	4.63 ± 0.50	4.93 ± 0.11A,a	6.33 ± 1.34A,a	6.37 ± 0.55A,a	5.88 ± 0.22A,a	0.679	0.497	2.810	0.236	10.902	0.081	10.306	0.085
Ornithine (Orn)	3.94 ± 0.06	2.11 ± 0.17A,a	2.21 ± 0.12A,a	2.78 ± 1.06A,a	2.35 ± 0.58A,a	211.844	<b>0.005</b>	352.922	<b>0.003</b>	2.387	0.262	14.797	0.061
Isoleucine (Ile)	3.55 ± 0.15	3.82 ± 0.32A,a	5.33 ± 1.33A,a	4.45 ± 0.15A,a	4.00 ± 0.17A,a	1.127	0.400	3.502	0.202	34.357	<b>0.028</b>	7.523	0.111
Glycine (Gly)	3.15 ± 0.25	3.21 ± 0.08A,a	3.96 ± 0.03B,a	4.83 ± 0.35A,b	3.81 ± 0.26A,a	0.085	0.798	20.898	<b>0.045</b>	30.920	<b>0.031</b>	6.644	0.123
Lysine (Lys)	2.46 ± 0.30	2.96 ± 0.79A,a	3.55 ± 0.05A,a	3.13 ± 0.28A,a	3.69 ± 0.30A,a	0.685	0.495	26.475	<b>0.036</b>	5.441	0.145	17.247	<b>0.053</b>
Total amino acids	1882.95 ± 157.33	1858.09 ± 220.12A,a	2118.27 ± 201.00A,a	2107.53 ± 49.24A,a	1952.67 ± 86.53A,a	0.017	0.908	1.700	0.322	3.712	0.194	0.301	0.638
Total amino acids without Pro	1453.24 ± 144.48	1408.67 ± 159.96A,a	1612.87 ± 176.81A,a	1625.87 ± 42.59A,a	1493.91 ± 107.09A,a	0.086	0.798	0.978	0.427	2.627	0.246	0.102	0.779

All parameters are listed with their standard deviation (n = 3). For each amino acid, different capital letters indicate significant differences between Phe1 & Phe2 and between Ur1 & Ur2 (dose effect) treatments and small letters indicate significant differences between Phe1 & Ur1 and between Phe2 & Ur2 (nitrogen source effect) (p ≤ 0.05). The mean values (n = 3) are shown with their standard deviation. Significant values between control and each of the treatments are in bold (p ≤ 0.05).

**Effect of treatments on grape amino acid content:** The most abundant amino acids were Arg, Pro, and Gln, while the least abundant were Ile, Gly, and Lys. Compared to control, it was observed that foliar application of Phe1 increased the synthesis of Trp and Phe, decreasing Cys, Cit and Orn (Tab. 2). Trp and Phe are precursors of positive compounds for the wine aroma, such as tryptophol and 2-phenylethanol, respectively. Phe2 increased the Trp, Phe Tyr, Gly and Lys, decreasing Orn content. Ur1 improved the synthesis of Pro, Glu, Ser, Trp, Val, Ile and Gly. However, Ur2 only increased Ser, Trp and Lys content. Ser, Trp, Val and Ile are precursors of some higher alcohols such as 1-butanol, 1-hexanol, isoamyl alcohols, and isobutanol, while Ser and Trp are good nitrogen sources for *Saccharomyces cerevisiae* (BELL and HENSCHKE 2005).

As for the effect of dosage with respect to the application of Phe, the concentration of GABA and Gly increased with the dose applied. In Ur, the concentration of GABA, Ser and Val decreased proportionally to the concentration applied. Comparing the nitrogen source using the lowest dose, the concentration of GABA, His, Cys and Gly in the must was higher when Ur was applied than Phe, while the concentration of Phe in grapes was greater when Phe was applied to vineyards. For the highest dose, GABA, Phe and Tyr increased when Phe was applied compared to Ur. The observed effect on grape nitrogen composition after the application of Phe and Ur was lower than in a previous study (GARDE-CERDÁN *et al.* 2014), in which the lowest dose of both nitrogen sources (0.9 kg N·ha<sup>-1</sup>) was used. This could be due to the total concentration of amino acids in the control being smaller (981 mg·L<sup>-1</sup>) than in the present study (1883 mg·L<sup>-1</sup>), so that the plant had higher nitrogen requirements.

### Conclusions

Both treatments with phenylalanine (Phe1 and Phe2) and the highest dose of urea (Ur2) enhanced the plant synthesis of total positive compounds and decreased the levels of C6 compounds in the 'Tempranillo' grapes. Grape content of total amino acids was not modified by any of the treatments carried out in the grapevine. The foliar application that most affected the amino acids was the Ur1, increasing the content of seven free amino acids. The applications of Phe increased the concentration in the grapes of Phe. Phe and Ur foliar applications were a good tool to enhance grape volatile composition without affecting its nitrogen content.

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