Scientia Horticulturae 239 (2018) 9–16

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Towards the definition of optimal grape harvest time in Grenache grapevines: Nitrogenous maturity

T. Garde-Cerdán*, G. Gutiérrez-Gamboa, J. Fernández-Novales, E.P. Pérez-Álvarez, M.P. Diago*

Instituto de Ciencias de la Vid y del Vino (CSIC, Universidad de La Rioja, Gobierno de La Rioja), Carretera de Burgos Km. 6, 26007, Logroño, Spain

ARTICLE INFO

Keywords:
Nitrogen
Optimal harvest
Amino acids
Arginine accumulator
Yeast assimilable nitrogen

ABSTRACT

Must nitrogen composition plays a key role on wine quality, affecting the development of alcoholic fermentation and the formation of volatile compounds. In order to provide additional information about the optimal time of harvest, we studied grape amino acid evolution pattern in relation to the accumulation of soluble solids along ripening. Additionally, we evaluate the amino acid profile of Grenache (*Vitis vinifera* L.) grapevines. The results showed that the degree of berry maturity strongly influence the evolution patterns of all amino acids. Grenache behaved as an arginine accumulator variety. This amino acid was found to model the total amino acid concentration in Grenache musts. Generally, soluble solids maturity coincided with the nitrogen maturity at 25 ºBrix. Therefore, in Grenache grapevines, when amino acid composition is aimed to be maximized, the optimal grape harvest time, in terms of nitrogenous maturity, matches the timing at which berries reach their sugar maturity at 25 ºBrix.

1. Introduction

Sugar and nitrogen composition of grape berries are key determinants of must composition (Bell and Henschke, 2005). Grape, and consequently, must nitrogen composition are essential for yeast growth, fermentation kinetics and flavor metabolism (Bisson and Butzke, 2000; Bell and Henschke, 2005). Some amino acids are precursors of important volatile compounds in wines, which are formed from the yeast enzymatic metabolism during the alcoholic fermentation process (Bell and Henschke, 2005). The volatile compounds derived from sugar and amino acid metabolism by yeast are the higher alcohols, esters, carbonyl compounds, volatile fatty acids, and sulphur compounds which contribute widely to the wine aroma (Swiegers and Pretorius, 2007; Garde-Cerdán and Ancín-Azpilicueta, 2008). Therefore, a close relationship between the must amino acid composition and several volatile compounds in wines has been reported (Garde-Cerdán and Ancín-Azpilicueta, 2008; Martínez-Gil et al., 2012). On the other hand, musts with deficient nitrogen levels may lead to stuck or sluggish fermentations, which is a persistent problem in wine production (Bisson and Butzke, 2000) that affects the operational logistic in the winery.

Many factors can influence the grape and subsequently must nitrogen composition, such as viticultural practices, soil management, nitrogen type, timing or rate of applications and rootstock, among others (Bell and Henschke, 2005; Pérez-Álvarez et al., 2015; Gutiérrez-Gamboa et al., 2017a,b). However, grape maturity and variety have been reported as the most determinant variables in the content of amino acids that accumulate in grape berries tissues (Huang and Ough, 1991; Stines et al., 2000; Garde-Cerdán et al., 2009). Therefore, to provide additional information on the optimal time of harvest it is relevant to assess the evolution of the amino acid concentration during grape ripening. Hernández-Orte et al. (1999) studied the changes in the concentration of eighteen amino acids during ripening in different varieties from the Somontano appellation of origin (Spain), reporting that the predominant amino acid in Riesling and Cabernet Sauvignon was proline and in Tempranillo arginine. In addition, these authors reported a good correlation between the arginine synthesis and the accumulation of soluble solids through berry maturation in this variety. Stines et al. (2000) studied proline and arginine accumulation in developing berries of six grapevine varieties showing that vine variety, berry maturity and tissue type had an important effect on amino acid accumulation. In a more recent work, Garde-Cerdán et al. (2009) studied the evolution of amino acids and ammonium during grape ripening in different varieties and cultivation systems (organic and conventional) reporting that technological maturity of the grapevines coincided with the maximum composition of nitrogen compounds. This work is the first approach that studies grape amino acid accumulation in parallel with the soluble solids through berry maturity, even until overripe (from 10 to 30 ºBrix). The aforementioned studies have focused on studying the accumulation of amino acids during grape ripening in terms of a data period (Hernández-Orte et al., 1999; Garde-

* Corresponding author.
E-mail addresses: Teresa.GardeCerdan@gmail.com (T. Garde-Cerdán), maria-paz.diago@unirioja.es (M.P. Diago).

https://doi.org/10.1016/j.scienta.2018.05.014
Received 17 February 2018; Received in revised form 4 May 2018; Accepted 7 May 2018

Cerdán et al., 2009) or during a short period in the vine phenological stage, from veraison to harvest (at 18.6, 20.5, and 25.1 ºBrix), as it has been showed by Stines et al. (2000). On the other hand, several studies have reported that the most abundant amino acids found in grapevines are proline and arginine (Huang and Ough, 1991; Hernández-Orte et al., 1999; Stines et al., 2000; Garde-Cerdán et al., 2014; Gutiérrez-Gamboa et al., 2017a,b; Pérez-Álvarez et al., 2017). The proline to arginine ratio reflects the proportion of non assimilable (proline) to assimilable nitrogen (arginine) and provides a useful indication of the likely nutritional value of the grape must of a particular variety to yeast (Bell and Henschke, 2005). Thus, the main goal of this work was to study the evolution pattern of the twenty proteic amino acids together with γ-aminobutyric acid and citrulline in Grenache *(Vitis vinifera* L.) variety, in relation to its accumulation of soluble solids along ripening in order to define the optimal grape harvest time to maximize grape quality in terms of its amino acid composition. Besides, a second goal of this work was to study the nitrogenous behavior of the Grenache variety regarding the proline to arginine ratio.

2. Materials and methods

2.1. Berry sampling

Clusters of grapevines of *Vitis vinifera* L. cv. Grenache were collected in a commercial vineyard "Vistahermosa" (160 ha) located in Tudelilla, La Rioja, Spain (Lat. 42°18′ 52.26″, Long. -2°7′ 59.15″, Alt. 582 m) during five dates (17/08, 01/09, 29/09, 14/10 and 20/10) from early August to October 2015. Grapevines were planted in 1970, using a Rupestris du Lot rootstock, vine spacing was 3 m between rows and 1.10 m in the row in a north-south orientation. The vine training system used was Gobelet system, that is a form of head training. No irrigation was applied during the growing season (rain-fed vineyard).

The sampling was randomized and between 4 and 5 kg of grapes were collected for each stage. Afterwards, they were transported in portable refrigerators to the Instituto de Ciencias de la Vid y del Vino (ICVV, Logroño, Spain) and kept in a cold room until processing. A total of 128 grape clusters were processed, and each cluster was split into upper, mid, and bottom zones to select 50 representative berries of each sample. Each 50 berry-sample was measured for soluble solids content (SSC) and immediately stored at −20 °C until the analysis of amino acids by HPLC.

2.2. Soluble solids content

Berries were passed through a hand-operated food mincer, which enabled constant pressure to be maintained during juice extraction with minimal seed and skin shearing. The soluble solids content (SSC) was measured as the refractometer reading taken for grape juice, using a temperature-compensated digital Quick-Brix 60-type refractometer (Mettler Toledo, Schwerzenbach, Switzerland).

2.3. Analysis of amino acids by HPLC

Prior to HPLC analysis of amino acids, the berry subsamples were subjected to a process of sample preparation. Berries were allowed to partially thaw prior to homogenization, and temperature was kept under 5 °C at all times. Each sub-sample of 50 berries was homogenized using an Ultra Turrax grind mixer (IKA, Staufen, Germany) at high speed (11.93 m s^{-1} for 1 min). The amino acid analysis was performed by the method described by Garde-Cerdán et al. (2014). Free amino acids were analysed by HPLC (Agilent, Palo Alto, USA). Each sample (5 mL of supernatant) was mixed with 100 µL of norvaline and 100 µL of sarcosine (internal standards). The mixture was submitted to an automatic precolumn derivatisation with *o*-phthaldialdehyde (OPA Reagent, Agilent) and with 9-fluorenylmethylchloroformate (FMOC Reagent, Agilent). The injected amount from the derived sample was 10 µL, and a constant temperature of 40 °C was maintained. All separations were performed on a Hypersil ODS (250 x 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). Detection was performed by fluorescence FLD detector, and DAD detector. Identification of compounds was performed by comparison of their retention times with those of pure reference standards. The pure reference compounds and internal standards were from Sigma-Aldrich (Madrid, Spain). Concentration of all amino acids, total amino acids and total amino acids without proline were expressed as mg N L^{-1}.

The amino acids analyzed were aspartic acid (Asp), glutamic acid (Glu), asparagine (Asn), serine (Ser), glutamine (Gln), histidine (His), glycine (Gly), threonine (Thr), citrulline (Cit), arginine (Arg), alanine (Ala), γ-aminobutyric acid (GABA), tyrosine (Tyr), cysteine (Cys), valine (Val), methionine (Met), tryptophan (Trp), phenylalanine (Phe), isoleucine (Ile), leucine (Leu), lysine (Lys), and proline (Pro).

3. Results

3.1. Amino acid profile of Grenache grapes

The profile of grape amino acids of Grenache grapevines along ripening is shown in Table 1. The most abundant compounds shown as a mean of the amino acid concentration during grape maturity were arginine, proline, glutamine, γ-aminobutyric acid (GABA), and histidine, while the least abundant amino acids were methionine, isoleucine, tyrosine, valine, and citrulline (Table 1). Arginine reached a 54% of total amino acids, while proline a 17% of total amino acids along grape ripening. Methionine reached a 0.20% of total amino acids, while isoleucine a 0.30% of total amino acids. Arginine and proline made up the greatest proportion of the total amino acids content found in grapes and our results respect to these two amino acids match those reported in previous works in others varieties (Hernández-Orte et al., 1999; Stines et al., 2000; Bell and Henschke, 2005). In relation to our results, arginine ranged from 4.41 to 341.92 mg N L^{-1}, while proline varied from 1.23 to 121.16 mg N L^{-1} along ripening.

3.2. Evolution pattern of amino acids

The evolution of the concentration of aromatic (phenylalanine, tryptophan, and tyrosine), neutral polar (asparagine, glutamine, serine, and threonine), and acid (aspartic acid and glutamic acid) amino acids are summarized in Fig. 1. Phenylalanine and tryptophan (Fig. 1a and b) showed a progressive increase in their concentration during grape ripening presenting their maximum content at 25 ºBrix. Tyrosine (Fig. 1c) showed the same pattern of evolution but its maximum content was reached at 20 ºBrix. Neutral polar and acid amino acids showed similar trends in their content during ripening, depending on the specific compound. Thus, asparagine and aspartic acid (Fig. 1d and h) exhibited an early decrease in their content during grape ripening (from 10 ºBrix), while glutamine and glutamic acid (Fig. 1e and i) showed also an early diminishment in their concentration, but from 10 to 15 ºBrix. These four amino acids shared a remarkable reduction in their content during ripening with a slight increase at 25 ºBrix. Serine (Fig. 1f) content showed a slight increase from 10 to 25 ºBrix with a decrease during the final maturation stage (at 30 ºBrix), while threonine (Fig. 1g) concentration showed a progressive enhancement, that reached the maximum level around 20 ºBrix, which subsequently decreased.

The evolution of the concentration of basic (arginine, histidine, and lysine), and aliphatic (alanine, glycine, leucine, isoleucine, and valine) amino acids is shown in Fig. 2. The evolution of arginine concentration during grape ripening showed a progressive increase showing a maximum level between 20 and 25 ºBrix which subsequently decreased in