Amino acid content in red wines obtained from grapevine nitrogen foliar treatments: consumption during the alcoholic fermentation

Javier Portu, Rosa López, Lucía González-Arenzana, Isabel López-Alfaro, Pilar Santamaría, Teresa Garde-Cerdán

Instituto de Ciencias de la Vid y del Vino, Gobierno de La Rioja-Consejo Superior de Investigaciones Científicas, Universidad de La Rioja, Logroño, Spain

Abstract

Nitrogen is an important element for grapevine and winemaking which affects the development of the plant and yeast, and therefore it is important for wine quality. The aim of this work was to study the influence of foliar application to vineyard of proline, phenylalanine and urea and two commercial nitrogen fertilizers, without and with amino acids in their formulation, on the wine amino acid content and their consumption during the alcoholic fermentation. The results showed that these treatments did not affect the amino acid composition in wines. The differences observed for certain amino acids were so small that the concentration of total amino acids was not significantly different among wines. Moreover, it was observed that the higher the content of amino acids in the medium, the greater their consumption during the alcoholic fermentation.

Introduction

Nitrogen fertilization entails a correct development of grapevine and might guarantee an appropriate grape composition[1] avoiding stuck or sluggish fermentations caused by nitrogen deficiencies.[2] Fertilizers are normally added to the soil in order to be absorbed by plant roots. Nowadays, the polution problems arised from the excessive use of soil fertilizers make necessary to develop new fertilization methods, more precise and efficient. One of these methods is foliar fertilization, which entails a fast and efficient assimilation of applied products by plant,[3] reducing costs besides contributing to sustainable and eco-friendly agriculture. Previous studies have shown that foliar application of oak extracts and urea modify grape and wine composition.[3-6]

Ammonium and amino acids are essential in the metabolism of yeast and their content affects the fermentation kinetic, as nitrogen-deficient musts can cause slow and stuck fermentations.[2,7] Some amino acids are precursors of higher alcohols, and esters, compounds that contribute to wine aroma.[8-10] However, wines with higher amounts of residual nitrogen have more risk of microbiological instability, with the possible formation of ethyl carbamate and biogenic amines, which are negative compounds for wine quality.[11,12]

Therefore, nitrogen fertilization must ensure grapevine growth and an adequate grape composition, allowing then a correct vinification and the obtaining of wines with low levels of residual nitrogen avoiding possible microbial alterations. By reason of the matters aforesaid, the aim of this work was to study the influence of foliar application of proline, phenylalanine, and urea and two commercial nitrogen fertilizers, without and with amino acids in their formulation, on the wine amino acid content and their consumption during the alcoholic fermentation.

Materials and Methods

Samples, commercial nitrogen fertilizers, grapevine treatments and vinification

Red grapes from *Vitis vinifera* Tempranillo variety grown in the experimental vineyard of the Research Centre of the Spanish northern region of La Rioja (CIDA) during the year 2012 were used. The soil was classified as *Typic Haplocambids*.[13] The vineyard was planted in 1999, grafted on 110-Richter rootstock. Planting density was 3000 plants/ha with vine and row spacings of 1.3 and 2.6 m, respectively. Vines were trained to a vertical shoot position trellis system on a double cordon Royat, and spur pruned to twelve buds per vine. The vineyard had an unirrigated pattern and an average yield about 6500-7000 kg/ha. Weather conditions were recorded by a meteorological station belonging to the Riojan Agroclimatic Service (SIAR) installed near the experimental vineyard (altitude 342 m asl). In 2012, the annual precipitation was 337.2 mm, and the average annual temperature was 13.5ºC.

In this study, five treatments were carried out using several nitrogen sources: proline (Pro), phenylalanine (Phe), urea (Ur), and two commercial nitrogen fertilizers, without (Cp) and with amino acids (Cpaas) in their composition. The nitrogen composition of commercial products was: 103 g total N/L and 48.62 g ammonium/L (Cp); 104 g total N/L and 31.37 g ammonium/L (Cpaas). The content of free amino acids in the nitrogen fertilizer with amino acids (Cpaas) was (in g N/L): 0.24 (Asp), 0.21 (Glu), 0.21 (Ser), 0.97 (Gly), 0.10 (Thr), 1.3 (Arg), 1.1 (Ala), 0.02 (Tyr), 0.12 (Val), 0.03 (Met), 0.03 (Ile), 0.11 (Leu), 0.27 (Lys), and 3.6 (Pro).

To carry out the treatments, aqueous solutions were prepared with a concentration of 750 mg total N/L of Pro, Phe, Ur (Sigma-Aldrich, St. Louis, MO, USA), and commercial nitrogen products (Cp and Cpaas), using Tween 80 as wetting agent (1 mL/L). Control plants were sprayed with water solution of Tween 80 alone.

The treatments were applied to grapevine twice, at veraison and one week later on. For each application, 200 mL/plant was sprayed over leaves, so the total amount applied in each treatment was 900 g total N/ha, assuming 3000 plants/ha. Treatments were carried out in triplicate and were arranged in a complete randomized block design, with 3 vines for each replication.

Grapes were harvested at their optimum technological maturity, *i.e.* when the weight of 100 berries remained constant and the probable alcohol reached 13 (% v/v) for control sample, and then were destemmed and crushed. Aliquots of each sample were frozen in order to

Correspondence: Teresa Garde-Cerdán, Instituto de Ciencias de la Vid y del Vino, Gobierno de La Rioja-Consejo Superior de Investigaciones Científicas, Universidad de La Rioja, Ctra. Mendavia-Logroño NA 134 Km 90, 26071 Logroño, Spain.
Tel. +34.941.291833 - Fax: +34.941.291392.
E-mail: teresa.gardecerdan@gmail.com; teresa.garde@icvv.es

Tel. +34.941.291833 - Fax: +34.941.291392.
E-mail: Teresa.GardeCerdan@gmail.com; teresa.garde@icvv.es

Key words: grape, nitrogen foliar applications, amino acids, alcoholic fermentation, wine.

Acknowledgments: many thanks for the financial support given by the Gobierno de La Rioja to R-11-12 project. TGC, LG-A and IL-A thank INIA-Gobierno de La Rioja for the contracts and JP thanks Gobierno de La Rioja for the formation grant.

Presented at the 3rd International Conference on Wine Active Compounds (WAC) – March 26th-28th 2014, Beaune, France.

Received for publication: 30 June 2014.
Revision received: 5 August 2014.
Accepted for publication: 13 August 2014.

Wine Studies 2014; 3:4475
doi:10.4081/ws.2014.4475

determine their amino acid composition.

To carry out the alcoholic fermentation, 3 kg of pomace (must, seed, skin) were introduced into glass bottles of 4 L.[14] Potassium metabisulfite was added to the samples to give a final total SO_2 concentration of 50 mg/L, and then the alcoholic fermentation was induced with the commercial *Saccharomyces cerevisiae* strain Uvaferm VRB (Lallemand, Montreal, Canada). The fermentations were performed at controlled temperature of 25°C. The end of alcoholic fermentation was determined by measuring the reducing sugars (<2.5 g/L). Aliquots of each wine were frozen in order to determine their amino acid content.

Analysis of amino acids by high performance liquid chromatography

The amino acids analysis was performed by the method described by Garde-Cerdán *et al*.[15] Free amino acids were analyzed by reverse phase high performance liquid chromatography using an Agilent 1100 Series (Agilent, Santa Clara, CA, USA) equipped with an ALS automatic liquid sampler, a fluorometric detector and a DAD detector. Five mL of sample (previously centrifuged, 3077 g, 10 min) was mixed with 100 µL of norvaline (internal standard to quantify all amino acids except proline) and 100 µL of sarcosine (internal standard to quantify proline). The mixture was filtered through a 0.45 µm OlimPeak pore filter (Teknokroma, Barcelona, Spain) and submitted to an automatic precolumn derivatization with *o*-phthaldialdehyde (OPA Reagent; Agilent) for primary amino acids and with 9-fluorenylmethylchloroformate (FMOC Reagent; Agilent) for secondary amino acid. The injected amount from the derivated sample was 10 mL and a constant temperature of 40ºC was maintained. All separations were performed on a Hypersil ODS (250 4.0 mm, I.D. 5 µm) column (Agilent). Solvents and gradient conditions for amino acids analysis are described below.

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). All reagents were first filtered with Millipore filters (0.45 µm). The gradient profile was: 0-15 min, 0-47.5% B, 1.63 mL/min; 15-15.01 min, 47.5% B, 0.80 mL/min; 15.01-25 min, 47.5-60% B, 0.80 mL/min; 25-25.01 min, 60% B, 1.63 mL/min; 25.01-26.01 min, 60-100% B, 1.63 mL/min; 26.01-26.51 min, 100% B, 2.50 mL/min; 26.51-34.01 min, 100% B, 1.63 mL/min; 34.01-36.01 min, 100-0% B, 1.63 mL/min. Detection was performed by fluorescence detector (λ excitation=340 nm, λ emission=450 nm for primary amino acids, and λ excitation=266 nm, λ emission=305 nm for secondary amino acid) and DAD detector (λ=338 nm for primary amino acids and λ=262 nm for secondary amino acid). Identification of compounds was carried out by comparison of their retention times with those of pure reference standards. The pure reference compounds and internal standards were from Sigma-Aldrich. Water was obtained from a Milli-Q purification System (Millipore, Billerica, MA, USA). Quantification of amino acids was performed with an internal standard method. The treatments were performed in triplicate, so the results of free amino acids correspond to the average of 3 analyzes (n=3). The free amino acid content of the commercial product with amino acids (Cpaas) was also determined by this method.

The study about the possible relationship between the concentration of free amino acids in the different initial musts (control samples and musts from the five foliar treatments carried out with proline, phenylalanine, urea, and commercial products without and with amino acids) and their consumption during the corresponding alcoholic fermentations was performed. The consumption was calculated for each amino acid as the difference between its concentration in the initial must and its concentration in the final wine.

Oenological parameters analysis

Musts were physico-chemically characterized by determining probable alcohol, pH, total acidity, malic acid, and potassium according to the European Commission methods.[16]

Statistical analysis

The statistical elaboration of the data was performed using SPSS Version 21.0 statistical package for Windows. The data for amino acids were processed using the variance analysis (ANOVA). Differences between means were compared using the Duncan test at 0.05 probability level. Discriminant analysis was performed with the amino acid concentration in the different musts and wines.

Results

The musts presented a balanced physico-chemical composition, usual for Tempranillo grapes from La Rioja region. No significant differences for any of the physico-chemical parameters studied were found between control and treated samples. In control must, the weight of 100 berries was 185 g, the probable alcohol was 13.2 (% v/v), total acidity was 6.24 g/L, malic acid content was 3.01 g/L, and potassium was 1658 mg/L. Lasa *et al*.[3] described that the urea application to Merlot grapevines affects neither the pH nor the weight of 100 berries, but it caused a decrease in total acidity, and an increase of probable alcohol.

In a previous work,[15] the effect of foliar application of these same nitrogen sources was studied. The results showed that the most effective treatments were phenylalanine and urea, followed by commercial nitrogen fertilizers, whereas proline treatment did not affect the must nitrogen composition. Phenylalanine and urea foliar application enhanced the synthesis of most of the amino acids by the plant, being their effect similar. Moreover, the spray of commercial nitrogen fertilizers over leaves also caused a rise in grape amino acid concentrations, regardless amino acids presence or absence in their formulation.

Table 1 shows the amino acid composition of wines made from the different grapevine nitrogen foliar treatments. The concentrations of glutamic acid, citrulline, arginine, alanine, tryptophan, total amino acids without proline, and proline did not show significant differences between samples. Threonine content in wines from treated grapevines did not show significant differences with respect to the control, although the wine elaborated with grapes from commercial product with amino acids (Cpaas) had a higher content of this amino acid in comparison with the wines from the other treatments (Table 1). Regarding tyrosine concentration, wines from foliar treatments showed similar levels of this amino acid to that found in control; among treated samples, the wines from Pro treatment had lower concentration of tyrosine than wines from Cpaas application, showing no significant differences with respect to the wines from Phe, Ur, and commercial product without amino acis (Cp) treatments.

Aspartic acid content in wines from Cpaas treatment was lower than in wines from control, Pro, and Phe foliar applications, showing no differences with wines from Ur, and Cp treatments (Table 1). Asparagine and lysine concentration was higher in the wines from Cpaas treatment than in the wines from control, Pro, and Ur applications. Wines made from grapevines applied with Cpaas showed the highest content of serine, followed by the wines obtained with Cp application. Finally, wines from control, Pro, Phe, and Ur were the ones that show the lowest concentration of this amino acid. Histidine concentration was higher in the wines from Cpaas application than in the ones from control and Pro treatments, while no signficant differences were observed for Phe, Ur, and Cp wines (Table 1). With respect to glycine, the highest concentration was found in wines from the commercial products applications (Cp and Cpaas). Methionine content was higher in wines from control, Phe, and Ur treatments than in the wines from Cp application, showing no significant differences with respect to Pro, and Cpaas wines.

Phenylalanine was found in higher concentration in wines from control, and Cpaas application than in the wines from Pro, and Cp

treatments, with no significant differences with respect to the other wines (Table 1). Leucine highest concentration was found in control wines, while in wines from treated grapevines, it was found in higher concentration in wines from Phe, Ur, and Cpaas applications than in wines from Cp application, with no differences with Pro wines. Wines from Pro, Ur, and Cp applications showed a lower content of leucine than control wines, with no differences with the wines from Phe, and Cpaas treatments (Table 1).

The relationship between initial must amino acid composition and amino acid consumption during the alcoholic fermentation is shown in Figure 1. In general, a high correlation existed between the initial amino acid concentration and their consumption during this process, which means that amino acid consumption by yeasts was directly proportional to their concentration at the beginning of the alcoholic fermentation, with the exception of glycine, citrulline, methionine, tryptophan, isoleucine, lysine, and proline. The major correlations between must amino acid content and amino acid consumption during the alcoholic fermentation were observed for serine (R^2=0.999), threonine (R^2=0.992), and alanine (R^2=0.989).

In order to classify different samples, the discriminant analysis was performed on data expressing as amino acid concentration in the different musts and wines (Figure 2). Function 1 explained 71.5% of the variance and function 2 explained 22.3% of the variance, so the total of variance explained by these two functions was 93.8%. The variables that contributed most to the discriminant model were phenylalanine, methionine, threonine, and aspartic acid (function 1) and alanine, phenylalanine, isoleucine, and proline (function 2). The two discriminant functions showed a very good separation among must from phenylalanine treatment (Phe-M) and the other samples. Moreover, there was a good separation among musts from the other treatments and the wines.

Discussion

Nitrogen foliar applications to grapevine did not have effect on the total amino acid content without proline in the wines. Despite that the application of phenylalanine, urea, and commercial nitrogen fertilizers increased nitrogen content in musts,[15] as amino acid consumption during alcoholic fermentation was directly proportional to their initial content (Figure 1), that is, the greater the initial concentration, the faster the consumption. This made wines not show significant differences in total amino acid content (Table 1). Lasa *et al*.[3] and Ancín-Azpilicueta *et al*.[6] carried out two studies about the effect on free amino acids of urea foliar applications to vineyard, but in these works only the results of the initial musts were showed; in these two papers, an improvement on nitrogen composition with urea applications was also observed. Smit *et al*.[17] found that the application of nitrogen, as ammonium nitrate, to the soil of the vineyard at two fertilization levels (60 and 150 kg N/ha) in two vintages affected total amino acids in wine only when the highest level of nitrogen was applied. In the case of the lowest nitrogen dose, the amount of total amino acids in the wines was similar in the control wine and in the wine from nitrogen fertilization, being the quantities of amino acids, in the first vintage of study, similar to those found in our work. Therefore, it can say that the foliar application of different nitrogen sources affected the grape nitrogen composition, but the alcoholic fermentation, carried out in the same conditions, did these differences were minimized. The consumption of nitrogen compounds during fermentation mainly depends on the physico-chemical properties of the must (pH, acidity, sugars, *etc*.), on the grape variety, on yeast and on the fermentation temperature, among other factors.[8,18-22] In our study, the fermentation conditions were the same, so that the only difference being the different initial nitrogen must composition,[15] and as already indicated,

Table 1. Amino acid concentrations (mg/L) in control wine and in wines from the treatment with proline, phenylalanine, urea, commercial product without and with amino acids.

Amino acid	Wine group					
	Control	Pro	Phe	Ur	Cp	Cpaas
Asp	2.20±0.27^{bc}	2.39±0.53^{bc}	3.02±1.20^{c}	2.03±0.43^{abc}	1.039±0.38^{ab}	0.71±0.22^{a}
Glu	14.57±4.05^{a}	17.80±8.95^{a}	26.02±14.38^{a}	20.95±3.33^{a}	21.79±0.04^{a}	27.79±8.04^{a}
Asn	8.58±1.89^{a}	8.22±1.44^{a}	11.34±4.05^{ab}	9.82±0.06^{a}	11.64±0.31^{ab}	15.66±2.72^{b}
Ser	1.47±0.56^{a}	0.97±0.02^{a}	1.52±0.30^{a}	0.99±0.12^{a}	3.10±0.66^{b}	4.82±0.61^{c}
His	3.86±0.21^{a}	4.09±0.09^{a}	7.33±3.97^{ab}	5.98±1.05^{ab}	6.29±0.86^{ab}	9.38±2.56^{b}
Gly	3.51±0.66^{a}	3.18±1.06^{a}	3.65±1.05^{a}	3.46±0.53^{a}	6.30±0.49^{b}	8.36±1.76^{b}
Thr	8.02±2.16^{ab}	6.42±0.12^{a}	7.06±0.86^{a}	6.67±0.40^{a}	6.86±0.75^{a}	9.99±1.15^{b}
Cit	2.23±0.20^{a}	6.58±6.49^{a}	16.19±17.31^{a}	4.32±3.07^{a}	9.02±2.43^{a}	8.19±4.94^{a}
Arg	20.66±2.11^{a}	19.99±0.05^{a}	60.44±45.60^{a}	52.91±37.13^{a}	38.06±17.89^{a}	35.07±2.73^{a}
Ala	17.15±1.43^{a}	20.48±10.33^{a}	24.90±12.55^{a}	23.44±7.35^{a}	21.73±0.67^{a}	20.08±0.88^{a}
Tyr	7.31±1.17^{ab}	6.11±0.83^{a}	7.11±2.18^{ab}	6.67±0.99^{ab}	7.54±0.06^{ab}	9.18±0.81^{b}
Val	10.80±1.73^{b}	7.74±0.12^{a}	9.14±0.10^{ab}	9.04±1.35^{ab}	7.34±0.79^{a}	10.24±0.08^{b}
Met	4.90±1.11^{b}	3.66±0.22^{ab}	4.61±0.60^{b}	4.56±0.22^{b}	2.77±0.02^{a}	3.89±0.49^{ab}
Trp	5.36±0.72^{a}	8.15±3.93^{a}	12.29±6.12^{a}	7.86±2.43^{a}	5.41±0.04^{a}	6.24±0.42^{a}
Phe	9.68±1.79^{c}	6.52±0.76a	8.26±0.59^{abc}	7.34±0.00^{ab}	7.19±0.13^{a}	9.27±0.51^{bc}
Ile	10.89±1.67^{c}	7.13±0.90^{ab}	8.08±0.46^{b}	8.11±0.57^{b}	5.28±0.93^{a}	7.86±0.84^{b}
Leu	16.22±2.78^{b}	10.27±2.16^{a}	13.19±2.59^{ab}	11.36±0.12^{a}	10.45±0.56^{a}	14.62±0.55^{ab}
Lys	3.90±0.77^{a}	2.67±0.20^{a}	7.44±3.14^{ab}	4.14±2.84^{a}	6.76±0.03^{ab}	9.70±2.16^{b}
Total aas without Pro	151.32±7.25^{a}	142.37±15.96^{a}	231.69±53.28^{a}	189.94±38.37^{a}	180.21±18.18^{a}	210.60±11.09^{a}
Pro	696.73±43.26^{a}	826.35±239.69^{a}	981.23±128.78^{a}	856.54±36.02^{a}	769.60±72.67^{a}	890.58±42.26^{a}

Asp, aspartic acid; Glu, glutamic acid; Asn, asparagine; Ser, serine; His, histidine; Gly, glycine; Thr, threonine; Cit, citrulline; Arg, arginine; Ala, alanine; Tyr, tyrosine; Val, valine; Met, methionine; Trp, tryptophan; Phe, phenylalanine; Ile, isoleucine; Leu, leucine; Lys, lysine; Pro, proline; Ur, urea; Cp, commercial nitrogen products without amino acids; Cpaas, commercial nitrogen products with amino acids. All parameters are listed with their standard deviation (n=3). For each amino acid, different letters indicate differences among samples (P≤0.05).

the amino acids consumption was proportional to their initial concentrations (Figure 1), explaining that the wines showed a similar composition in amino acids (Table 1).

Regarding free amino acid content in wines, the differences observed in some cases were not important (Table 1) because amino acid consumption was proportional to their initial content for most of the amino acids studied, besides the fact that their sum was significantly equal for all the wines (Table 1). Moreover, discriminant analysis shows all wines grouped (Figure 2). Garde-Cerdán *et al*.[23] also observed that amino acid consumption was proportional to initial must amino acid content, but in the latter work the study was conducted considering the consumption during the first half of the fermentation, unlike this work in which the complete alcoholic fermentation has been studied.

Lysine and glycine are not considered as good nitrogen sources for *S. cerevisiae*,[24] which may explain the fact that there has not been a correlation between the initial content of these two amino acids and their consumption during alcoholic fermentation (Figure 1). Proline was released in all the fermentations (Figure 1) due to the fact that it is an intermediate product in the degradation of arginine.[8,11] For this reason, proline is the major amino acid in wine samples.[25,26] Isoleucine concentration in wines was higher than that found in musts, since its consumption was negative for every fermentation (Figure 1), or in other words, it was released into the medium despite being a good nitrogen source for *S. cerevisiae*. Moreover, it was also observed that other amino acids were released in some of the fermentations. This could be due to yeast autolysis that occurs at the end of fermentation[11,27] and/or due to toxic effect of ethanol.[28] During the autolysis, different compounds are released into the medium as amino acids,[29] and the ethanol inhibits the amino acid transport systems and causing their excretion. Despite certain amino acids were released to the medium, their high consumption during the alcoholic fermentation (Figure 1) caused that they were not present in high concentration in wine (Table 1), avoiding then possible risks of microbial instability derived from high concentrations of residual nitrogen.

Conclusions

Foliar application of various nitrogen sources to the vineyard did not affect wine nitrogen composition. The differences observed for certain amino acids were so small that the sum of all amino acids was not significantly different among wines. It was observed that the initial content of amino acids in the medium and their consumption during the alcoholic fermentation were related: the higher the content, the greater the consumption.

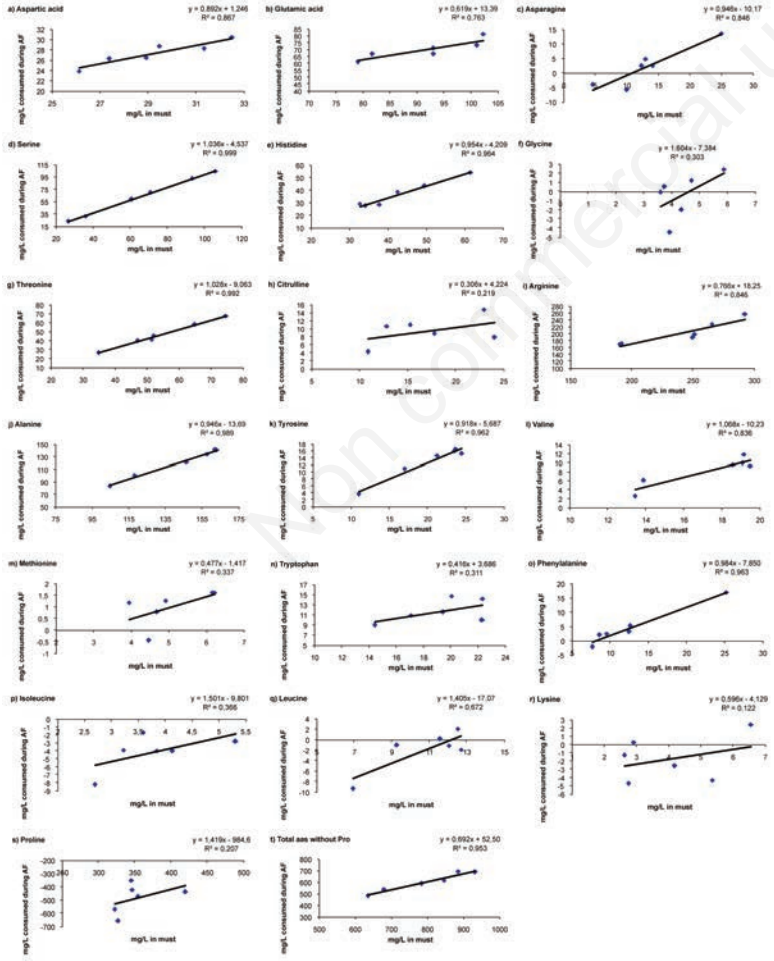

Figure 1. Relationship between the concentrations (mg/L) of each amino acid in the control must and in the musts from vineyard treated with proline, phenylalanine, urea, commercial products without and with amino acids, and their consumption (mg/L) during the corresponding alcoholic fermentations.

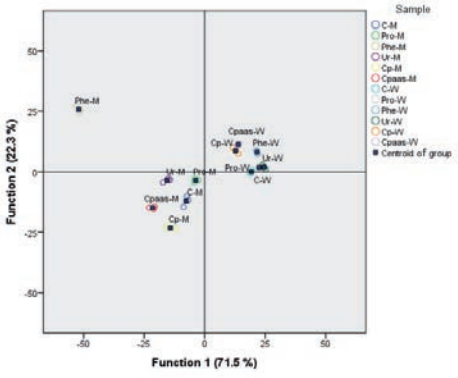

Figure 2. Application of discriminant analysis to the data expressing as concentration (mg/L) of amino acids in the different musts (C-M=control; Pro-M=proline; Phe-M=phenylalanine; Ur-M=urea; Cp-M=commercial product without amino acids; Cpaas-M=commercial product with amino acids) and wines (C-W=control; Pro-W=proline; Phe-W=phenylalanine; Ur-W=urea; Cp-W=commercial product without amino acids; Cpaas-W=commercial product with amino acids) samples.

References

1. Stockert CM, Bisson LF, Adams DO, Smart DR. Nitrogen status and fermentation dynamics for Merlot on two rootstocks. Am J Enol Viticult 2013;64:195-202.
2. Arias-Gil M, Garde-Cerdán T, Ancín-Azpilicueta C. Influence of addition of ammonium and different amino acid concentrations on nitrogen metabolism in spontaneous must fermentation. Food Chem 2007;103:1312-8.
3. Lasa B, Menendez S, Sagastizabal K, et al. Foliar application of urea to "Sauvignon Blanc" and "Merlot" vines: doses and time of application. Plant Growth Regul 2012;67:73-81.
4. Martínez-Gil AM, Garde-Cerdán T, Zalacain A, et al. Application of an oak extract on Petit Verdot grapevines. Influence on grape and wine volatile compounds. Food Chem 2012;132:1836-45.
5. Martínez-Gil AM, Garde-Cerdán T, Martínez L, et al. Effect of oak extract application to Verdejo grapevines on grape and wine aroma. J Agr Food Chem 2011;59:3253-63.
6. Ancín-Azpilicueta C, Nieto-Rojo R, Gómez-Cordón J. Effect of foliar urea fertilisation on volatile compounds in Tempranillo wine. J Sci Food Agr 2013;93:1485-91.
7. Bisson LF. Influence of nitrogen on yeast and fermentation of grapes. In: Rantz J, ed. Proceedings of the International Symposium on Nitrogen in Grapes and Wine, 1991 June 18-19, Seattle, Washington, DC, USA. Davis: American Society for Enology and Viticulture; 1991. pp 78-89.
8. Bell S-J, Henschke PA. Implications of nitrogen nutrition for grapes, fermentation and wine. Aust J Grape Wine R 2005;11:242-95.
9. Garde-Cerdán T, Ancín-Azpilicueta C. Effect of the addition of different quantities of amino acids to nitrogen-deficient must of the formation of esters alcohols, and acids during wine alcoholic fermentation. LWT-Food Sci Technol 2008;41:501-10.
10. Hernández-Orte P, Ibarz MJ, Cacho J, Ferreira V. Effect of the addition of ammonium and amino acids to must of Airen variety on aromatic composition and sensory properties of the obtained wine. Food Chem 2005;89:163-74.
11. Moreno-Arribas MV, Polo MC. Wine chemistry and biochemistry. New York: Springer; 2009.
12. Uthurry CA, Suárez Lepe JA, Lombardero J, García del Hierro JR. Ethyl carbamate production by selected yeasts and lactic acid bacteria in red wine. Food Chem 2007;94:262-70.
13. USDA. Keys to soil taxonomy, USDA-Natural resources conservation service. Available from: http://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/survey/class/?cid=nrcs142p2_053580
14. Sampaio TL, Kennedy JA, Vasconcelos MC. Use of microscale fermentations in grape and wine research. Am J Enol Viticult 2007;58:534-9.
15. Garde-Cerdán T, López R, Portu J, et al. Study of the effects of proline, phenylalanine, and urea foliar application to Tempranillo vineyards on grape amino acid content. Comparison with commercial nitrogen fertilizers. Food Chem 2014;163:136-41.
16. European Commission. Commission Regulation concerning the establishment of common analytical methods in the sector of wine, 2676/90/CE. In: Official Journal, L 272, 03-10-1990, pp 1-192.
17. Smit I, Pfliehinger M, Binner A, et al. Nitrogen fertilisation increases biogenic amines and amino acid concentrations in Vitis vinifera var. Riesling musts and wines. J Sci Food Agr 2014;94:2064-72.
18. Bouloumpasi E, Souflerops EH, Tsarchopoulos C, Biliaderis CG. Primary amino acid composition and its use in discrimination of Greek red wines with regard to variety and cultivation region. Vitis 2002;4:195-202.
19. Héberger K, Csomós E, Simon-Sarkadi L. Principal component and linear discriminant analyses of free amino acids and biogenic amines in Hungarian wines. J Agr Food Chem 2003;51:8055-60.
20. Valero E, Millán C, Ortega JM, Mauricio JC. Concentration of amino acids in wine after the end of fermentation by Saccharomyces cerevisiae strain. J Sci Food Agr 2003;83:830-5.
21. Barbosa C, Falco V, Mendes-Faia A, Mendes-Ferreira A. Nitrogen addition influences formation of aroma compounds, volatile acidity and ethanol in nitrogen deficient media fermented by Saccharomyces cerevisiae wine strains. J Biosci Bioeng 2009;108:99-104.
22. Barrajón-Simancas N, Giese E, Arévalo-Villena M, et al. Amino acid uptake by wild and commercial yeasts in single fermentations and co-fermentations. Food Chem 2011;127:441-6.
23. Garde-Cerdán T, Martínez-Gil AM, Lorenzo C, et al. Implications of nitrogen compounds during alcoholic fermentation from some grape varieties at different maturation stages and cultivation systems. Food Chem 2011;124:106-16.
24. Cooper TG. Transport in Saccharomyces cerevisiae. In: Strathern JN, Jones EW, Broach JB, eds. The molecular biology of the yeast Saccharomyces. Metabolism and gene expression. New York: Cold Spring Harbor Laboratory; 1982. pp 399-461.
25. López R, Tenorio C, Gutiérrez AR, et al. Elaboration of Tempranillo wines at two different pHs. Influence on biogenic amine contents. Food Control 2012;25:583-90.
26. Gómez-Alonso S, Hermosín-Gutiérrez I, García-Romero E. Simultaneous HPLC analysis of biogenic amines, amino acids, and ammonium ion as aminoenone derivatives in wine and beer samples. J Agr Food Chem 2007;55:608-13.
27. Fornairon-Bonnefond C, Camarasa C, Moutounet M, Salmon J-M. New trends on yeast autolysis and wine ageing on lees: a bibliographic review. J Int Sci Vigne Vin 2002;36:49-69.
28. Ancín-Azpilicueta C, Fraile-Jiménez de Maquirriain P, Garde-Cerdán T, Torrea-Goñi D. Influence of inoculation of selected yeast on the quality of rosé and white wines. In: Riley AP, ed. Food research, safety and policies. New York: Nova Science Publ.; 2005. pp 57-92.
29. Alexandre H, Guilloux-Benatier M. Yeast autolysis in sparkling wine: a review. Aust J Grape Wine R 2006;12:119-27.