Use of different waste waters from the leachate of the mushroom production process as foliar fertilizers: Effects on grape amino acids concentration

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Abstract

The production of edible mushrooms presents a serious problem for the environment, since about 5 kg of waste are produced for each kilogram of mushroom. These waste waters have nitrogenous matter. Thus, the aim was to investigate the effect of foliar applications of waste water from the mushroom production process on must amino acid composition during two seasons compared to other nitrogen sources. The treatments were applied to the vineyard at veraison and one week later at a total dose of 0.9 kg N/ha. Amino acids were analysed by HPLC. Results showed that treated mushroom water (Tmw) and mushroom water (Mw) improved the amino acid concentration in both seasons differentially. Tmw applied to the grapevines increased total amino acids concentration from 1479.58 to 1735.90 mg/L compared to untreated grapevines over the second study season. The effectiveness of the applications depends on grapevines nitrogen needs. Under moderate nitrogen conditions, Tmw and Mw applications seem to be more effective than urea and phenylalanine treatments. These results are important in relation to the sustainable management of the agri-food sector.

Additional keywords: mushroom waste water; foliar application; grapevines; Tempranillo.

Introduction

Nitrogen (N) composition of the must plays a key role in wine quality since it affects the growth and development of yeast during alcoholic fermentation. Its importance is related to the fermentation kinetics which results in a complete consumption of sugar by yeast and contribute to the formation of fermentative compounds, especially of higher alcohols and esters that constitute the “fermentation bouquet” of the wines (Bisson & Butzke, 2000; Bell & Henschke, 2005; Garde-Cerdán et al., 2009). A deficient N concentration in the must may cause stuck or sluggish fermentations, which is a persistent problem in wine production (Bisson & Butzke, 2000). An adequate alcoholic fermentation rate is reached when N concentration is approximately of 140 mg N/L (Bell & Henschke, 2005). On the other hand, it is important to note that viticultural practices may have a strong impact on grape amino acids composition. Amino acids concentration and composition can vary according to grape cultivar, rootstock, seasonal conditions, and level of maturity, among others (Bell & Henschke, 2005; Pérez-Álvarez et al., 2015; Gutiérrez-Gamboa et al., 2018). In addition, the application of chemical fungicides has been showed to reduce the concentration of several amino acids (Oliva et al., 2011). Soil N application to the vines increases the concentration of N compounds in must (Hilbert et al., 2003). However, through this type of application, there is a risk of N leaching and denitrification, so the use of organic amendments helps to avoid these environmental problems (Weier et al., 1992). Thus, the foliar N application is a recent technique which has allowed to improve the must quality in relation to amino acids concentration (Garde-Cerdán et al., 2014; Gutiérrez-Gamboa et al., 2017; Pérez-Álvarez et al., 2015).
2017), volatile composition (Garde-Cerdán et al., 2015a) and phenolic content (Garde-Cerdán et al., 2015b; Portu et al., 2015).

Currently, certain studies have been carried out applying urea as foliar fertilizer. Urea intrinsic characteristics, such as being a molecule of small size and its high solubility, allow its rapidly taken up through leaf cuticle (Lasas et al., 2012; Hannam et al., 2016). Furthermore, urea applications are cheaper than the applications carried out with different compounds, solutions or commercial products that are sold in the market, due to its easy chemical synthesis. However, the effect of foliar urea applications to the vineyard depends fundamentally on the N needs of the grapevines. Foliar phenylalanine (Phe) and urea (Ur) applications were the most effective treatments when the grapevines had a lower content of yeast assimilable N (YAN) (169 mg N/L). These treatments improved grape amino acids concentration (Garde-Cerdán et al., 2014). On the other hand, Gutiérrez-Gamboa et al. (2017) reported that, foliar Ur applications had a negative effect on the concentration of several amino acids in grapes, when the grapevines had a higher YAN concentration (251 mg N/L). However, commercial nitrogen products with amino acids in their composition increased grape amino acid content (Gutiérrez-Gamboa et al., 2017), being this type of solutions the most effective in order to increase grape amino acids concentration, when the grapevines have a high YAN concentration.

On the other hand, only one report studied the effect of foliar N applications other than urea, using commercial nitrogen products and amino acid such as proline (Pro), arginine (Arg) and Phe for this purpose. In this way, Khan et al. (2012) exhibited that foliar applications of a mixture of amino acids and a seaweed extract can be used effectively to improve growth and physicochemical berry quality of grapevine cv. Perlette. Therefore, it is considered of great interest the study of other N sources as foliar fertilizer with the aim to improve grape quality. In this sense, it would be interesting to study the use of an agri-food waste as foliar fertilizer. Water from the leachate of the mushroom production process contains nitrogenous matter that could be adequate to be used as foliar fertilizer to the vineyard. The use of this bioproduct (which is usually applied as compost) would help to reduce the consumption of inputs, contributing to the integral, efficient and sustainable management of the agri-food sector.

The leachate generated from the mushroom production is usually used as compost called spent mushroom substrates (SMS). Paredes et al. (2016) reported that, the application of SMS improved soil fertility and did not affect the lettuce yield. Morlat & Chaussod (2008) reported that, the SMS application to a vineyard planted in a sandy soil during a long-term experiment (28 years) increased soil organic carbon and mineral N, P and K, as well as improved the soil moisture content at field capacity and bulk density. Peregrina et al. (2012) showed that the application of SMS to a vineyard planted in semiarid soils facilities SMS recycling with simultaneous improvement in soil quality indicators related to labile organic matter and microbiologic activity. Thus, to our knowledge, there are no reports that show how the use of this singular waste from the mushroom industry affects the grape quality, neither its use as a foliar fertilizer.

Due to the lack information about the use of waste waters in the wine industry and its possible effects on grape quality, the purpose of this work was to investigate the impact of foliar applications of different waters from the leachate of the mushroom production process compared to Ur and Phe applications on must amino acids composition from Tempranillo grapes during two consecutive seasons.

Material and methods

Study site

The experiment was conducted over two consecutive years (2014 and 2015) in a commercial vineyard located in Alfaro (warmest and driest area of La
Rioja, Spain). The altitude of the location was 335 m.a.s.l. Tempranillo grapevines (Vitis vinifera L.) were grafted onto 1103-Paulsen rootstock, planted in 1999 and trained to a VSP (vertical shoot positioned) trellis system. Planting density was 2,976 plants/ha with grapevines spacing at 2.80 m between rows and 1.20 m within the row. Weather conditions were recorded using an automatically meteorological station belonging to the Agroclimatic Information Service of La Rioja (SIAR) installed at 5 km from the experimental field. Accumulated rainfall from the beginning of April to October was 174 mm in 2014 and 127 mm in 2015, while the average temperatures for the same period were 18.7 and 19.8 °C, respectively.

Grapevine treatments and harvest

The trial involved the foliar application of water as a control and four N fertilizers: two of them were waste waters from the leachate of the mushroom production process. Mushroom water (Mw) corresponds to a water extract from the leachates of the mushroom production process. Treated mushroom water (Tmw) corresponds to a mushroom water treated with a concentrate liquid containing microorganisms, such as phototrophic bacteria, yeast extract, lactic acid producing bacteria and fermentation fungi. Some of these microorganisms can fix N from the environment. The other two N treatments applied to the grapevines were commercial urea (Ur) (Sigma-Aldrich, Madrid, Spain) and commercial phenylalanine (Phe) (Sigma-Aldrich). During the 2014 season, Tmw, Mw, Ur and Phe were applied. During the 2015 season, Mw, Ur and Phe were applied. Also, the application of water as a control was carried out in the grapevines in both seasons.

Mw and Tmw solutions contained 316 mg N/L in 2014 year; Mw solution contained 466 mg N/L in 2015. Ur and Phe solutions were prepared at a concentration of 750 mg N/L, according to the reported by Garde-Cerdán et al. (2014). All solutions were prepared using Tween 80 as wetting agent (0.1% v/v). Control plants were sprayed with water solution of Tween 80 alone. The treatments were applied to the grapevines twice, at veraison and one week later. For each application, 200 mL/plant was sprayed over leaves for Phe, Ur, and control treatments; and 400 mL/plant and 300 mL/plant, in 2014 and 2015, respectively, for mushroom applications; so, the total amount applied in each treatment was 900 g total N/ha, assuming 3,000 plants/ha. Treatments were applied in triplicate and were arranged in a complete randomized block design with ten vines per replicate.

Grapes were harvested at their optimum technological maturity, and then they were destemmed and crushed. The oenological parameters were determined in the musts obtained. Aliquots of each sample were frozen in order to determine their free amino acids content.

Oenological parameters and yeast assimilable nitrogen (YAN)

Musts were physicochemically characterized through the determination of probable alcohol, pH, titratable acidity, malic acid, and potassium according to the stated by ECC (1990) and tartaric acid according to the Rebelein method (Lipka & Tanner, 1974). Yeast assimilable nitrogen (YAN) was determined according to the method described by Aerny (1996). Since the treatments were performed in triplicate in the vineyard, the results of these parameters were showed as the average of three analyses (n = 3).

Analysis of amino acids by HPLC

The amino acids analysis of the musts was performed using the method described by Garde-Cerdán et al. (2014). Free amino acids were analysed by reversal-phase HPLC using an Agilent 1100 Series (Palo Alto, CA, USA), equipped with an automatic liquid sampler (ALS), a fluorescence detector (FLD) and a diode array detector (DAD). Each sample was centrifuged at 3,200×g for 10 min at 20°C and then, 5 mL of the sample was mixed with 100 μL of norvaline, internal standard for quantify all the amino acids except proline and 100 μL of sarcosine, internal standard for quantify only proline. This mixture was filtered through 0.45 μm OlimPeak pore filter (Teknokroma) and submitted to an automatic precolumn derivatization with o-phthalaldehyde (OPA Reagent, Agilent) and with 9-fluorenylmethylchloroformate (FMOC Reagent, Agilent). The injected amount from the derivatized sample was 10 μL at 40 °C. All separations were performed on a Hypersil ODS (250 × 4.0 mm, I.D. 5 μm) column (Agilent).

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

A statistical analysis on oenological parameters, yeast assimilable nitrogen (YAN) and amino acids was performed using variance analysis (one-way ANOVA), by Statgraphics Centurion XVI.I (Statgraphics Technologies, Inc, VA, USA). Differences between samples were compared using the Duncan test ($p \leq 0.05$). Principal component analysis (PCA) was performed with the amino acids concentration in the different samples (InfoStat, www.infostat.com.ar).

Results and discussion

Oenological parameters

The oenological parameters for Tempranillo samples during 2014 and 2015 seasons are summarized in Table 1. During the 2014 season, the different treatments did not affect the must probable alcohol, pH, tartaric acid and malic acid, while titratable acidity and potassium (K) were affected. In 2015 season, pH, titratable acidity and potassium were affected by the treatments. Previous studies showed different results in oenological parameters after N foliar applications. Lasa et al. (2012) studied the effect of foliar application of urea at different dosages and times during two growing seasons in Sauvignon Blanc and Merlot grapevines. In the first year of study, these authors exhibited that, the urea treatment showed low content of titratable acidity and high probable alcohol, in Merlot grapes. On the other hand, the authors found no statistical differences in any of the studied oenological parameters in Sauvignon blanc grapes in the same year of study. For the two cultivars, no statistical differences were found in the second year of study. In another report, Ancín-Azpilicueta et al. (2013) showed statistical differences in the content of total acidity and pH on must when urea was applied in increasing doses. For its part, Garde-Cerdán et al. (2014), Gutiérrez-Gamboa et al. (2017) and Pérez-Álvarez et al. (2017) did not find statistical differences in must oenological parameters after urea applications to the grapevines.

In our samples, foliar application of waste water from Mw in 2015 increased must pH and K respect to control samples, while Ur decreased pH, respect to control, Mw and Phe samples. This decrease in the must pH values was evidenced in a high concentration of total acidity, although without statistical differences

Table 1. Oenological parameters and yeast assimilable nitrogen (YAN) in musts from untreated (Control) and treated grapevines with different nitrogen sources as foliar fertilizer: mushroom water (Mw), treated mushroom water (Tmw), urea (Ur) and phenylalanine (Phe), during 2014 and 2015 seasons.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Mw</th>
<th>Tmw</th>
<th>Ur</th>
<th>Phe</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2014 Season</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probable alcohol (%) v/v</td>
<td>14.66 ± 0.28 a</td>
<td>14.49 ± 0.25 a</td>
<td>14.50 ± 0.37 a</td>
<td>14.15 ± 0.61 a</td>
<td>14.33 ± 0.03 a</td>
</tr>
<tr>
<td>pH</td>
<td>3.44 ± 0.04 a</td>
<td>3.47 ± 0.07 a</td>
<td>3.45 ± 0.02 a</td>
<td>3.41 ± 0.02 a</td>
<td>3.40 ± 0.05 a</td>
</tr>
<tr>
<td>Titratable acidity (g/L tartaric acid)</td>
<td>5.25 ± 0.07 ab</td>
<td>5.12 ± 0.19 a</td>
<td>5.40 ± 0.13 ab</td>
<td>5.55 ± 0.17 b</td>
<td>5.25 ± 0.34 ab</td>
</tr>
<tr>
<td>Tartaric acid (g/L)</td>
<td>7.49 ± 0.10 a</td>
<td>7.61 ± 0.09 a</td>
<td>7.52 ± 0.08 a</td>
<td>7.34 ± 0.19 a</td>
<td>7.41 ± 0.29 a</td>
</tr>
<tr>
<td>Malic acid (g/L)</td>
<td>2.26 ± 0.39 a</td>
<td>2.05 ± 0.09 a</td>
<td>2.44 ± 0.32 a</td>
<td>2.07 ± 0.09 a</td>
<td>2.05 ± 0.17 a</td>
</tr>
<tr>
<td>Potassium (mg/L)</td>
<td>1785.95 ± 111.37 ab</td>
<td>1862.34 ± 122.66 b</td>
<td>1935.61 ± 40.50 b</td>
<td>1652.10 ± 20.15 a</td>
<td>1693.98 ± 78.26 a</td>
</tr>
<tr>
<td>YAN (mg N/L)</td>
<td>221.40 ± 25.50 a</td>
<td>217.93 ± 26.65 a</td>
<td>231.00 ± 12.83 a</td>
<td>245.47 ± 5.83 a</td>
<td>245.00 ± 25.24 a</td>
</tr>
<tr>
<td><strong>2015 Season</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probable alcohol (%) v/v</td>
<td>12.34 ± 1.22 a</td>
<td>13.48 ± 1.05 a</td>
<td>12.47 ± 1.13 a</td>
<td>12.85 ± 0.18 a</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>3.46 ± 0.05 b</td>
<td>3.56 ± 0.04 c</td>
<td>3.39 ± 0.03 a</td>
<td>3.46 ± 0.02 b</td>
<td></td>
</tr>
<tr>
<td>Titratable acidity (g/L tartaric acid)</td>
<td>4.63 ± 0.11 ab</td>
<td>4.40 ± 0.15 a</td>
<td>5.03 ± 0.29 b</td>
<td>4.72 ± 0.26 ab</td>
<td></td>
</tr>
<tr>
<td>Tartaric acid (g/L)</td>
<td>6.88 ± 0.18 a</td>
<td>6.86 ± 0.12 a</td>
<td>7.06 ± 0.18 a</td>
<td>6.91 ± 0.26 a</td>
<td></td>
</tr>
<tr>
<td>Malic acid (g/L)</td>
<td>1.33 ± 0.25 a</td>
<td>1.41 ± 0.08 a</td>
<td>1.39 ± 0.18 a</td>
<td>1.46 ± 0.30 a</td>
<td></td>
</tr>
<tr>
<td>Potassium (mg/L)</td>
<td>1401.00 ± 152.29 a</td>
<td>1604.00 ± 22.34 b</td>
<td>1419.67 ± 62.75 a</td>
<td>1389.67 ± 78.77 a</td>
<td></td>
</tr>
<tr>
<td>YAN (mg N/L)</td>
<td>175.00 ± 10.10 a</td>
<td>159.60 ± 14.00 a</td>
<td>156.33 ± 26.80 a</td>
<td>171.27 ± 31.75 a</td>
<td></td>
</tr>
</tbody>
</table>

All parameters are given with their standard deviation (n = 3). For each parameter, different letters in the same row indicate significant differences between treatments ($p \leq 0.05$).
compared to control samples (Table 1). It is important to note that, a high concentration of K in the must may lead to a high must pH, and consequently to a decrease in the wine quality (Kodur, 2011). Along with this, an imbalance of pH and K concentration may cause stuck fermentations (Kudo et al., 1998). On the other hand, to our knowledge, there are no reports that show the effect of foliar applications of waste waters from the mushroom production on oenological parameters neither in grape quality. However, Sánchez-Gómez et al. (2016) exhibited that foliar application of a grapevine-shoot waste aqueous extract to Airén grapevines did not affect must and wine oenological parameters.

Grape amino acids content

Fig. 1 and Table 2 show the amino acids concentration in the different samples from 2014 and 2015 seasons. Fig. 2 shows the concentration of total amino acids and total amino acids without Pro in 2014 and 2015 seasons. The most abundant amino acids found in all samples were proline (Pro), arginine (Arg), glutamine (Gln), glutamic acid (Glu) and γ-aminobutyric acid (Gaba), representing around of 80% of total amino acids (Fig. 1a). These five amino acids are the most abundant in several cultivars such as Airén, Cabernet Sauvignon and Tempranillo (Garde-Cerdán et al., 2014, 2016; Sánchez-Gómez et al., 2016; Gutiérrez-Gamboa et al., 2017; Pérez-Alvarez et al., 2017). However, Pro, Arg, Gln, Gaba and serine (Ser) were the most abundant amino acids found in Merlot and Pinot gris in a report by Hannam et al. (2016). Asparagine (Asn), glycine (Gly), citrulline (Cit), isoleucine (Ile) and lysine (Lys) were the amino acids quantified in lower concentration in grapes accounting around 0.87 to 1.65% of total amino acids content as has been mentioned by Garde-Cerdán et al. (2016). In the first season, Arg was the most abundant amino acid and in the second season, the most abundant amino acid was Pro. Arg is an important N source for yeasts necessary for a correct development of alcoholic fermentation. In 2014 season, the concentration of this amino acid varied from 560.11 to 737.18 mg/L (28 to 33% of total amino acids), while in 2015 season Arg concentration ranged from 229.60 to 408.04 mg/L (21 to 28% of total amino acids). In 2014 season, the concentration of Pro ranged from 523.56 to 699.69 mg/L (28 to 31% of total amino acids), while in 2015 season ranged from 376.61 to 581.85 mg/L (29 to 34% of total amino acids). All amino acids were found in the normal concentrations described for these compounds as has been reviewed by Bell & Henschke (2005). The treatments had no effect on must yeast assimilable nitrogen (YAN) concentration in the studied seasons. In 2014 and 2015 seasons, must YAN content in all treatments, reached at a level above of 140 mg N/L (Table 1), considered as a “moderate” N level to ensure a complete alcoholic fermentation. During 2014 season, the must YAN concentration was higher than in 2015.

The differential accumulation of Pro and Arg by different grape cultivars provides a characteristic index based on the ratio of these amino acids (Bell & Henschke, 2005). This index reflects the proportion of non assimilable (Pro) to assimilable N (Arg) and provides an useful indication of the likely nutritional value of the grape must (Bell & Henschke, 2005). Pro to Arg ratio had a different behavior in both study seasons. In 2014 season, Tempranillo grapes from untreated grapevines

![Figure 1](image.png)

**Figure 1.** Glutamic acid (Glu), glutamine (Gln), arginine (Arg), γ-aminobutyric acid (Gaba) and proline (Pro) concentration (mg/L) in musts from untreated (Ctr) and treated grapevines with different nitrogen sources as foliar fertilizer: mushroom water (Mw), treated mushroom water (Tmw), urea (Ur) and phenylalanine (Phe), during the a) 2014 and b) 2015 seasons. All parameters are shown with the standard deviation (n = 3). For each amino acid, different letters indicate significant differences between treatments (p ≤ 0.05).
Table 2. Amino acids concentration (mg/L) in musts from untreated (Control) and treated grapevines with different nitrogen sources as foliar fertilizer: mushroom water (Mw), treated mushroom water (Tmw), urea (Ur) and phenylalanine (Phe), during 2014 and 2015 seasons.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>2014 Season</th>
<th>2015 Season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Mw</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>22.63±2.65a</td>
<td>19.11±1.86a</td>
</tr>
<tr>
<td>Asparagine</td>
<td>5.50±0.61b</td>
<td>6.07±0.09b</td>
</tr>
<tr>
<td>Serine</td>
<td>53.01±0.89b</td>
<td>49.71±6.76a</td>
</tr>
<tr>
<td>Histidine</td>
<td>40.92±3.21ab</td>
<td>44.77±7.65b</td>
</tr>
<tr>
<td>Glycine</td>
<td>7.17±0.48b</td>
<td>7.71±1.37b</td>
</tr>
<tr>
<td>Threonine</td>
<td>45.54±3.27bc</td>
<td>38.01±3.09a</td>
</tr>
<tr>
<td>Citrulline</td>
<td>6.72±1.93a</td>
<td>7.29±2.60a</td>
</tr>
<tr>
<td>Alanine</td>
<td>93.62±2.27a</td>
<td>89.64±17.81a</td>
</tr>
<tr>
<td>Methionine</td>
<td>8.95±0.74a</td>
<td>8.06±0.33a</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>23.38±4.98a</td>
<td>19.87±0.79a</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>7.96±0.54a</td>
<td>8.28±1.24a</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>4.59±0.93a</td>
<td>4.05±0.17a</td>
</tr>
<tr>
<td>Leucine</td>
<td>12.02±1.44c</td>
<td>9.91±0.60a</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.87±0.58a</td>
<td>2.53±0.13a</td>
</tr>
</tbody>
</table>

All parameters are given with their standard deviation (n = 3). For each parameter, different letters in the same row indicate significant differences between treatments (p ≤ 0.05).
tended to behave as an Arg accumulator variety, except in Phe treatment, in contrast to those mentioned extensively by several authors (Garde-Cerdán et al., 2016; Pérez-Álvarez et al., 2017). It seems that Mw, Tmw and Ur foliar applications had a positive effect in Pro to Arg ratio during 2014 season, evidencing a positive effect of N application in this ratio as has been mentioned by Rodríguez-Lovelle & Gaudillère (2002) and reviewed by Bell & Henschke (2005). In 2015 season, Mw, Ur and Phe foliar applications increased Pro to Arg ratio, therefore Tempranillo grapevines tended to behave as Pro accumulator variety. Respect to the effect of the different treatments on grape amino acids concentration, in 2014, 11 of the 22 studied amino acids were not affected by the foliar applications, among them were Glu, Gln, Gaba, aspartic acid (Asp), Cit, alanine (Ala), methionine (Met), tryptophan (Trp), phenylalanine (Phe), Ile and Lys. Urea (Ur) and Phe foliar applications to Tempranillo grapevines had not a positive effect on grape amino acids content. Ur treatment decreased Pro (25%), Arg (20%) and Gly (20%) concentrations, being decreased total amino acids concentration, respect to control samples. Phe application decreased the concentration of several amino acids such as Arg (21%), Asn (36%), Ser (15%), Gly (20%), threonine (Thr) (16%), tyrosine (Tyr) (15%) and leucine (Leu) (16%) (Fig. 1a and Table 3), being not affected total amino acids concentration (Fig. 2a), respect to control samples. Respect to the use of the waste waters from the mushroom production process as foliar fertilizers, its effect on grape amino acid concentration appears to be more positive than the Ur and Phe treatments decreasing only three amino acids compared to the nine amino acids decreased in the must by Ur and Phe applications. Thus, Mw treatment decreased Thr (17%), Tyr (16%), Valine (Val) (12%) and Leu (18%) concentrations, respect to control samples. However, total amino acids concentration was not affected by this treatment (Fig. 2a). Tmw application only strongly increased Asn must concentration (58%). On the other hand, comparing the effectiveness of the waters, Tmw exhibited high must concentration of Arg (24%), Pro (25%), Asn (52%), His (30%), Gly (25%), Thr (12%) and Cys (30%) compared to Ur treatment and showed high must concentration of Arg (24%), Asn (59%), histidine (His) (30%), Gly (26%), Thr (17%) and Tyr (19%) respect to Phe applications. In addition, Mw exhibited high must concentration of Arg (24%), Pro (24%), His (28%), Gly (24%) and cysteine (Cys) (28%) compared to Ur treatment and showed high must concentration of Arg (24%), Asn (42%), His (27%) and Gly (26%) respect to Phe applications.

The concentration of total amino acids ranged from 1,903.85 to 2,418.88 mg/L (corresponding to Ur and Tmw treatments, respectively). Also, total amino acids concentration without Pro varied from 1,380.29 to 1,721.24 mg/L (corresponding to Ur and Tmw treatments, respectively). These both total concentrations were higher than to those obtained in the 2015 season (Fig. 2). Thus, it was observed that Tmw samples reached a higher concentration of total amino acids compared to Ur.

In 2015 season, 5 of the 22 analysed amino acids were not affected by foliar applications (Arg, Gaba, Cit, Cys and Trp). There is a differentiated response by season, possibly due to the lower concentration of YAN reached by the grapevines in 2015 season (Table 1). The concentration for the most amino acids was lower in 2015 than to those obtained in 2014 season (Fig. 1 and Table 2). On the other hand, respect to the effect of Ur and Phe foliar applications, there was a similar trend to 2014 season. Ur treatment decreased Glu (17%), Asp (12%), Ser (14%) and Met (32%) concentration, respect to control samples however, these decreases did not affect the concentration of total amino acids, respect to control samples (Fig.
Phe application decreased the concentration of several amino acids such as Glu (17%), Gln (29%), Asp (24%), Asn (46%), Ser (24%), Thr (29%), Ala (26%), Val (13%) Met (21%) and Ile (31%) while increased Phe (23%) concentration, respect to control samples. This increase was also observed by Garde-Cerdán et al. (2014) when Phe was applied to Tempranillo vines. The decrease in several amino acids led to a decrease of total amino acids concentration, respect to control samples (Fig. 2b). On the other hand, Mw had a positive effect on the synthesis of must amino acids, strongly increasing the concentration of Pro (17%), His (164%) and Val (14%). These results led to an increase of total amino acids concentration, respect to control, Ur and Phe samples during 2015 season (Fig. 2b). Comparing the effectiveness of Mw respect to the others N treatments studied, Mw exhibited high must concentration of Glu (22%), Gln (24%), Pro (27%), Ser (21%), His (38%), Thr (21%), Val (21%), Met (27%) and Ile (20%) compared to Ur treatment. Mw also showed high must concentration of Glu (21%), Gln (36%), Pro (35%), Asp (17%), Asn (45%), Ser (30%), His (65%), Gly (43%), Thr (31%), Ala (27%), Tyr (34%), Val (24%), Met (14%), Ile (38%), Leu (30%) and Lys (25%) respect to Phe applications. Therefore, it seems that, when grapevines reached low must YAN concentration (2015 season), the effect of Mw foliar application resulted in an improve of grape amino acids concentration.

Garde-Cerdán et al. (2016) reported that Ur and Phe foliar application to Tempranillo grapevines with a dosage of 0.9 kg total N/ha had a stronger effect on must N composition, increasing the content of several amino acids. These significant differences may be attributed to the low YAN concentration (169 mg N/L) and total amino acids (around of 950 mg/L) obtained in untreated grapevines. On the other hand, Gutiérrez-Gamboa et al. (2017) reported that, Ur foliar application to Cabernet Sauvignon grapevines with a dosage of 2 kg total N/ha had a negative effect on must N composition, decreasing the concentration of several amino acids compared to control samples. It should be noted that, YAN concentration (251 mg N/L) and total amino acids (2,358 mg/L) in untreated grapevines were higher than those showed by Garde-Cerdán et al. (2016). In our results, YAN content (175 and 221 mg N/L, in 2014 and 2015 seasons, respectively) and total amino acids concentration (1,480 and 2,365 mg/L, in 2014 and 2015 seasons, respectively) obtained in control samples, were higher than those reported by Garde-Cerdán et al. (2014). Due to this, it seems to be that the effectiveness of foliar N applications depends fundamentally on grapevines N needs, despite of the better absorption via the leaf cuticle in veraison compared to soil application (Lasa et al., 2012; Hannam et al., 2016). Thus, from moderate to a high grapevine N status, the N foliar applications may disrupt grapevine balance, leading to a limited supply of carbohydrates if the grapevine becomes overcropped or excessively vegetative due to further applications of N (Bell & Henschke, 2005). For this reason, in vineyards with moderate or high N content is important to consider other N sources to be applied foliarly to the grapevines, which can be better absorbed by the vines than Ur and Phe applications, in order to improve the grape amino acids composition. In this way, the foliar application of different waters from the leachate of the mushroom production process were the most effective treatments in both vine growing seasons.

As mentioned previously, the leachate generated from the mushroom production is usually used in grapevines to improve soil fertility (Morlat & Chaussod, 2008; Peregrina et al., 2012). However, to our knowledge, there are no reports about the use of waste waters from the mushroom production process as foliar fertilizer to increase grape amino acids concentration. Meanwhile, Sánchez-Gómez et al. (2016) studied the influence of foliar applications of a vine-shoot waste aqueous extracts on grape amino acids concentration reporting that, this extract can enhance amino acids concentration. Therefore, the use of agro-industry bioproducts as foliar fertilizer can be a sustainable alternative with the environment.

Treatments classification

To classify the different treatments and assess its effect on grape amino acids concentration in Tempranillo musts during 2014 season, a PCA was performed (Fig. 3a). Principal component 1 (PC 1) explained 64.8% of the variance and principal component 2 (PC 2) explained 20.0%, representing 84.8% of all variance. PC 1 was strongly correlated with glutamic acid (Glu), glutamine (Gln), arginine (Arg), γ-aminobutyric acid (Gaba), serine (Ser), histidine (His), glycine (Gly) and alanine ( Ala), while PC 2 was correlated only with tryptophan (Trp). Both components allowed to separate the different samples. Control and Tmw treatment were correlated with major content of several amino acids. Control was correlated with high concentration of aspartic acid (Asp), threonine (Thr), tyrosine (Tyr), methionine (Met), Trp and leucine (Leu), while Tmw treatment was correlated with high concentration of Glu, Gaba, asparagine (Asn), Ser and Ala. Urea and phenylalanine (Phe) treatments were correlated with low content of several amino acids except Phe. For its part, Mw treatment was correlated only positively with lysine (Lys).
For 2015 season (Fig. 3b), PC 1 explained 80.9% of the variance and PC2 explained 11.5%, representing 92.4% of all variance. PC 1 was strongly correlated with all amino acids except Asp, His, Met and Phe, while PC 2 was correlated only with Phe. Both components allowed to separate the different samples. Control was correlated with high concentration of Arg, Asp, Asn, Ala and Met. Mw treatment was correlated with high concentration of Glu, Gaba, Ser, Gly, Tyr, valine (Val), Leu and Lys. Ur and Phe treatments were correlated with low concentration of most amino acids.

In conclusion, the use of different waters from the leachate of the mushroom production process such as treated mushroom water (Tmw) and mushroom water (Mw) applied as foliar fertilizer allowed to improve the grape amino acids concentration in grapevines growing under moderate N levels. For Mw treatment, this effect was improved in 2015 season, under lower YAN concentration than to that obtained in 2014 season. Therefore, under moderate N conditions of the grapevines, Tmw and Mw applications were more effective compared to urea (Ur) and phenylalanine (Phe) treatments. Tmw and Mw were correlated with high concentration of several amino acids as seen in both PCAs.

The use of this bioproduct would help to reduce the consumption of inputs, contributing to the sustainable management of this agri-food sector. Based on the aforementioned, it would be interesting to evaluate the effect of Tmw and Mw applications to grapevines growing under low, moderate and high N conditions and compare them with other nitrogen treatments in order to improve grape quality being more sustainable with the environment.

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References

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