Free amino acid composition of must from 7 *Vitis vinifera L.* cv. in Latium (Italy)

Composition des aminoacides libres dans le moût de 7 *Vitis vinifera L.* cv. en Latium (Italie)

Francesca CECCHINI[1]*, Massimo MORASSUT[1] and Emilia GARCIA MORUNO[2]

1: CRA Istituto Sperimentale per l'Enologia Via Cantina Sperimentale, 00049 Velletri (Roma), Italy
2: CRA Istituto Sperimentale per l'Enologia Via Pietro Micca, 35, 00141 Asti, Italy
*Corresponding author: pbal0196@panservice.it

Abstract: Free amino acid concentrations in must of 7 *Vitis vinifera* cultivars (Cabernet Franc, Syrah, Merlot, Montepulciano, Sangiovese, Cesanese d'Affile, Carmenere) grown in the Latium region (Italy) were monitored from 2003 to 2005. The cultivars were located in a homogeneous soil and climatic zone and with the same training system (Cordon Spur). The influence of climate was assessed with year-to-year variations of maximum and minimum temperatures and rainfall. The amino acids were compared individually to show year-to-year variations. The concentrations of individual amino acids differed considerable between years, whereas the amino acids profile did not differ significantly from one year to another in all 7 cultivars studied. Arginine, proline, varied between years, while proline: arginine ratio did not change. Arginine, proline, proline: arginine ratio, total free α–amino acid and total free α–amino N concentration were not correlated with soluble solids concentration at harvest. The sum total of amino acids in musts differed significantly from one variety to another and was used as an index to differentiate between varieties from the same area.

Key words: cultivar, must, amino nitrogen

Introduction

Nitrogen is very important nutrient in the must, necessary for correct yeast growth. Ammonia and free α-amino acids are major nitrogen sources that can be assimilated and used by oenological yeast (Albers *et al*., 1996, Bisson *et al.*, 1991, Bisson *et al.*, 1999; Jarenek *et al*., 1995 a). The sum of N contributed by free α-amino acids and ammonia can be considered a good definition of assimilable nitrogen (Spayd *et al.*, 1995, Hernandez-Orte, 1999, Weeks and Henschke, 1999). Nitrogen deficiency slows down yeast growth and the fermentation or may even result in a stuck fermentation, possibly due to the inhibition of the synthesis of proteins which carry sugar to the interior of the cells. The amino acids also plays a important role in the formation of fermentation flavours, hydrogen sulphide, organic acids, higher alcohols, and esters (Jaranek *et al*., 1995b, Hernandez-Orte *et al*., 2002) Arginine is also an important precursor of the formation of ethyl carbamate (Bertrand *et al.*, 1991; Ough, 1919), a mild carcinogen found in fermented food products.
Nitrogen fertilization of vineyards influences the concentrations of nitrogen compounds in the must. The amino acids composition of the grape varies from one cultivar and growing region to another. When vineyard soil nitrogen is low, arginine and proline are usually the major amino acids in grapes. When vineyard are fertilized with nitrogen, the concentration of all nitrogenous compounds in the must, including the individual amino acids increases (Bertrand *et al*., 1991; Spayd *et al*., 1995)
The amino acid content of wines may also be used for wine characterization (Haung *et al.*, 1991; Lehtonen 1996), such that for a given grape variety, from the same region, it has been found that amino acid profile has a common pattener in spite of considerable variation in the amount of some amino acids from year to year (Spayt, 1991; Hernandez-Orte, 1999). This suggests that climatological conditions play a fundamental role in the amino acid content in the must.
Bearing in mind the importance for vinification of the total content of amino acids in the must, it is surprising that this parameter have never been established as index of grape quality. Even in the most recent papers concerning quality factors the °Brix, berry weight, and titratable acidity continue to be the main parameters taken into account.

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There are very few data that regard to the climatic conditions of vineyards, particularly temperature and rainfall, on amino acid accumulation in grape. Without these data, no conclusion can be made between the different cultivars of *Vitis vinifera*.
Aim of this work was to value the amino acids profiles of musts, derived from different cultivars of *Vitis vinifera*, located in a homogeneous soil and climatic zone, and with same training system.

Materials and methods

The study was carried out on an experimental vineyard 12 years old of the Institute of Enology, located in Velletri (Rome) in the Lazio region (Italy) (41° 40.5' N latitude, 12° 50.7' E longitude) at 355 m up the sea level. The trials were made using V*itis vinifera* L. cultivars Merlot, Syrah, Sangiovese, Cesanese d'Affile, Carmenere, Montepulciano, and Cabernet Franc. Grapes were harvested over three consecutive years, 2003, 2004, and 2005 at technological maturation. The cultivars had the same training system to Cordon Spur. Seven Kg of grapes for each grape cultivar were crushed and pressed using a basket press. Four independent replications were done for each of the seven musts.

Climatological data: Climatological data were supplied by the weather station in Velletri belonging to the Regional Institute of Meteorology. This weather station is located in Institute of Enology and is representative of the weather in the area.

Chemical analysis of musts and amino acid determination: Soluble solid (reducing sugars) were measured as °Brix, the titratable acidity and pH were determined according to standard methods (O.I.V., 1991) (table 1). The determination of amino acids was performed with a high-pressure liquid chromatograph (HP 1100 Hewlett Packard, Waldbronn, Germany) equipped with pump (HP G1311A Hewlett Packard, Waldbronn, Germany), autosampler (HP G1313A Hewlett Packard, Waldbronn, Germany) and fluorimetric detector (HP G1321A Hewlett Packard, Waldbronn, Germany). O-phthaldialdehyde (OPA reagent, Agilant Technologies, Palo Alto, Calif.) was used to derivatize amino acids. The separation was performed on an Alltima C18 column. Samples were injected in duplicate onto the column after being filtered through a 0.2 µm filter. As mobile phases, two eluents were used: eluent A (1.224 g of sodium acetate trihydrate, 500 ml of water, 0.09 mL of triethylamine, and 1.5 mL of tetrahydrofuran) and eluent B (1.088 g of sodium acetate trihydrate, 100 mL water, 200 mL of acetonitrile, and 200 mL of methanol). A 1-h program began with an initial concentration of 10% of eluent B at flow of 0.450 mL/min. and terminated with 100% of eluent B at a flow of 0.700 mL/min. Fluorescence wavelengths for excitation and emission were 340 and 450 nm, respectively. The quantification of amino acids was performed using amino acids standard.

Statistical analysis: The statistical analysis of the amino acid content determined in musts was carried out with the single-factor analysis of variance (ANOVA); the means were compared by the LSD analyses. For the data analyses, StatSoft statistical package (version 5.1, Inc. Tulsa, OK USA) was used.

Table 1 - Composition of the musts. The values represent the average of duplicate determinations.
*** sugar concentration as °Brix, ** Titratable acidity (TA) expressed in g/L of tartaric acid.**

Cultivar	Year 2003			Year 2004			Year 2005		
	°Brix g/L	TA g/L	pH	°Brix g/L	TA g/L	pH	°Brix g/L	TA g/L	pH
Merlot	230	6.60	3.28	193	7.20	3.40	195	7.50	3.31
Syrah	200	7.00	3.16	190	7.80	3.24	190	8.20	3.19
Cabernet Franc	220	7.20	3.26	188	6.00	3.25	200	6.00	3.30
Carmenere	220	6.18	3.35	192	7.20	3.50	192	6.67	3.39
Sangiovese	210	7.20	3.15	193	7.95	3.18	197	8.15	3.15
Montepulciano	200	7.25	3.10	197	8.00	3.16	191	8.20	3.13
Cesanese di affile	210	7.12	3.16	197	8.20	3.21	190	8.10	3.18

Results and discussion

Free α amino acids: The amino acids profiles of seven cultivars followed similar patterns during the three year period, although there were considerable variations in the concentrations of some amino acids from year to year). In figure 1 are reported the total free α- amino acid concentration determined by summing all of the free α- amino acid in each must sample. The data showed a significant differences ($p < 0.05$) in amino acid content between different cultivars.

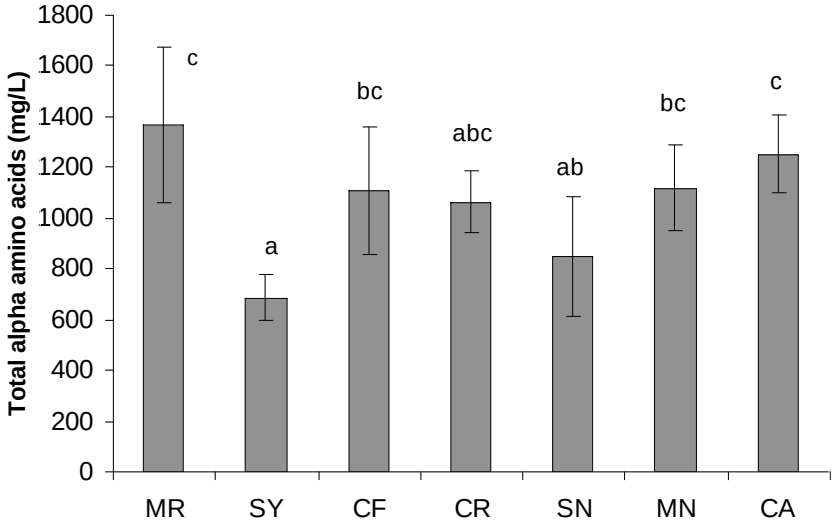

Figure 1 - Mean total free α amino acid concentration of Merlot (MR), Syrah (SY), Cabernet Franc (CF), Carmenere (CR), Sangiovese (SN), Montepulciano(MN) and Cesanese di Affile (CA) musts, 2003, 2004 and 2005.
Error bars (vertical line with horizontal cap above the bar) represent the standard deviation the mean. Means were pooled across years within a given cultivar. Letters a-c indicate significant differences ($p < 0.05$). There is no significant difference between samples with the same letter.

Arginine and proline were present in highest concentrations. Arginine was the predominant amino acid in Carmenere and Cesanese di Affile juices, proline was predominant amino acid in Merlot, Syrah, Cabernet Franc, Sangiovese and Montepulciano juices.
Proline/arginie ratio did not differ significantly over three years for all of the cultivars (fig. 2), This ratio was less than 1 for Carmenere and Cesanese di. Affile juices.
The mean concentrations of the other individual amino acids, over three years for all cultivars (data not shown) showed that: isoleucine, leucine, pheylalanine were the amino acids with the lowest concentrations. Their concentration were lower than 15 mg/L, and they accounted for less than 3% of the total amino acid contents over three years for all cultivars. Cysteine, tryptophan and ornithine were not detected in any of the juices. Alanine was the third highest of the amino acid in concentration after arginine and proline, for Cabernet Franc, Carmenere and Cesanese Di Affile. Glycine was the third highest amino acid in concentration for Merlot juice and valine was found most predominant in concentration for Montepulciano. Aspartic acid and glutamic acid were predominant in concentration in Sangiovese juices. Serine, threonine and histidine concentrations were higher in Cesanese di Affile, Carmenere and Merlot juices. In this cultivar juices Histidine was greater than 80mg/L. Histidine concentration was less than 80 mg/L of juice in the remaining cultivars. Syrah had the lowest concentration of the all amino acids.

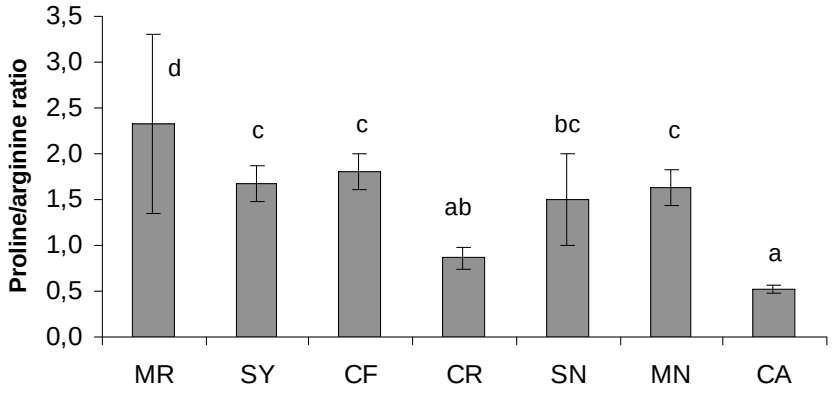

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Figure 2 - Mean proline/arginine ratio of Merlot (MR), Syrah (SY), Cabernet Franc (CF), Carmenere (CR), Sangiovese (SN), Montepulciano (MN), and Cesanese di Affile (CA) musts, 2003, 2004 and 2005.

Error bars (vertical line with horizontal cap above the bar) represent the standard deviation of the mean. Means were pooled across years within a given cultivar. Letters a-d indicate significant differences ($p < 0.05$). There is no significant difference between samples with the same letter.

Total free α-amino N: Total free α-amino N concentration was calculated using the percent composition of α-amino nitrogen for each free α-amino acid. Of the seven cultivars surveyed MR was the only cultivar that had a mean concentration, over three years, of total free α-amino N greater than 400 mg/L (fig. 3).SY produced juice with the lowest mean concentration, over three years, of total free α-amino N at only 230 mg/L. Other five cultivars had intermediate concentrations

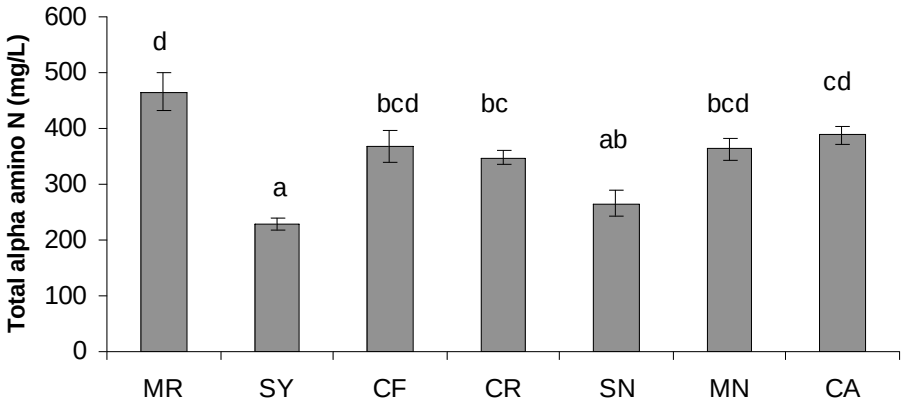

Figure 3 - Mean total free α-amino N concentration of Merlot (MR), Syrah (SY), Cabernet Franc (CF), Carmenere (CR), Sangiovese (SN), Montepulciano(MN), and Cesanese di Affile (CA) musts, 2003, 2004 and 2005.

Error bars (vertical line with horizontal cap above the bar) represent the standard deviation of the mean. Means were pooled across years within a given cultivar. Letters a-d indicate significant differences ($p < 0.05$). There is no significant difference between samples with the same letter.

The highest total amino acid and nitrogen values were reached over 2003 years. The annual rainfall of 2003 year was very favourable for fruit ripening. Rainfall was heavy in the autumn. In the spring, rainfall was plentiful, but the ripening period was dry. The mild evolution of the temperature with few fluctuation. In the2004-2005 cycle, there was a little rain in autumn and spring and summer there was a considerable rain, whit high fluctuation of temperature.

Authors (Oug, 1968; Spayd, 1999) have reported that fruit proline concentrations increased as °Brix increase. Whether arginine increased or decreased with increased grape maturity was dependent upon the cultivar. In the present study, arginine, proline, total free α-amino acids, and total free α-amino N concentrations, as well as the proline/arginine ratio, were poorly related to fruit maturity, using soluble solids concentration as the indicator (data no shown).

Conclusion

Free amino acid concentrations in must of 7 *Vitis vinifera* cultivars (Cabernet Franc, Syrah, Merlot, Montepulciano, Sangiovese, Cesanese d'Affile, Carmenere) grown in the Latium region (Italy) were monitored from 2003 to 2005.
The concentration of individual amino acids differed between years, whereas the amino acids profile did not differ significantly from one years to another in all seven cultivars studied.
The concentration the proline was always higher than that arginine, with exception of the Carmenere and Cesanese di Affile juices. Proline/arginine ratio remained nearly the same, although their values differed during the three years. The sum total of amino acids in musts differed significantly from one variety to another and this might be used as an index to differentiate between varieties from the same area. Arginine, proline, proline/arginine ratio, total free α–amino acid and total free α–amino N concentration were not correlated with soluble solids concentration at harvest.
The years (2003) with the mildest temperatures, without extreme temperatures, and with little rainfall during the ripening process produced the grapes with the highest concentration of amino acids.

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