Distribution and Effect of Grape Maturity on Organic Acid Content of Red Muscadine Grapes

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Changes in organic acid contents of red muscadine grapes with fruit maturity and their distribution within mature berries were determined by HPLC. The major acids identified are succinic, tartaric, and malic acids. Succinic acid was the most abundant immediately after fruit, set but its concentration dropped sharply as fruits matured. Tartaric acid was the most prominent acid from verasion until the fruits were fully mature. Malic acid content increased gradually until verasion, after which it decreased with fruit ripening. Distribution of acids within the berries was uneven. Grape skins had the highest acid content, whereas the amount of acids in the seeds was the lowest.

Keywords: *Vitis; muscadine; organic acids; carbohydrates*

INTRODUCTION

Organic acids significantly affect the nature and content of other organic compounds in grapes, as well as grape and wine flavor, color, and stability. Information regarding the nature and amounts of acids of *Vitis vinifera* grapes has been known for many years (Amerine and Joslyn, 1970; Winkler et al., 1974), but limited reports (Carroll et al., 1971; Carroll and Marcy, 1982; Marcy and Carroll, 1982) are available for *Vitis rotundifolia* (muscadine) grapes.

Tartaric and malic acids typically account for >90% of acids in grapes. In *V. vinifera* grapes, the acids are not evenly distributed throughout the flesh of the grape (Winkler et al., 1974). The zone near the skin has the lowest titratable acidity and that around the seeds has the highest. The relative amounts of acids are also influenced by environmental factors such as temperature, light, and humidity. Concentration of malic acid, for example, is dependent on the extent of malic enzyme and malic dehydrogenase activities (Hawker, 1969) and appears to have a respiratory quotient that is temperature dependent (Amerine and Joslyn, 1970).

The objective of this study was to determine the nature and amount of organic acids present in some red muscadine grapes (Noble, Regale, and Chief cultivars), and variations that occur in these acids with fruit maturity by HPLC. Most of the reports in the literature were conducted before the advances in HPLC technology (Romero et al., 1993). The distribution of these acids in mature fruits is also reported.

MATERIALS AND METHODS

Collection and Preparation of Grape Samples. Grape sample clusters were harvested from the university's trial vineyard at different stages of maturity. Harvest dates correspond to 9, 25, 45, 65, and 95 days after fruit set. Four clusters, one from each arm of vines trained on a Geneva Double Curtain system, were removed from a vine for sampling. The clusters were destemmed, and the grapes were combined. A set of clusters was removed from another as a duplicate. Mean values of soluble solids in four randomly selected fruits from each sampling were determined on the field with a hand temperature-compensated refractometer. The fruits were lyophilized immediately and stored at −20 °C until analyzed. Dry fruits were crushed into powder, and extraction of organic acids was carried out by stirring the powder (400 mg) in acetic acid solution (pH 2.5; 20 mL) for 4 h. (Wicks and Kliewer, 1983). The mixture was centrifuged at 5000*g*, and the supernatant was filtered through a membrane filter (0.45 μ). The supernatant was lyophilized, redissolved in H_2SO_4 (0.1 M), and analyzed for organic acid by HPLC. Additional samples of ripe fruits were collected and analyzed for the distribution of organic acids within the berries. The grapes were sliced separated into three components; that is, skins, pulp (includes juice), and seeds. These components were lyophilized separately, and samples for HPLC analysis were prepared by the same procedure.

Table 1. Changes in Sugar Content of Grapes with Number of Days after Fruit Set

cultivar	harvest time (days)	°Brix
Noble	9	5.0
	25	5.0
	45	6.5
	65	13.0
	95	17.0
Regale	9	6.2
	25	6.9
	45	7.5
	65	16.7
	95	17.3
Chief	9	5.0
	25	5.5
	45	8.0
	65	15.8
	95	20.5

Analysis of Organic Acids. Organic acids were analyzed on an ISCO HPLC system interfaced with an HP 3394A integrator. The system was equipped with an Interaction ION 300 polymeric ion-exclusion column (300 × 7.8 mm i.d. column Interaction Corp., San Jose, CA) and an LDC Specromonitor II UV monitor. The acids were monitored at 213 nm. Isocratic elution was performed with a 0.01 N H_2SO_4 solution at a flow rate of 0.4 mL/min. Identification and quantitation were done by comparison of sample peaks with those of external standards.

RESULTS AND DISCUSSION

Changes in soluble solids of the berries with maturity are shown in Table 1. The major organic acids present in the cultivars are tartaric, malic, and succinic acids (Figure 1). The total acid level, based on the sum of the amounts of individual acids, decreased with fruit maturity. Succinic and tartaric acid contents of the

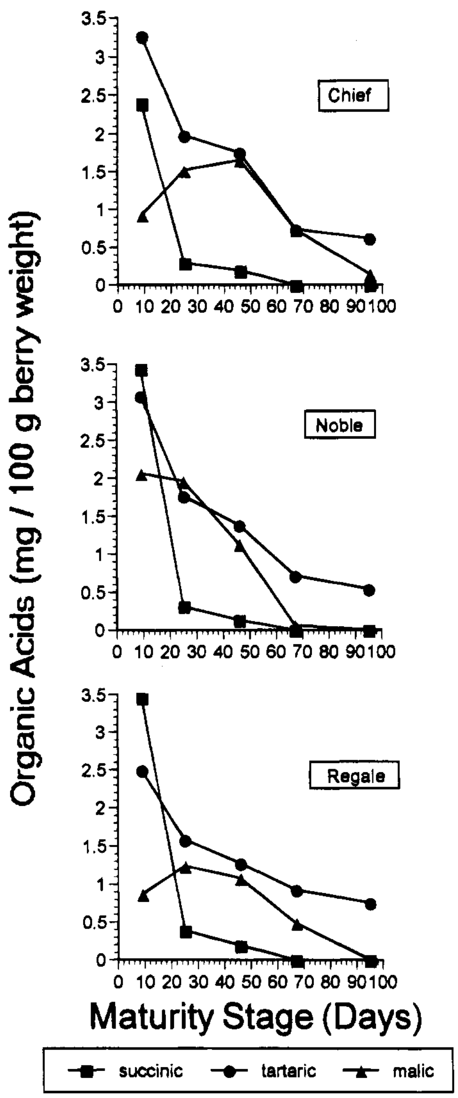

Figure 1. Changes in organic acid contents of muscadine cultivars with grape maturity (number of days after fruit set).

three cultivars used were comparable at fruit set. The rate of succinic acid loss was considerably higher than those of tartaric and malic acids. Grapes harvested 3 weeks after fruit set had lost >90% of their initial succinic acid content compared with an average of 30% loss of tartaric acid during the same period of time. Malic acid contents at fruit set were relatively lower than those of tartaric and succinic acids. Also, unlike the decrease in concentrations observed for the latter two acids with grape maturity, malic acid increased until verasion in both Chief and Regale cultivars and then decreased with ripening. The malic acid content of the Noble cultivar was much higher at fruit set than the contents of the other two cultivars, but decreased with fruit maturity.

Due to the relative ease of separation of grapes into skins, seeds, and pulp in mature versus unripe fruits, analysis of grape components for their organic acid contents was limited to fruits harvested after verasion (65 and 95 days after fruit set; Table 2). Approximately 70% of the acids was located in the skins, and the content of acids in the seeds was negligible. The ratios of malic to tartaric acid in the skins and seeds appear to be comparable with those for the whole fruits.

During the development of berries, a progressive increase in tartaric and malic acid is expected until verasion, after which their concentrations decrease with ripening. Tartaric acid loss is usually more gradual than that of malic acid. The decrease in acidity with ripening results from a number of factors, such as increased respiration, reduced translocation of acids from leaves, transformation of acids to other compounds, dilution effect due to increased volume of fruit, and reduced ability of the fruit to synthesize acids with maturity (Kliewer, 1971; Winkler et al., 1974). With the exception of malic acid in Chief and Regale cultivars, in which increases in concentration occurred until verasion, the maximum concentration of the acids occurred at fruit set and decreased with maturity. This physiological trend is different from that of the *V. vinifera* cultivars.

The relatively high content of succinic acid at fruit set has not been previously observed. Succinic acid is believed to be formed primarily from the glyoxylate cycle (Thoukis et al., 1965) and has been reported to be present in trace quantities in both *V. vinifera* and *V. rotundifolia* grape species (Kliewer, 1966; Johnson and Carroll, 1973). The presence of this acid at such high levels in muscadine grapes at fruit set and the trend in acid concentrations with fruit maturity has also been confirmed during other growing seasons and at other locations in Florida (Kassa, 1995). The presence of succinic acid at concentrations that are comparable to that of tartaric acid in pre-bloom muscadine xylem exudate was also reported by Anderson and Brodbeck (1969). The high levels of succinic acid observed in this study at fruit set followed by its rapid loss with fruit maturity imply that it is rapidly converted to other compounds after fruit development on the vine.

The possible involvement of succinic acid as a precursor or intermediate product in the formation of other compounds during berry development has received very little attention, apparently because of the low concentrations of such products in grapes (Kliewer, 1967). Succinic acid, like malic acid, is involved in the citric

Table 2. Organic Acid Content of Muscadine Grape Seeds, Juice, and Skins

		organic acid content[a] (mg/100 g of fresh berry weight)					
		Chief		Noble		Regale	
component	acid	65 days[b]	95 days[b]	65 days[b]	95 days[b]	65 days[b]	95days[b]
seeds	tartaric	1.15a	0.01a	0.03a	0.00a	0.02a	0.03a
	malic	0.00a	3.10a	0.13a	0.00a	0.00a	0.09a
pulp	tartaric	1.15b	1.33b	0.67c	0.55c	1.8a	2.0a
	malic	1.40a	0.18b	0.11b	0.07b	0.58b	0.07b
skins	tartaric	5.8ab	4.34bc	6.34a	5.88ab	7.59b	5.24c
	malic	4.85a	0.70b	1.24b	1.25b	1.51ab	0.74b
	succinic	tr[f]	tr	tr	tr	tr	tr

[a] Means were separated using Duncan's multiple range test; values followed by different letters are significantly different ($p < 0.05$). [b] Days after fruit set. [c] tr, < 0.01 mg/100 g.

acid and glyoxylic acid cycles. Research to determine changes in biochemical compounds, such as enzymes involved in the biosynthesis of organic acids during the development and ripening of muscadine grapes, should provide the necessary information regarding the production and utilization of succinic acid in this grape species. It is also interesting to note that succinic acid esters are major flavor compounds in muscadine wines (Lamikanra, 1987; Lamikanra et al., 1995). The amount of succinic acid present in muscadine wines is also relatively high, and the acid is produced primarily during active must fermentation (Lamikanra, 1993). The production of succinate esters in muscadine wines seem to result from biosynthesis of succinate to its esters. Research is needed to determine factors that favor the formation of succinic acid and its derivatives in muscadine grapes and wines.

CONCLUSION

It is evident from this study that during the development of berries, significant differences in physiological trends that affect organic acid production exist between muscadine and non-muscadine grape species. The maximum acid concentration occurs at fruit set in muscadines and decreases with grape maturity and ripening. Acids in muscadine grapes are also predominantly located in the skin. To our knowledge, the formation of succinic acid at relatively high quantities has not been reported and does not appear to occur in non-muscadine cultivars. Studies designed to determine the presence and extent of activities of enzymes known to be involved in the biosynthesis of organic acid in grapes will provide a better insight to the factors that influence organic acid formation in muscadines.

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