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Changes in the Free Amino Acid Composition with Maturity of the Noble Cultivar of *Vitis rotundifolia* Michx. Grape

O. Lamikanra* and A. K. Kassa

Center for Viticultural Science and Small Farm Development, Florida A&M University, Tallahassee, Florida 32307

The changes in amino acid composition that occur with maturity of the Noble cultivar of the *Vitis rotundifolia* Michx. (muscadine) grape were determined by HPLC. Eighteen amino acids were identified. Histidine was the most prominent amino acid followed by alanine. The concentrations of most of the major amino acids (alanine, glycine, histidine, valine, isoleucine, aspartic acid, and serine) were highest at verasion. Glutamine and threonine contents dropped sharply after fruit set, while those of arginine and proline increased gradually with maturity and ripening. Tyrosine content increased gradually with maturity and ripening following a slight drop after fruit set. In ripe grapes, seeds contained most of the amino acids in mature grapes (50%) followed by the pulp (23%), the juice (15%), and the skin (11%). Alanine, histidine, and arginine were the principal amino acids identified in the juice. Alanine, histidine, arginine, valine, glutamine, aspartic acid, proline, serine, and threonine accounted for about 90% of the amino acids in the pulp. In seeds, alanine, proline, asparagine, and histidine accounted for over 55% of the amino acids, while alanine and histidine were found to be the predominant free amino acids in the skin. The profile indicates some differences in the changes in amino acid composition with berry maturity and relative amounts of amino acids present in muscadine compared to those in nonmuscadine grape species.

Keywords: *Vitis; muscadine; amino acids*

INTRODUCTION

Amino acids account for the majority of nitrogenous compounds found in grapes (Kliewer, 1969, 1970). The quality of grape products can be significantly influenced by the amount and composition of their free amino acids. They are used as nutrients for yeast and bacteria during alcoholic and malo-lactic fermentations and are precursors to some flavor compounds and higher alcohols (Nykanen, 1986; Huang and Ough, 1989; Bisson, 1991; Ough et al., 1991; Jinarek, 1995). Because they stimulate the citric acid cycle, they have a role to play in the amount of organic acids produced during fermentation.

Amounts of individual amino acids found in grapes could vary with variety, location, maturity, cultural practices, and method of analysis (Kliewer, 1969; Huang and Ough, 1989b). Most reports indicate an increase in free amino acid content of grapes with maturity (Ough, 1968; Nasser and Kliewer, 1970; Kliewer, 1970). About one-fifth of the total nitrogen is found in the juice and the remaining four-fifths in the skins and seeds. Nitrogenous compounds are also released from the seeds to the pulp at maturity, and nearly half of the juice nitrogen can usually be accounted for by their amino acid content (Winkler et al., 1974). The majority of the amino acids have been reported to be synthesized during the last 6−8 weeks of berry ripening (Kliewer, 1968).

Most reports on grape amino acids have been on nonmuscadine grape cultivars. Muscadine grapes are primarily grown in the Southeastern United States where they are consumed as fresh fruit and processed into wines, juices, and preserves. Marcy et al., (1981), using an amino acid analyzer, investigated the free amino acids of muscadine grapes grown in North Carolina by ion-exchange chromatography. It was not possible for the authors to quantify some amino acids such as histidine and methionine with the method used. The objective of this study is to quantitatively determine the free amino acids present in the Noble cultivar of the muscadine grape and changes in their relative amounts that occur with grape maturity using HPLC. Their distribution within the grape components (seeds, pulp, skin, and juice) was also quantified.

MATERIALS AND METHODS

Grape Sampling. Grape samples were collected from a small commercial vineyard located at Lloyd, FL. Cultural management of the vineyard was based on recommended practices (Bourne et al., 1990). The vines were trained on a Geneva-Doubled system. Ten vines were randomly selected from over 1000 vines of the Noble cultivar vines on the vineyard. Fruit sampling began right after fruit set (mid June) and continued on a biweekly basis until the grapes were fully ripe. Six sets of samples (H1−H6), corresponding to the days shown in Figure 1, were collected. Each harvest consisted of

* To whom correspondence should be addressed. Present address: Food Processing and Sensory Quality Research Unit, USDA-ARS-SRRC, 1100 Robert E. Lee Boulevard, New Orleans, LA 70179. Tel: 504-286-4278. Fax: 504-286-4419. E-mail: sola@nola.srrc.usda.gov.

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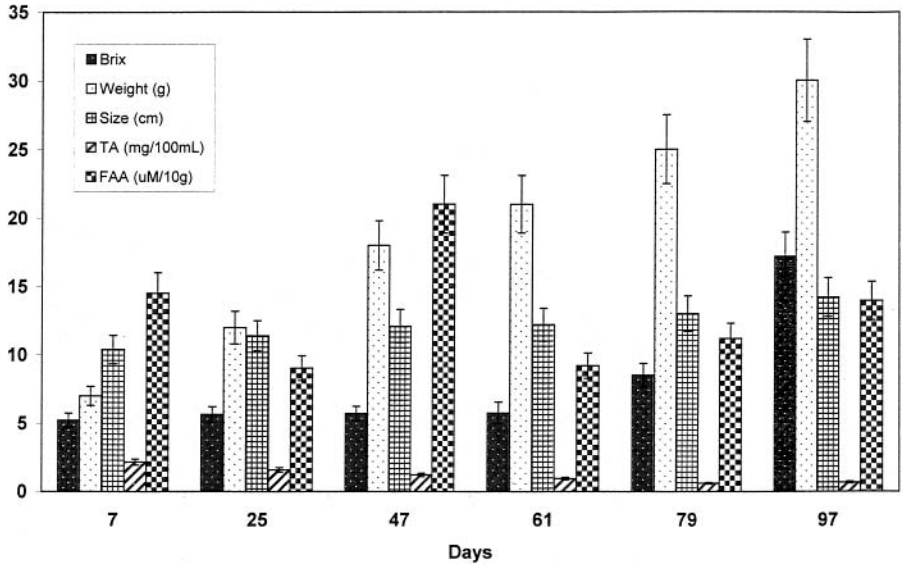

Figure 1. Effect of grape maturity level on sugar, berry weight, acidity, and total free amino acids content. Time points correspond to harvests H1, H2, H3, H4, H5, and H6, respectively.

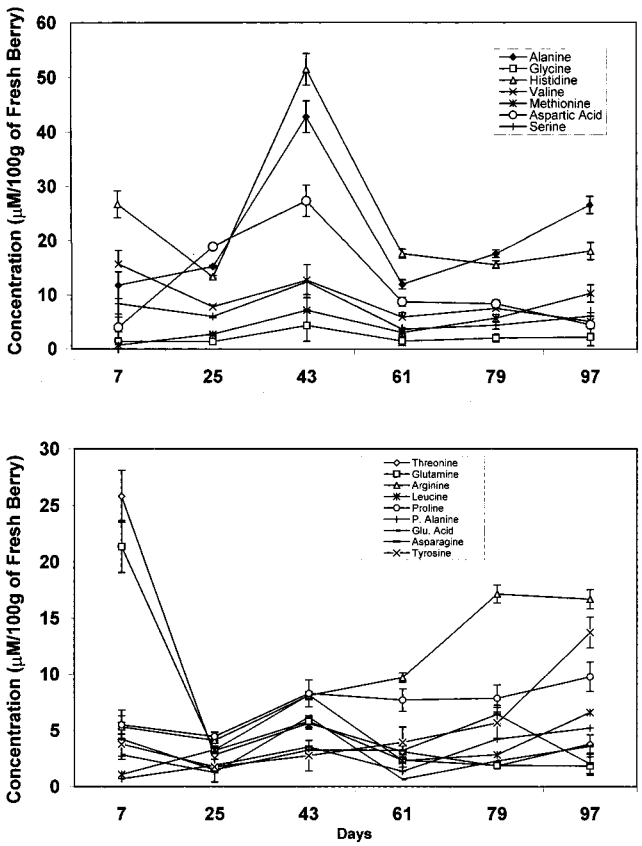

Figure 2. Changes in free amino acid concentrations with maturation of the Noble muscadine grape.

three replicates of approximately 500 g per vine. The °Brix was measured using a temperature-compensated hand refractometer, and pH of extracted juice was determined with a pH meter. Total titratable acidity was determined as tartaric acid. Mean berry size for each harvest was determined using a vernier caliper. The fruit samples were immediately treated in liquid nitrogen, lyophilized, and stored at −70 °C until analyzed.

Separation of Grape Components. Freshly picked mature grapes (100 g) were harvested at the same maturity level as H6 and manually separated into skin, seeds, pulp, and juice. Skins were separated from the rest of the fruit by squeezing individual berries to expel the pulp, juice, and seeds. The seeds were then hand picked from expressed pulp and juice and placed in a separate container. Juice was obtained by blending the mixture containing the pulp and juice. The juice was then separated from the mixture by filtration on a Whatman No. 4 filter paper under suction. These grapes were lyophilized separately and stored at −70 °C until analyzed.

Amino Acid Analysis. All reagents and chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise stated. Ground dry grape samples (0.4 g) were dissolved in a mixture (20 mL) of methanol, chloroform, and water (60:25:12) and stirred for 1 h. The resulting mixture was centrifuged at 15000*g* for 20 min. The residue was allowed to dry overnight in a hood and then dissolved in citrate borate buffer (10 mL; pH 2.2) and centrifuged at 8000*g* for 10 min. To the supernatant (4 mL) was added sulfosalicylic acid (50% w/v; 1 mL). The mixture was centrifuged for 20 min at 50000*g*. Polyvinylproyrrilodone (2%; 4 mL) was added to the supernatant and recentrifuged at 20000*g* for 20 min. The resulting supernatant (7 mL) was lyophilized and resuspended in Milli-Q water (3.5 mL; Millipore Corp., Milford, MA). The mixture (100 μL) was passed through a 5000 mw Millipore filter. To this was added a mixture (100 μL) of ethanol/trimethylamine/water (2:2:1) and dried. Cyanide derivatization of amino acids was done by the addition of ethanol/triethylamine/water/phenol-isothiocynate (7:1:1:1; 40 μL) to the residue, and the mixture was allowed to sit at room temperature for 20 min. This was then added to buffer A (sodium acetate (pH 6.4; 140 mM) with triethlyamine (0.5 mL) added) and used for HPLC analysis.

HPLC Analysis. Samples were analyzed on a Waters HPLC system on a Gradient Controller model 680, pump model M6000, and the MAXIMA software. Solvent systems were sodium acetate buffer (buffer A) and a mixture of acetonitrile and water (3:2; solvent B). Samples (20 μL) were injected on to the Waters physiological fluids column (3.9 × 300 mm). The initial solvent mixture used for elution was 89% buffer A and 11% solvent B. The solvent composition was changed to 48% buffer A and 52% solvent B after 20 min and then to 15% buffer A and 85% solvent B after 29 min using gradient curve 6 in both cases. Peak detection and quantitation by UV at 254 nm were confirmed by the use of external standards (Amino Acid H; Pierce, Rockford, IL).

RESULTS AND DISCUSSION

Changes in °Brix, total acidity, berry weight, berry size, and total free amino acids are shown in Figure 1. The onset of ripening (verasion) often indicated by the accumulation of soluble solids and reduction of acidity was evident. A drop in the total free amino acids

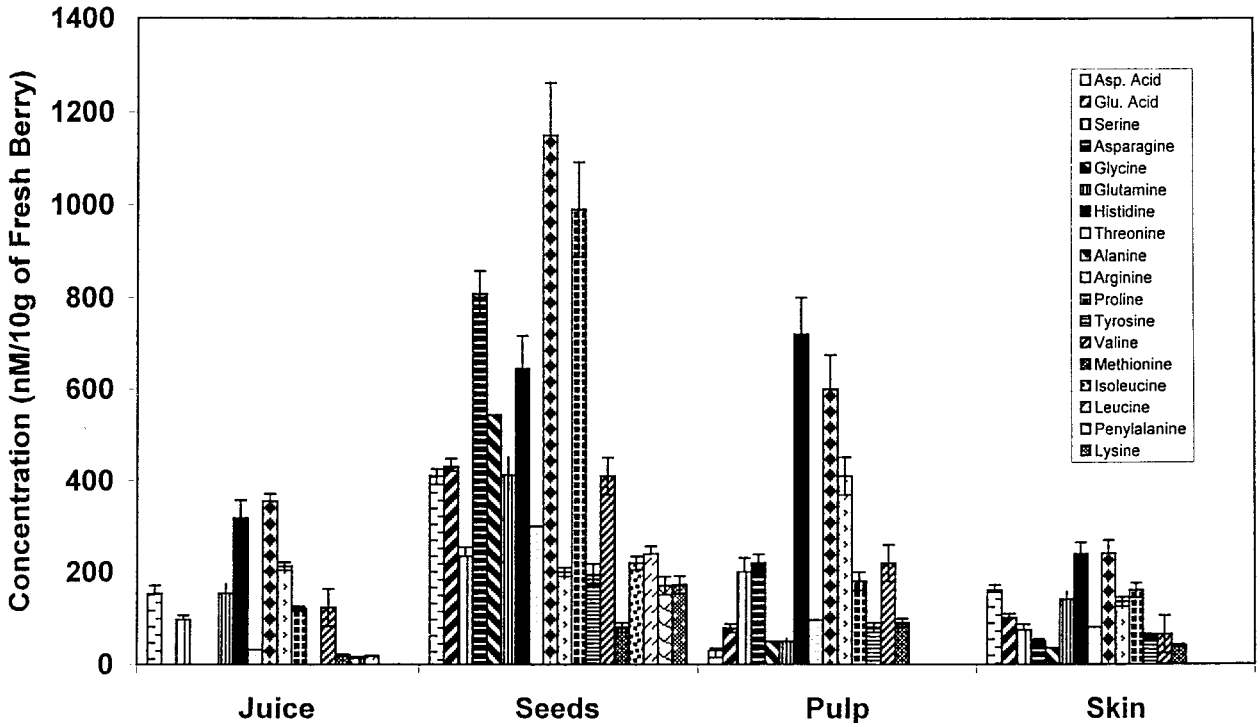

Figure 3. Distribution of free amino acids among grape components in mature Noble muscadine grape. Grapes used were harvested at the same maturity level as H6.

occurred during the first weeks after fruit set, but a rapid increase occurred around verasion. The ripening process (after H3) occurred with an initial drop in total free amino acid content, followed by a gradual increase until full maturity.

Eighteen amino acids were detected. These include aspartic acid, glutamic acid, serine, asparagine, glycine, glutamine, histidine, threonine, alanine, arginine, proline, tyrosine, valine, isoleucine, leucine, and phenylalanine. Histidine, alanine, aspartic acid, valine, proline, threonine, arginine, serine, and glutamine, however, accounted for over 80% of the total free amino acids. Methionine and lysine were only present in grapes harvested at fruit set (H1) and those obtained immediately after verasion (H4). The concentrations of methionine were 0.7 (H1) and 43.0 (H4), while those of lysine were 0.67 (H1) and 0.78 (H4) μM/100 g of fresh grapes, respectively.

Concentrations of most of the individual amino acids reached their peak during verasion. Changes in the amount for the amino acids with maturity can be classified on the basis of their relative amounts at verasion. The concentrations of alanine, glycine, histidine, valine, isoleucine, aspartic acid, and serine were highest at verasion, while trends for the other amino acids were different (Figure 2). The concentrations of glutamine and threonine dropped sharply after fruit set and continued to decrease with fruit maturity. Arginine and proline increased gradually with maturity but increased rapidly with the ripening process. Tyrosine content increased gradually with maturity and ripening following a slight drop after fruit set.

Grape components consisted of juice (48%), skin (26%), pulp (17%), and seeds (10%) by weight. The seeds, however, contributed most of the amino acids in mature grapes (50%) followed by the pulp (23%), the juice (15%), and the skin (11%). Alanine, histidine, and arginine were the principal amino acids identified in the juice with total percentages of 23, 20, and 14%, respectively (Figure 3). Nine amino acids (alanine, histidine, arginine, valine, glutamine, aspartic acid, proline, serine, and threonine) accounted for about 90% of the amino acids found in the pulp. In the seeds, alanine, proline, asparagine, and histidine accounted for over 55% of the amino acids. Alanine (17.3%) and histidine (14.5%) were found to be the predominant free amino acids in the skin. Proline, aspartic acid, glutamine, arginine, and glutamic acid contents were also relatively high when compared to those of threonine, serine, valine, tyrosine, asparagine, methionine, and glycine.

Arginine, proline, glutamic acid, and alanine have been reported to be the major free amino acids in many *V. vinifera* table grapes (Kliewer, 1969; Huang and Ough, 1991; Sarimento et al., 1992; Guitart et al., 1998). These acids along with butyric acid, aspartic acid, serine, and threonine accounted for over 90% of the amino acids present in these grapes. Their concentration generally increased with berry maturity. In *V. labrusca*, alanine and arginine are the predominant acids (Kluba et al., 1978). In both *V. vinifera* and *V. labrusca*, proline and arginine showed the most noticeable increase in concentration with grape maturity (Kliewer, 1970; Kluba et al., 1978). The profile obtained in this study also shows these amino acids as being dominant in the Noble muscadine grape with the exception of histidine. Histidine was the most prominent amino acid at almost every stage of grape maturity. This observation was not reported for the same muscadine cultivar by Marcy et al. (1981). This difference is apparently due to the fact that the method used by the authors was unable to resolve histidine as a separate peak. The fraction that contained a mixture of histidine and γ-aminobutyric acid was consistently higher than most amino acid fractions in that study. The amino acid profile obtained in this study is in general consistent with those reported by Marcy et al. (1981). In that study, however, grapes were deseeded prior to analysis unlike in this study, in which free amino acids of whole berries were deter-

mined. *V. labrusca* grapes (Kluba et al., 1978) contained large quantities of glutamine and asparagine that are not dominant in the muscadine grape.

A significant difference between the change in concentrations of amino acids in muscadines and nonmuscadine cultivars is that while in the former, a majority of amino acids increase until verasion and decrease with ripening, in nonmuscadine grapes most amino acids increase with berry development and ripening, with no postverasion decrease (Kliewer, 1968; Kluba et al., 1978). The reason for the postverasion decrease in amino acid concentrations with ripening in muscadines is unclear. This could be related to a greater demand in muscadines for amino acids in protein synthesis during the active physiological changes that occur during the ripening of grapes (Marcy et al., 1981). The average size of muscadine grape proteins is higher than most proteins isolated from nonmuscadine grape species (Lamikanra, 1986; Lamikanra and Inyang, 1988). The relative ease of converting amino acids in muscadines to flavor compounds has also been suggested. Muscadine grapes, for example, appear to convert phenylalanine to 2-phenylethanol with relative ease inside the grapes, while such conversions only occur in nonmuscadines during active fermentations (Lamikanra et al., 1996). Muscadine grape seeds are also relatively bigger than those of nonmuscadines. There could also be an increased demand for amino acids in seed formation. Synthesis of glutamine, the precursor of other amino acids such as histidine and proline, proceeded by way of glutamate and glutamine synthase intermediates (Yeaman, 1986; Mathews and Van Holde, 1996). The rapid decrease in the concentration of glutamine from the amount present at fruit set might be indicative of its role in synthesizing some of the amino acids whose concentrations increased with grape maturity.

The expected composition of the grape juice based on the profile reported for *V. vinifera* grapes (Kliewer et al., 1978; Spayd and Andersen-Bagge, 1996; Huang and Ough, 1991; Guitart et al., 1998) is one in which arginine and/or proline would be the most dominant amino acid(s) and cystine content would be very low. While the concentrations of arginine and proline in muscadine juice were relatively high and the cystine level was below the detection limit in this study, alanine was the most prominent amino acid followed by histidine. In the *V. labrusca* cultivars (Kluba et al., 1978), alanine was also the most prominent amino acid, but the histidine level was low compared with that of the other amino acids present. Kliewer (1969, 1970) noted that grape varieties with predominant alanine levels had some American species in their parentage. The high levels of alanine in both *V. labrusca* and *V. rotundifolia* from this study are in agreement with this observation. Glycine concentrations were low for both muscadine and nonmuscadine juices. Igartuburu et al. (1991a) reported that lysine, tryptophan, and the sulfur-containing amino acids appear to be the limiting amino acids in the synthesis of seed proteins of some *V. vinifera* cultivars. Histidine, methionine, cystine, and alanine were also found in low amounts, while aspartic acid, glutamic acid, and tyrosine were present in the highest concentrations. The composition of *V. vinifera* grape skin proteins also includes relatively small quantities of tryptophan, cystine, methionine, and histidine and relatively large amounts of glutamic acid aspartic acid and leucine (Igartuburu et al., 1991b). These are in contrast to the muscadine seed and skin, where alanine, histidine, and proline are the more dominant amino acids.

The most significant difference between the free amino acid composition of muscadine and nonmuscadine grapes is the dominance of histidine at every stage of maturity and in its distribution within the grape berry. The relatively high amounts of histidine would normally be expected to have a significant impact on the fusel alcohol composition of the muscadine wine. In the fermentation of *V. vinifera* juice, for example, arginine and/or proline, the dominant free amino acids, are utilized by yeast strains more than the other amino acids and are usually involved in the formation of key nitrogen compounds in wines (Ough et al., 1991; Jiranek et al., 1995;). Histidine utilization should thus be high in muscadines. Jiranek et al. (1995) demonstrated that on a percent residual basis the removal of histidine by *Saccharomyces cerevisiae* strain 77 was comparable to other amino acids. *S. cerevisiae* strain 72 had a slower rate of removal of histidine. The expected product from the decarboxylation and deamination of histidine by way of 2-keto acid intermediates during fermentation (Nykanen, 1986) is imidazole ethanol. It is interesting to note that neither this compound nor other imidazole derivatives have been identified in muscadine wines (Lamikanra, 1987; Lamikanra et al., 1996). They also do not appear to be present in nonmuscadine wines (Amerine and Joslyn, 1970; Nykanen, 1986). Their absence could be due to a number of reasons including other reactions during fermentation that break down the imidazole ring and the stability of these compounds at high temperatures encountered in gas chromatograph analysis. Research to determine histidine uptake during alcoholic fermentation of muscadine grapes and compounds produced as a result of such histidine utilization would be beneficial.

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