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RESEARCH

Improvement of wine volatile composition through foliar nitrogen applications to 'Cabernet Sauvignon' grapevines in a warm climate

Gastón Gutiérrez-Gamboa[1], Teresa Garde-Cerdán[1], Marioli Carrasco-Quiroz[1], Ana María Martínez-Gil[2], and Yerko Moreno-Simunovic[3*]

[1]Instituto de Ciencias de la Vid y del Vino (CSIC-CAR-UR), Carretera de Burgos km 6, 26007 Logroño, España.
[2]Universidad de Valladolid, Departamento de Química Analítica, Avda. de Madrid 50, 34004 Palencia, España.
[3]Universidad de Talca, Facultad de Ciencias Agrarias, Av. Lircay S/N, Talca, Chile.*Corresponding author (ymoreno@utalca.cl).

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ABSTRACT

Volatile compounds play a key role on wine quality due to their importance in wine aroma. The improvement of these compounds in Cabernet Sauvignon wines may be of oenological relevance because these are usually defined by strong character and somewhat herbaceous. The effect of different foliar N applications on 'Cabernet Sauvignon' grapevines (*Vitis vinifera* L.) was studied in order to improve wine volatile composition. Five treatments were applied: urea (Ur), urea plus sulfur (Ur+S), arginine (Arg), and two commercial products: Nutrimyr Thiols (NT) and Basfoliar Algae (BA). Volatile compounds were analyzed by GC-MS. Results showed that wines elaborated with grapes treated with arginine (Arg) exhibited the lowest content of total higher alcohols (322.29 mg L^{-1}). Wine concentrations of ethyl hexanoate, octanoate, and decanoate from Ur+S and BA treatments were the highest (385.28, 590.46, 214.14, and 416.00, 600.50, 227.96 μg L^{-1}, respectively). No effects were found on vanillin derivatives content in wines by foliar treatments. Total terpenes content in wines from Ur and Arg grapevine treatments were higher than in control (17.87 and 20.36 μg L^{-1} than 14.99 μg L^{-1}). Thus, N foliar applications can improve wine volatile composition, mainly by Ur+S and BA treatments. The importance of this study is to add knowledge about the effects of foliar N applications on Cabernet Sauvignon wine volatile composition in warm climates.

Key words: Cabernet Sauvignon, esters, foliar application, grapevines, *Vitis vinifera*, volatile compounds.

INTRODUCTION

Cabernet Sauvignon is one of the most important grapevine varieties cultivated worldwide. It is originally from Bordeaux (France), but currently, it is planted in vineyards of different regions. It has been essential in countries of the "New World". Wines from 'Cabernet Sauvignon' grapevines have been awarded in various competitions worldwide, which has allowed these countries to position themselves in the world wine market. The aroma that has been usually described for this variety is fruity or floral with roasted, wood-smoke, cooked meat nuances, red/blackberry, and usually herbaceous due to its important levels of methoxypyrazines (Tao et al., 2008; Cai et al., 2014). However, the rise of temperatures and the decrease of precipitations due to climate change trigger advanced maturation. This will affect grape composition, in particular with respect to its aroma composition (van Leeuwen and Darriet, 2016). Due to this, it has shown that some viticultural practices such as defoliation or foliar N applications can improve aromatic composition in grapes or wines (Ancín-Azpilicueta et al., 2013; Pascual et al., 2017).

One of the main factors that determine wine quality is aroma. Thus, several volatile compounds have been found in wines with a wide concentration range (Cai et al., 2014). These compounds belong to a various chemical family of volatiles such as higher alcohols, C6 compounds, ethyl esters, acetate esters, terpenes, among others, which contribute to the desirable wine aroma. As reviewed by Bell and Henschke (2005), N compounds contribute to the formation of some of these volatile compounds, especially higher alcohols and ethyl esters during alcoholic fermentation and regulate the formation of other volatiles, such as hydrogen sulfide or thiols. In addition, Martínez-Gil et al. (2012) presented a close correlation between the synthesis of ethyl esters during alcoholic fermentation and the must amino acid content. Thus, must N composition affects yeast growth and fermentation processes, which is involved in the final quality of wine, especially in the aroma (Garde-Cerdán and Ancín-Azpilicueta, 2008). In order to improve must N composition, different studies have been carried out applying N to the vineyard. However, a high rate of N fertilization can result in the accumulation of negative compounds in wine, such as biogenic amines and ethyl carbamate (Smit et al., 2014). A traditional way to improve wine volatile composition has been the soil N fertilization. However, the results exposed by different authors are quite varied. Linsenmeier et al. (2005) reported that the synthesis of wine volatile compounds was slightly affected by soil N fertilization. On the other hand, Webster et al. (1993) showed that, as N soil fertilization increased, wine concentrations of isoamyl alcohols (3-methylbutan-1-ol and 2-methylbutan-1-ol), and 2-phenylethan-1-ol decreased. In contrast, wine concentrations of butan-1-ol, (*E*)-hex-3-en-l-ol, phenylmethanol, and the majority of ethyl esters increased. Another innovative strategy, used in recent years, is to increase N vine status by foliar N applications. This technique allows a quick and efficient assimilation of applied products by the plants (Lasa et al., 2012). Previous studies have shown that urea foliar application improved wine volatile composition (Lacroux et al., 2008; Ancín-Azpilicueta et al., 2013). This N source is of low price and easily assimilated by the plants, compared to urea soil applications (Lasa et al., 2012). On the other hand, N application with sulfur facilitates their assimilation in grains (Tea et al., 2007). Due to this, urea plus S application was performed in grapevines, which improved the concentration of amino acids in 'Cabernet Sauvignon' grapes (Gutiérrez-Gamboa et al., 2017a).

Due to the aforementioned, some amino acids were applied to the grapevines in order to improve grape quality. Garde-Cerdán et al. (2014) reported that the use of phenylalanine increased the concentration of some amino acids compared to control samples. To our knowledge, only one study researched the effect of arginine as a new fertilizer. In a recent report, Gutiérrez-Gamboa et al. (2017a) exhibited slight differences in the must concentration of amino acids by arginine foliar application. However, the concentration of glutathione in the must was increased considerably regarding control samples. The function of this molecule in winemaking processes is to act as an antioxidant, preventing the appearance of browning pigments in musts and protecting anthocyanins from oxidation (Gambuti et al., 2015). In addition, in relation to wine volatile compounds, glutathione also exerts a protective effect in their concentration (Ugliano et al., 2011) but until now, there had been no studies in the literature reporting the effects of arginine foliar applications on wine volatile concentration.

For these reasons, the aim of this work was to study the influence of foliar application of urea, urea plus S, arginine, and two commercials N fertilizers to 'Cabernet Sauvignon' grapevines on wine volatile composition.

MATERIALS AND METHODS

Study site

The field study was conducted at a commercial vineyard located in a warm climate location of the Maule Valley (Pencahue, 35°20' S, 71°46' W; 87 m a.s.l.), Chile, during the 2015 growing season. A 4-yr-old 'Cabernet Sauvignon' vineyard, grown in 1103 Paulsen rootstock, trained to a vertically shoot positioned system (2.3 × 1.0 m) with a plant density of 4347 plants ha^{-1} was used. The vineyard was equipped with a drip irrigation system using 4 L h^{-1} drippers, to assure the plant water needs. The vines were irrigated when the midday leaf water potential (ψ_l) reached 1.0 to 1.2 MPa. The vineyard plot was homogeneous on its vegetative expression and fruit load. The annual average temperature was 14.5 °C with a minimum of -2.5 °C (July) and a maximum of 36.7 °C (January), and an average annual rainfall of 583.8 mm. The vineyard soil is clay loam classified as Cunculén series Vertic Haploxeralfs (CIREN, 1997).

Grapevines treatments

Five treatments were carried out using several N sources according to Gutiérrez-Gamboa et al. (2017a): urea (Ur), urea plus S (Ur+S), arginine (Arg), and two commercial products, Nutrimyr Thiols (NT) (Italpollina Spa, Casalmenini, Italy) and Basfoliar Algae (BA) (Compo Agro, Santiago, Chile). Treatments were made in triplicate and were distributed as a complete randomized block design. Each replicate was carried out on 20 vines, so 60 plants were used for each treatment, leaving 18 untreated plants in the same row and two rows between replicates to avoid contamination. Treatments consisted of a 2 kg N ha^{-1} dose, divided in two moments, the first at the beginning of veraison and the second 2 wk later. In the Ur+S treatment besides the urea N, 0.5 kg S ha^{-1} for each application were added; 200 mL of each formulation were applied evenly per plant by spraying over the full canopy. Additionally, 60 plants, distributed in the same form, were kept as untreated control.

Commercial products N content

Basfoliar Algae is a concentrated extract of Chilean natural algae (*Durvillaea antarctica*), supplemented with several nutrients, minerals and phytohormones (auxins and cytokinins). On the other hand, Nutrimyr Thiols is a foliar fertilizer, which is sold as a wine flavor enhancer that improves grape quality. The nutritional content of both products is Basfoliar Algae (BA): 6.9% total N content, of which 0.9% is from the following amino acids (g L^{-1}): 0.76 alanine, 1.31 glycine, 0.51 valine, 0.29 threonine, 0.35 serine, 0.73 leucine, 0.34 isoleucine, 0.69 proline, 0.06 cysteine, 0.54 histidine, 0.23 methionine, 0.69 aspartate, 0.45 phenylalanine, 0.93 glutamate, 0.57 lysine, 0.30 tyrosine, 0.38 arginine, and 0.09 histidine; Nutrimyr Thiols (NT): 16% total N content, with 1.2% organic N and 14.8% ureic N.

Samples and winemaking

Harvest was carried out when grapes reached their optimal technological maturity, when the weight of 200 berries remained constant, the concentration of soluble solids was around 24-25 °Brix, and the titratable acidity remained between 5 and 6 g L^{-1}. Following the harvest, grapes were stored 1 d in a cold chamber at 6 °C before processing and then were destemmed and crushed to obtain the must. The winemaking process was performed according to Gutiérrez-Gamboa et al. (2017b). Each replicate was introduced into 20 L tanks, so 18 tanks were filled (three tanks for each treatment and three for control wine). Tanks were stored at 6 °C for pre-fermentative maceration during 2 d. Subsequently, must was inoculated with a commercial yeast *Saccharomyces cerevisiae* strain BO 213 (Laffort, Bordeaux, France) to carry out the alcoholic fermentation, which took place at 22 ± 1 °C. Alcoholic fermentation was considered finished when the must reached 2.5 g L^{-1} residual sugar. After 16 d maceration-fermentation, skins and seeds were manually removed, and then the tanks were carried to cold at 6 °C to eliminate lees. Wines were inoculated with 4 g hL^{-1} of *Oenococcus oeni* strain, B28 PreAc (Laffort). The malolactic fermentation was carried out in 5 L tanks. Malic acid analyses were performed weekly to determine the end of malolactic fermentation (bellow 100 mg L^{-1} malic acid). Then, wine samples were frozen at -20 ºC until the analysis of oenological parameters and volatile compounds.

Oenological parameter analysis

Alcohol degree, pH, total acidity (g L^{-1} tartaric acid) and volatile acidity (g L^{-1} acetic acid), total and free sulfur dioxide and reducing sugars were analyzed according to the methodology established by OIV (2003). Wine color intensity and total polyphenol index were analyzed according to the methodology published by Bordeu and Scarpa (1998). Malic acid was analyzed weekly by an enzymatic equipment (Biowine 300, Biolan, Bilbao, Spain). All treatments were performed in triplicate, so the results were the average of three analyses (n = 3).

Analysis of wine volatile compounds by GC-MS

Volatile compounds were extracted following a previously reported methodology (López et al., 2002). Prepacked cartridges (total volume 3 mL) filled with 200 mg LiChrolut EN resin (Merck, Darmstadt, Germany) were used for this extraction. Before passing the wine through the cartridge, 500 µL internal standard (2-octanol) were added in absolute ethanol solution (Merck). The separation, identification and quantification of volatile compounds from the wine were carried out using an Agilent 7890A gas chromatograph, coupled with a 5975C mass spectrometer (Agilent Technologies Inc., Santa Clara, California, USA). The unit was equipped with a fused silica capillary column (30 m × 0.25 mm id, and

0.5 μm phase thickness, DB-Wax, J & W Scientific, Agilent). The carrier was helium applied at a flow rate of 1 mL min^{-1}. The temperature of the injector was 250 ºC and 2 μL wine extract were injected. The oven temperature was initially held at 40 ºC for 5 min, then increased linearly at a rate of 2 ºC min^{-1} up to 130 ºC and held at that temperature for 5 min; after that, temperature was again increased at a rate of 2 ºC min^{-1} up to 180 ºC and held at that temperature for 2 min; finally, the temperature was increased linearly at a rate of 4 ºC min^{-1} up to 230 min. The analysis was carried out with two injections: in split mode (50:1) for isoamyl alcohols, benzyl alcohol, 2-phenylethanol, 1-hexanol, ethyl hexanoate, ethyl lactate, diethyl succinate and ethyl octanoate, and in splitless mode for the rest of the analyzed volatile compounds. Ionization was carried out by electron impact at 70eV. The operating method was a scan mode at m/z between 30 and 300. Identification was carried out using the NIST library (Merck, Darmstadt, Germany). When standards were available, the quantification was based on seven-point calibration curves of the respective standards (Sigma-Aldrich, Steinheim, Germany) ($R^2 > 0.93$) in a 12% (v v^{-1}) ethanol solution with 6 g L^{-1} tartaric acid at pH 3.6; otherwise, semi-quantitative analyses were carried out using the calibration curves of the most similar compound.

Statistical analysis

The statistical analysis of oenological parameters and volatile compounds was performed using one-way ANOVA by Statgraphics Centurion XVI.I (Statgraphics Technologies, The Plains, Virginia, USA). The Duncan test at 95% probability level was used to compare differences between samples. Principal component analysis (PCA) was performed using InfoStat (www.infostat.com.ar).

RESULTS AND DISCUSSION

Wine oenological parameters

Table 1 shows the values of oenological parameters, color intensity (CI) and total polyphenol index (TPI) in Cabernet Sauvignon wines elaborated from grapevines untreated (control) and treated with different N sources: urea (Ur), urea plus S (Ur+S), arginine (Arg), Nutrymir Thiols (NT) and Basfoliar Algae (BA). Significant differences were found in pH, total acidity, volatile acidity, and CI among the treatments. Regarding pH, wines from Ur applications presented the lowest values (Table 1). The wines from Ur+S and Arg treatments showed lower total acidity than control and Ur wines. The wines made from grapevines treated with NT presented lower volatile acidity than Arg wines, and along with BA, higher values of CI than Ur and Ur+S wines. As several authors have extensively studied, pH plays an important role in wine color, especially in phenolic stability and the process of copigmentation (Zhang et al., 2016). In wines, the phenolic compounds have an important role in copigmentation, aging, oxygen-depleting compounds, and the bitter and a stringent feature, which are determining factors for the wine taste and character (Cetó et al., 2012). TPI in wines was similar to those showed by Cetó et al. (2012).

Table 1. Oenological parameters, color intensity (CI), and total polyphenol index (TPI) in wines from untreated (control) and treated 'Cabernet Sauvignon' grapevines with different N sources as foliar fertilizers: urea (Ur), urea plus S (Ur+S), arginine (Arg), and different commercial products Nutrimyr Thiols (NT) and Basfoliar Algae (BA).

	Control	Ur	Ur+S	Arg	NT	BA
Alcohol degree, % v v^{-1}	14.73 ± 0.74a	15.07 ± 0.25a	14.60 ± 0.60a	15.00 ± 0.30a	14.23 ± 0.65a	14.27 ± 0.31a
pH	4.13 ± 0.11b	3.99 ± 0.06a	4.16 ± 0.02b	4.21 ± 0.08b	4.18 ± 0.05b	4.15 ± 0.08b
Total acidity, g L^{-1} [(a)]	3.67 ± 0.26bc	3.83 ± 0.08c	3.42 ± 0.06a	3.41 ± 0.06a	3.46 ± 0.11ab	3.45 ± 0.02ab
Volatile acidity, g L^{-1} [(b)]	0.39 ± 0.04ab	0.40 ± 0.03ab	0.40 ± 0.03ab	0.43 ± 0.02b	0.36 ± 0.04a	0.42 ± 0.04ab
CI	8.22 ± 0.95ab	6.47 ± 0.29a	6.88 ± 0.85a	8.39 ± 0.97ab	9.57 ± 0.91b	9.57 ± 1.75b
TPI	43.20 ± 7.73a	38.27 ± 2.16a	41.77 ± 4.42a	46.13 ± 3.91a	46.20 ± 2.41a	43.87 ± 1.23a

All parameters are given with their standard deviation (n = 3). Different letters in the same row indicate significant differences among treatments ($p \leq 0.05$).
[(a)] As g tartaric acid L^{-1}.
[(b)] As g acetic acid L^{-1}.

The differences obtained may be attributed to the N treatment applied to the grapevines since the variety studied, application dosage, climate condition, among others parameters, were similar. Lacroux et al. (2008) found few differences in most of oenological parameters studied in wines from 'Sauvignon blanc' grapevines treated with Ur and Ur+S with a dose of 10 kg N ha^{-1}. Ancín-Azpilicueta et al. (2013) showed that urea treatments at 2 kg N ha^{-1} to 'Tempranillo' grapevines decreased the values of wine alcohol degree and total acidity, and increased pH and volatile acidity in wines. When these authors applied urea to the grapevines at 4 kg N ha^{-1}, these differences in wines were higher than the application of 2 kg N ha^{-1}, except in volatile acidity, which did not differ from the control samples. Portu et al. (2015) found that urea and phenylalanine applications to the 'Tempranillo' grapevines treated with doses of 0.9 and 1.5 kg N ha^{-1} did not affect the wine oenological parameters in respect to control samples.

Wine volatile composition

Table 2 shows the wine volatile compounds concentration from 'Cabernet Sauvignon' grapevines untreated (control) and treated with Ur, Ur+S, arginine (Arg) and two commercial N products as Nutrimyr Thiols (NT) and Basfoliar Algae (BA). Table 3 shows the odorant activity values (OAV) of the Cabernet Sauvignon wine volatile compounds. OAV was calculated as the ratio of the concentration to the odor detection thresholds reported by different authors for each volatile compound (Guth, 1997; Culleré et al., 2004; Cai et al., 2014; Noguerol-Pato et al., 2014).

The volatile compounds found in most abundant concentration in wines were isoamyl alcohols (3-methyl-1-butanol + 2-methyl-1-butanol), 2-phenylethanol and isobutanol (Table 2). As expected, the higher alcohols were the family of compounds found in most abundant concentration in wines, ranging from 97% to 98% of total volatile composition. On the other hand, the volatile compounds found in the lowest amount in wines were geraniol, linalool, citronellol, vanillin and *cis*-3-hexen-1-ol. Thus, the terpenes were the group of compounds found in least abundant concentration in wines, ranging from 0.002% to 0.006% of total volatile compounds content. These results were similar to those shown by Falcao et al. (2008) in a characterization of young Cabernet Sauvignon wines from Brazil. However, the results found in our research were in discrepancy to those reported by Tao et al. (2008) in young Cabernet Sauvignon wines from China. These authors found that higher alcohols represented about of 46% of the total volatile compounds, while ethyl and acetate esters reached 51% of the total analyzed volatile compounds. Table 3 shows the odorant activity value (OAV) of each wine volatile compound analyzed. It is known that volatile compounds with OAVs higher than 1 contribute to wine aroma. However, the presence of other compounds, although exhibiting OAVs lower than 1 ($0.2 < OAV < 1.0$) could also contribute to the aroma of wines (Garde-Cerdán et al., 2008). The most odoriferous compounds in Cabernet Sauvignon wines were ethyl octanoate, isoamyl acetate, ethyl hexanoate, 2-phenylethanol, and ethyl butanoate; while the lowest odoriferous compounds in wines were benzyl alcohol, 1-propanol, 1-butanol, 1-pentanol, 4-methyl-1-pentanol, ethyl lactate, diethyl succinate, ethyl 3-hydroxybutanoate, and methyl vanillate. Ethyl octanoate, isoamyl acetate, ethyl hexanoate, and 2-phenylethanol are responsible for the fruity and floral sensory properties of wines (Tao et al., 2008). Regarding the family of volatile compounds, the most odoriferous was the ethyl esters, followed by higher alcohols and acetate esters. It is important to note that only two acetate esters were analyzed in wines. However, their OAVs were greater than most of the individual higher alcohols (Table 3).

Effects of foliar N applications to 'Cabernet Sauvignon' grapevines on higher alcohols content in wines

The applications of the different sources of N to 'Cabernet Sauvignon' grapevines have a differentiated effect on wine higher alcohols concentration (Table 2). Ur application had a slight effect on wine higher alcohols composition. The concentration of benzyl alcohol and 1-pentanol in wines elaborated from grapevines treated with Ur was lower than control wines although, these compounds may have no importance in the wine aroma ($OAV = 0$) (Table 3). In addition, wines from Ur+S grapevine treatment showed higher concentration of isoamyl alcohols (3-methyl-1-butanol + 2-methyl-1-butanol) and lower content of benzyl alcohol and 2-phenylethanol than control wines. The concentration of 2-methyl-1-butanol in Ur+S samples was higher than Ur, Arg and NT wines, while the content of 2-phenylethanol in Ur+S wines was lower than Ur, NT and BA wines. Isoamyl alcohols and 2-phenylethanol strongly contribute to the alcohol, cheese, roses and honey aroma descriptors to Cabernet Sauvignon wines ($OAV > 1$) (Table 3). The application of Arg to the grapevines had a detrimental effect on wine higher alcohols. The concentration of 2-methyl-1-butanol and 2-phenylethanol in wines from grapevines treated with Arg was lower than control, Ur, NT, and BA wines. Thus, total higher alcohols concentration

Table 2. Volatile compounds in Cabernet Sauvignon wines from untreated (control) and treated grapevines with different N sources as foliar fertilizers: urea (Ur), urea plus S (Ur+S), arginine (Arg), and different commercial products: Nutrimyr Thiols (NT) and Basfoliar Algae (BA).

	Control	Ur	Ur+S	Arg	NT	BA
Higher alcohols, mg L^{-1}						
3-Methyl-1-butanol	126.55 ± 22.46ab	123.02 ± 22.64ab	203.43 ± 44.98c	78.32 ± 3.74a	150.49 ± 14.78bc	203.14 ± 43.18c
2-Methyl-1-butanol	52.84 ± 8.64b	55.42 ± 9.11b	87.96 ± 17.39d	33.65 ± 1.59a	65.42 ± 6.44bc	83.98 ± 13.21cd
Benzyl alcohol	1.51 ± 0.29d	0.52 ± 0.05a	0.82 ± 0.20abc	0.73 ± 0.11ab	1.07 ± 0.12c	0.87 ± 0.16bc
2-Phenylethanol	207.32 ± 18.86b	227.47 ± 29.33b	175.49 ± 13.48a	174.43 ± 3.47a	238.92 ± 17.12b	211.58 ± 5.93b
1-Propanol	5.84 ± 0.70ab	6.73 ± 0.62b	6.33 ± 0.31b	6.70 ± 0.07b	4.89 ± 0.63a	5.83 ± 0.78ab
1-Butanol*	2.74 ± 0.30ab	3.22 ± 0.52bc	2.92 ± 0.54abc	3.56 ± 0.33c	2.42 ± 0.27a	2.66 ± 0.15ab
1-Pentanol*	0.32 ± 0.02b	0.26 ± 0.02a	0.35 ± 0.01b	0.33 ± 0.03b	0.31 ± 0.03b	0.35 ± 0.02b
4-Methyl-1-pentanol*	0.27 ± 0.02a	0.25 ± 0.03a	0.28 ± 0.02a	0.26 ± 0.00a	0.26 ± 0.02a	0.28 ± 0.02a
3-Methyl-1-pentanol *	0.65 ± 0.03a	0.77 ± 0.08a	0.66 ± 0.08a	0.77 ± 0.05a	0.67 ± 0.04a	0.64 ± 0.11a
Isobutanol	24.52 ± 2.23c	25.22 ± 0.41c	24.13 ± 0.46bc	23.54 ± 1.27bc	18.78 ± 1.31a	21.79 ± 1.69b
Total alcohols	422.57 ± 53.57b	442.88 ± 62.80b	502.38 ± 77.47b	322.29 ± 10.66a	483.22 ± 40.76b	531.19 ± 65.25b
C6 compounds, µg L^{-1}						
1-Hexanol	1633.13 ± 234.01ab	1545.76 ± 198.90ab	1839.62 ± 36.91abc	1477.46 ± 102.45a	1881.77 ± 112.58bc	2214.87 ± 370.39c
trans-3-Hexen-1-ol	51.14 ± 5.17ab	43.51 ± 5.09a	50.55 ± 2.51ab	52.68 ± 6.94ab	52.20 ± 5.79ab	56.12 ± 2.02b
cis-3-Hexen-1-ol	27.97 ± 3.25b	14.44 ± 0.93a	26.75 ± 6.88b	21.04 ± 1.78ab	25.02 ± 4.99b	16.46 ± 0.81a
Total C6 compounds	1712.24 ± 242.43a	1603.71 ± 204.92a	1916.92 ± 46.30ab	1551.18 ± 111.17a	1958.99 ± 123.36ab	2287.45 ± 373.22b
Ethyl esters, µg L^{-1}						
Ethyl hexanoate	324.42 ± 25.48b	328.60 ± 32.85b	385.28 ± 36.73c	254.40 ± 25.22a	386.49 ± 15.70c	416.00 ± 15.49c
Ethyl lactate*	2343.09 ± 104.09c	1504.66 ± 240.34b	1575.97 ± 315.80b	1091.91 ± 36.71a	1365.45 ± 159.55ab	1440.96 ±148.07ab
Diethyl succinate	4955.19 ± 732.10b	2907.79 ± 675.11a	3061.86 ± 1013.76a	4603.74 ± 222.09b	4088.12 ± 441.74ab	3848.76 ± 422.87ab
Ethyl butanoate*	171.54 ± 1.64bc	161.79 ± 7.28b	96.38 ± 15.05a	164.50 ± 7.10b	182.69 ± 8.18c	181.48 ± 5.58c
Ethyl octanoate	453.09 ± 38.42ab	396.37 ± 28.81a	590.46 ± 34.37c	416.33 ± 24.50a	503.88 ± 61.78b	600.50 ± 68.88c
Ethyl 3-hydroxybutanoate*	100.37 ± 2.93ab	97.22 ± 0.29a	103.46 ± 5.26ab	99.72 ± 0.93ab	103.57 ± 5.91ab	107.13 ± 5.36b
Ethyl decanoate	190.13 ± 14.95ab	157.06 ± 8.33a	214.14 ± 28.98c	170.48 ± 8.33a	187.90 ± 14.79ab	227.96 ± 38.96c
Total ethyl esters	8537.82 ± 919.62b	5553.49 ± 993.00a	6027.54 ± 1449.91a	6801.09 ± 324.88ab	6818.09 ± 707.65ab	6822.79 ± 705.23ab
Acetate esters, µg L^{-1}						
Isoamyl acetate	815.81 ± 51.52ab	702.73 ± 74.89a	951.31 ± 141.57b	661.43 ± 58.20a	1002.51 ± 160.93b	1026.75 ± 199.58b
2-Phenylethyl acetate	164.18 ± 28.80a	214.71 ± 34.26b	147.75 ± 23.86a	165.68 ± 10.86a	160.17 ± 12.17a	145.86 ± 26.31a
Total acetates	979.99 ± 80.32ab	917.44 ± 109.16ab	1099.06 ± 165.43ab	827.11 ± 69.06a	1162.68 ± 173.09b	1172.60 ± 225.88b
Vanillin derivatives, µg L^{-1}						
Vanillin	27.29 ± 0.23ab	26.72 ± 0.66a	28.42 ± 0.85ab	28.15 ± 1.31ab	28.77 ± 1.43b	28.56 ± 0.59b
Methyl vanillate	39.73 ± 0.72a	39.21 ± 3.58a	39.07 ± 1.22a	41.30 ± 2.33a	38.57 ± 1.31a	40.11 ± 0.56a
Ethyl vanillate*	37.59 ± 0.99ab	36.13 ± 0.35a	35.75 ± 1.07a	38.40 ± 0.99b	38.39 ± 1.80b	36.25 ± 0.85a
Acetovanillone	58.87 ± 4.11a	50.84 ± 6.34a	55.61 ± 6.21a	56.73 ± 7.15a	52.78 ± 1.34a	56.02 ± 8.24a
Total vanillin derivatives	163.47 ± 6.05a	152.90 ± 10.93a	158.84 ± 9.36a	164.58 ± 11.77a	158.51 ± 5.88a	160.94 ± 10.23a
Terpenes, µg L^{-1}						
Geraniol	3.32 ± 0.10a	3.35 ± 0.35ab	3.89 ± 0.11c	3.60 ± 0.07abc	3.82 ± 0.10c	3.76 ± 0.38bc
Linalool	3.38 ± 0.23a	6.42 ± 0.09d	3.93 ± 0.02ab	5.12 ± 0.82c	3.72 ± 0.44ab	4.26 ± 0.55b
Citronellol	8.29 ± 1.47b	8.10 ± 0.56b	6.51 ± 0.62a	11.64 ± 0.04c	8.74 ± 0.66b	8.09 ± 0.38b
Total terpenes	14.99 ± 1.80a	17.87 ± 1.01b	14.33 ± 0.75a	20.36 ± 0.93c	16.29 ± 1.20ab	16.11 ± 1.32ab

All parameters are given with their standard deviation (n = 3). Different letters in the same row indicate significant differences among treatments ($p \leq 0.05$).
*Semi-quantitative analyses were carried out using the calibration curves of the most similar compound.

in wines from Arg grapevine treatment was the lowest. NT applied to the grapevines had a slight effect on wine higher alcohols. The concentration of benzyl alcohol and isobutanol in wines from grapevines treated with NT was lower than control wines (Table 2). The application of BA improved the concentration of certain individual higher alcohols, especially compounds that contribute to wine aroma. Thus, the concentration of isoamyl alcohols in wines elaborated from grapes treated with BA was higher than control, Ur, and Arg wines, while the concentration of benzyl alcohol and isobutanol was lower than control wines.

In our samples, must yeast assimilable N (YAN) concentrations ranged from 251 to 282 mg N L^{-1} (Gutiérrez-Gamboa et al., 2017a). These values are considered as moderate N levels (Bell and Henschke, 2005). Ur, Arg, and NT treatments applied to the grapevines increased must YAN concentration respect to the control samples; however, the foliar application of Ur+S and BA treatments did not affect must YAN content (Gutiérrez-Gamboa et al., 2017a). Ancín-Azpilicueta et al. (2013) and Lasa et al. (2012) found an inverse correlation between the concentration of wine higher

Table 3. Odorant activity value (OAV) of the different volatile compounds found in Cabernet Sauvignon wines from untreated (control) and treated grapevines with different N sources as foliar fertilizer: urea (Ur), urea plus S (Ur+S), arginine (Arg), and different commercial products: Nutrimyr Thiols (NT) and Basfoliar Algae (BA).

	Control	Ur	Ur+S	Arg	NT	BA	Odor threshold	Aroma descriptor
Higher alcohols								
3-Methyl-1-butanol	4.22	4.10	6.78	2.61	5.02	6.77	30000[d]	Cheese, alcohol[bd]
2-Methyl-1-butanol	1.76	1.85	2.93	1.12	2.18	2.80	30000[d]	Alcohol[d]
Benzyl alcohol	0.01	0.00	0.00	0.00	0.01	0.00	200000[d]	Roasted, sweet, fruity[c]
2-Phenylethanol	20.73	22.75	17.55	17.44	23.89	21.16	10000[d]	Roses, honey[c]
1-Propanol	0.02	0.02	0.02	0.02	0.02	0.02	306000[c]	Alcohol, ripe fruit[c]
1-Butanol	0.02	0.02	0.02	0.02	0.02	0.02	150000[d]	Medicinal, phenolic[c]
1-Pentanol	0.00	0.00	0.01	0.01	0.00	0.01	64000[c]	Balsamic, bitter almond[c]
4-Methyl-1-pentanol	0.01	0.01	0.01	0.01	0.01	0.01	50000[c]	Almond, toasted[c]
3-Methyl-1-pentanol	1.31	1.54	1.32	1.53	1.33	1.28	500[c]	Pungent, solvent, greenc[c]
Isobutanol	0.33	0.34	0.32	0.31	0.25	0.29	75000[c]	Alcohol, solvent, green, bitter[c]
C6 compounds								
1-Hexanol	0.20	0.19	0.23	0.18	0.24	0.28	8000[d]	Herbaceous, grass, woody[c]
trans-3-Hexen-1-ol	0.05	0.04	0.05	0.05	0.05	0.06	1000[d]	Herbaceous, green[c]
cis-3-Hexen-1-ol	0.07	0.04	0.07	0.05	0.06	0.04	400[d]	Herbaceous, green, bitter, fatty[c]
Ethyl esters								
Ethyl hexanoate	23.17	23.47	27.52	18.17	27.61	29.71	14[b]	Banana, green apple[c]
Ethyl lactate	0.02	0.01	0.01	0.01	0.01	0.01	154636[b]	Fruity, buttery[c]
Diethyl succinate	0.02	0.01	0.02	0.02	0.02	0.02	200000[d]	Fruity, melon[c]
Ethyl butanoate	8.58	8.09	4.82	8.22	9.13	9.07	20[b]	Banana, pineapple, strawberry[c]
Ethyl octanoate	90.62	79.27	118.09	83.27	100.78	120.10	5[b]	Sweet, floral, fruity, banana, pear[c]
Ethyl 3-hydroxybutanoate	0.01	0.00	0.01	0.00	0.01	0.01	20000[d]	Grape[c]
Ethyl decanoate	0.95	0.79	1.07	0.85	0.94	1.14	200[d]	Fruity, fatty, pleasant[c]
Acetate esters								
Isoamy lacetate	27.19	23.42	31.71	22.05	33.42	34.22	30[a]	Banana[c]
2-Phenylethyl acetate	0.66	0.86	0.59	0.66	0.64	0.58	250[d]	Rose[d]
Vanillin derivatives								
Vanillin	0.45	0.45	0.47	0.47	0.48	0.48	60[d]	Vanillin[b]
Methyl vanillate	0.01	0.01	0.01	0.01	0.01	0.01	3000[b]	Vanillin[b]
Ethyl vanillate	0.04	0.04	0.04	0.04	0.04	0.04	990[d]	Pollen, flowery[b]
Acetovanillone	0.06	0.05	0.06	0.06	0.05	0.06	1000[d]	Flowery, clove, vanilla[b]
Terpenes								
Geraniol	0.11	0.11	0.13	0.12	0.13	0.13	30[d]	Citric, geranium[c]
Linalool	0.23	0.43	0.26	0.34	0.25	0.28	15[d]	Flowery, muscat[c]
Citronellol	0.08	0.08	0.07	0.12	0.09	0.08	100[d]	Rose, citrus[cd]

OAV was calculated from the odors threshold (μg L^{-1}) exhibited by different authors: [a]Guth (1997), [b]Culleré et al. (2004), [c]Cai et al. (2014), and [d]Noguerol-Pato et al. (2014).

alcohols and YAN in must. Thus, these authors showed that the higher alcohols concentration in wines decreased as the YAN content in must increased by the effect of urea foliar applications to the grapevines. Therefore, the low wine concentration of several individual higher alcohols in Ur, Arg, and NT wines were probably due to the increase in YAN concentrations through the N foliar applications.

Thus, Oshita et al. (1995) reported that under low amino acid availability related to low YAN concentrations, surplus keto acids, which are largely synthesized from sugars, are decarboxylated and reduced to higher alcohols, due to the lack of alpha-amino N availability from transamination reactions. In contrast, under the presence of sufficient amino acids in the medium, amino acid transamination reactions (needed for the biosynthesis of amino acids in short supply) lead to a relatively larger formation of higher alcohols essentially from corresponding amino acids by the Ehrlich pathway, relative to those formed from sugars (Bell and Henschke, 2005).

Effects of foliar N applications to 'Cabernet Sauvignon' grapevines on C6 compounds content in wines

Ur and BA applications to 'Cabernet Sauvignon' grapevines had effects on the wine concentration of C6 compounds. The C6 compounds at high levels can provide undesirable herbaceous flavors to the wines (Cai et al., 2014). These descriptors are usually given to define the wine Cabernet Sauvignon aroma (Cai et al., 2014). The wine concentration of *cis*-3-hexen-1-ol elaborated from grapes treated with Ur and BA was lower than control, Ur+S, and NT samples (Table 2). Wines

resulting from grapes treated with BA showed higher concentration of 1-hexanol than control, Ur, and Arg wines. The concentration of total C6 compounds in wines elaborated from grapes treated with BA was higher than control, Ur and Arg wines. Regarding OAV values, the high concentration of 1-hexanol found in BA wines can affect the wine aroma (OAV > 0.2) being able to contribute to a more herbaceous aroma in wine (Table 3). On the other hand, *cis*-3-hexen-1-ol did not contribute to the aroma of Cabernet Sauvignon wines (OAV < 0.1) (Table 3).

Effects of foliar N applications to 'Cabernet Sauvignon' grapevines on ethyl and acetate esters content in wines

The different foliar N applications to 'Cabernet Sauvignon' grapevines have an important effect on wine ethyl and acetate esters concentrations (Table 2). The concentration of ethyl hexanoate, ethyl octanoate, and ethyl decanoate in wines from Ur+S and BA grapevine treatments was higher than control, Ur, and Arg wines. These compounds greatly contribute to the fruity aroma of the wine with aromatic descriptors such as banana, green apple, pear, among others (Table 3). The concentration of ethyl lactate in wines elaborated from untreated grapes was the highest. This compound appears generally in relatively high amounts when lactic acid has formed in malolactic fermentation (Cortés-Diéguez et al., 2015). The wine concentration of diethyl succinate in Ur and Ur+S samples was lower than control and Arg wines. Diethyl succinate increases after the aging of the wine; however, its composition is representative of the wine distillate (Cortés-Diéguez et al., 2015). Ethyl lactate and diethyl succinate did not contribute to the Cabernet Sauvignon wine aroma (OAVs < 0.2) (Table 3). Due to the decrease in these compounds, the concentration of total ethyl esters in wines from Ur and Ur+ S treatments was lower than control wines. The application of Arg to grapevines had slight effect on wine ethyl esters concentration. Thus, the concentration of ethyl hexanoate in wines elaborated from grapes treated with Arg was the lowest. The commercial products applied to the grapevines had a differentiating effect on ethyl esters composition. The concentration of ethyl octanoate and ethyl decanoate in wines from NT grapevine treatment was lower than BA wines. The concentration of ethyl butanoate in wines from commercial products grapevine treatments (NT and BA) was higher than Ur, Ur+S, and Arg wines, contributing to the banana, pineapple, and strawberry wine aroma (Table 3) (Cai et al., 2014).

Regarding the acetate esters, the concentration of isoamyl acetate in wines from Ur+S, NT, and BA grapevine treatments was higher than Ur and Arg wines. However, there were nonsignificant differences for this compound among the treatments and control samples. This compound can contribute to banana aroma in wines (Cai et al., 2014). The concentration of 2-phenylethyl acetate in wines elaborated from grapes treated with Ur was the highest. This compound can contribute in a pleasant way to the Cabernet Sauvignon wines with roses aromas (OAV > 0.2) (Table 3). The wines from Arg treatment showed lower concentration of total acetate esters than NT and BA samples.

The principal esters of wine are synthesized enzymatically by yeast from alcohols and fatty acids, but esterases and wine acidity compete causing their hydrolysis (Bell and Henschke, 2005). Martínez-Gil et al. (2012) confirmed the possibility to estimate the concentration of ethyl esters in the wines with the concentration of N compounds in the must, exhibiting a strong relationship between the must N composition and the formation of ethyl esters in wines. In addition, Ancín-Azpilicueta et al. (2013) reported that the foliar urea application increased the concentration of ethyl hexanoate, ethyl octanoate and ethyl decanoate as the YAN concentration increases by incremental doses of urea. Regarding the grape amino acids data, Gutiérrez-Gamboa et al. (2017a) showed a strong correlation between a high concentration of several individual amino acids and the foliar applications of Ur+S and BA to the grapevines. Therefore, the highest concentration of ethyl hexanoate, ethyl octanoate and ethyl decanoate in wines from grapes treated with Ur+S and BA applications were probably due to the high concentration of grape amino acid reached through of these treatments. These compounds may contribute significantly to floral and fruity aroma to the Cabernet Sauvignon wines (OAV > 1) (Table 3).

Effects of foliar N applications to 'Cabernet Sauvignon' grapevines on vanillin derivatives and terpenes content in wines

The different N foliar applications to 'Cabernet Sauvignon grapevines' did not affect the wine concentration of any vanillin derivative compounds and only vanillin could contribute to the Cabernet Sauvignon wine aroma (OAV > 0.2) (Tables 2 and 3). Terpenes play an important role on the varietal aroma of wine contributing to its floral and citric character (Peinado et al., 2004). The treatments applied to the grapevines significantly affected the wine concentration of terpenes in Cabernet Sauvignon wines (Table 2). Thus, the concentration of geraniol in wines made from grapes treated with Ur+S, NT, and BA was higher than control wines. In addition, the concentration of linalool in wines elaborated from

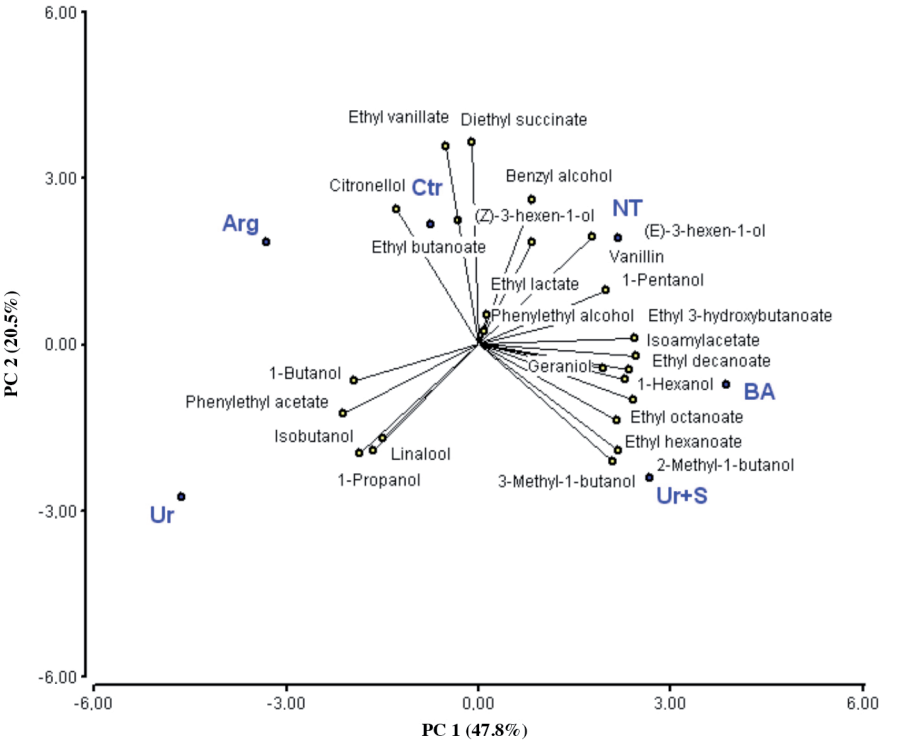
grapes treated with Ur was the highest. This compound can contribute to the wine with floral aromas according to the OAV values (OAV > 0.2) (Table 3). Moreover, the concentration of citronellol and total terpenes in wines elaborated from grapes treated with Arg was the highest.

Treatments classification

To classify the different treatments, PCA was performed (Figure 1) with the compounds, whose p-valor in the f-test was less than 0.5. Principal component 1 (PC1) explained 47.8% of the variance and principal component 2 (PC2) explained 20.5%, representing a 68.3% of the total variance. PC1 was strongly correlated with isoamyl alcohols, 1-hexanol, ethyl hexanoate, ethyl octanoate, ethyl 3-hydroxybutanoate, ethyl decanoate, isoamyl acetate, and 2-phenylethyl acetate. PC2 was strongly correlated with diethyl succinate and ethyl vanillate. PC1 allowed to separate control and the different treatments. Control samples were correlated with high content of diethyl succinate, ethyl butanoate, ethyl vanillate, and citronellol in the wine. These compounds except ethyl butanoate did not contribute the wine aroma (Table 3). Ur treatment was correlated with high wine concentration of 1-propanol, 1-butanol, isobutanol, 2-phenylethyl acetate, and linalool. Of these compounds, only 1-butanol did not affect the wine aroma (Table 3). The Ur+S and BA treatments were correlated with major content of several volatile compounds in the wine such as isoamyl alcohols, 1-hexanol, ethyl hexanoate, ethyl octanoate, ethyl 3-hydroxybutanoate, ethyl decanoate, isoamyl acetate, and geraniol in relation to the other treatments. Of these compounds, ethyl 3-hydroxybutanoate, and geraniol did not affect the wine aroma (Table 3). The NT treatment was correlated with high content of 1-pentanol, and vanillin in the wine. The latter can contribute to the wine aroma (Table 3). Arg was inversely correlated with high content of isoamyl alcohols, ethyl hexanoate, and ethyl octanoate.

As reported by Gutiérrez-Gamboa et al. (2017a), Ur+S and BA applications to the 'Cabernet Sauvignon' grapevines were correlated with high must concentration of alanine, isoleucine, valine, and threonine. Higher alcohols are formed from these amino acids (Bell and Henschke, 2005). On the other hand, these same treatments were also correlated with high must concentration of other several amino acids which could led to an important synthesis of wine ethyl esters

Figure 1. Principal component analysis (PCA) performed with wine volatile compounds, whose p-valor in the F-test was less than 0.5, in 'Cabernet Sauvignon' samples from untreated, control (Ctr), and treated vineyards with different foliar N applications, such as urea (Ur), urea plus S (Ur+S), arginine (Arg) and two different commercial products, Nutrimyr Thiols (NT) and Basfoliar Algae (BA).



(Bell and Henschke, 2005). The addition of amino acids and ammonium nitrogen increased both acetate and medium chain fatty acid esters to a greater extent and decreased higher alcohols to a lesser extent than ammonium nitrogen alone, whereas ammonium nitrogen substantially increased ethyl acetate and acetic acid (Torrea et al., 2011). Moreover, according to the results in cachaça fermentation, higher alcohol production was reduced by ammonium supplementation, and this can be correlated with a general downregulation of genes encoding decarboxylases and dehydrogenases of the Ehrlich pathway. The production of acetate esters was enhanced by mid-range ammonium supplementation while the production of acyl esters by high ammonium supplementation. The acyl esters could be correlated with expression of alcohol acyl-transferase *EEB1* and the acyl esterase *IAH1* (Espinosa Vidal et al., 2013). Therefore, the aforementioned volatile compounds may contribute to the desirable aroma to Cabernet Sauvignon wines, which exhibited OAV values higher than 0.2. Because of this, Ur+S and BA treatments application to the 'Cabernet Sauvignon' grapevines improved wine volatile composition, respect to the others treatments and control samples.

CONCLUSIONS

Nitrogen foliar applications to 'Cabernet Sauvignon' grapevines improved wine volatile compounds concentration from a vineyard located in a warm climate, mainly those that have varietal aroma, fruity and floral descriptors. The most relevant results were achieved through urea plus S and Basfoliar Algae treatments on some higher alcohols and esters. Besides, arginine and urea applications to the grapevines allowed a high content of certain terpenes in wines. Therefore, foliar N applications to the 'Cabernet Sauvignon' grapevines can enhance wine aroma. These findings have oenological and viticultural interest for improving wine quality, considering the negative impact that climate change have in warmer wine regions on the Cabernet Sauvignon wine production.

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