

# Changes in Organic Acid Composition during Fermentation and Aging of Noble Muscadine Wine

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The nature and concentration of organic acids during muscadine (*Vitis rotundifolia* Michx.) grape fermentation and wine aging were determined. Acids identified were tartaric acid, succinic acid, malic acid, lactic acid, and citric acid. Unlike non-muscadine wines, in which tartaric and malic acids normally account for over 90% of the total organic acid content, tartaric and succinic acids were the predominant acids of the muscadine wine studied. The concentration of succinic acid was almost negligible at the onset of fermentation, but increased to 15 mM at the end of this period. This level was maintained throughout the aging period. Malic acid decreased rapidly over 5 weeks while the loss of tartaric acid was gradual. Initial molar ratios of tartaric acid to malic, succinic, lactic, and citric acids were 1:0.39, 1:0.07, 1:0.00, and 1:0.01, respectively. After 7 months, the ratios had changed to 1:0.23, 1:0.77, 1:0.21, and 1:0.21, respectively. Formation of succinic acid appears to be responsible for the characteristic increase in total acidity during vinification of muscadine wines.

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

## INTRODUCTION

Acids impart stability to wines and are important for preserving their organoleptic qualities and color. Their importance in preventing undesirable byproduct formation during fermentation is also well-known (Amerine *et al.*, 1979). Tartaric and malic acids normally account for an overwhelming majority of fixed acids in wines, while other nonvolatile acids such as succinic, oxalic, citric, lactic, and pyruvic may be present in minor concentrations. Various reactions take place during fermentation and aging of wines that result in changes in the level of fixed acids (Amerine and Joslyn, 1970; Amerine *et al.*, 1979; Vine, 1981). Precipitation of acid salts, acid decarboxylation, and acid–microorganism interactions are some reactions that occur in wines. The overall effect of these reactions normally is the reduction of the amount of fixed acids with vinification. The total acidity in grape juice may be reduced by as much as 33% during normal wine-making procedures (Vine, 1981).

When fermented, *Vitis rotundifolia* grapes, unlike most grape species, have a tendency to increase their total acidity content. This unusual acid increase has been a topic of great concern and research in the southeastern United States (Vine, 1981). Fermentation of muscadine grapes, for example, could result in a total acidity increase of about 10% or higher. Recently, Lamikanra *et al.* (1995) and Kassa (1995) indicated possible physiological differences between *V. rotundifolia* grapes and other grape species that appear to impact on their organic acid biosynthetic pathways. For example, unlike the *Vitis vinifera* cultivars that have trace quantities of succinic acids, immature *V. rotundifolia* grapes exhibited very high levels of the acid. Samples of *V. rotundifolia* collected shortly after fruit set contained higher levels of the acid than tartaric acid, which predominates in most grape species. Succinic

acid concentration in the muscadine berries, however, decreased rapidly with maturity. Also, in other studies (Lamikanra *et al.*, 1987, 1996), it was demonstrated that the derivatives of succinic acid were very high in *V. rotundifolia* grape wines and that the odor of at least one of the derivatives of the acid is characteristic of that of *V. rotundifolia* wines.

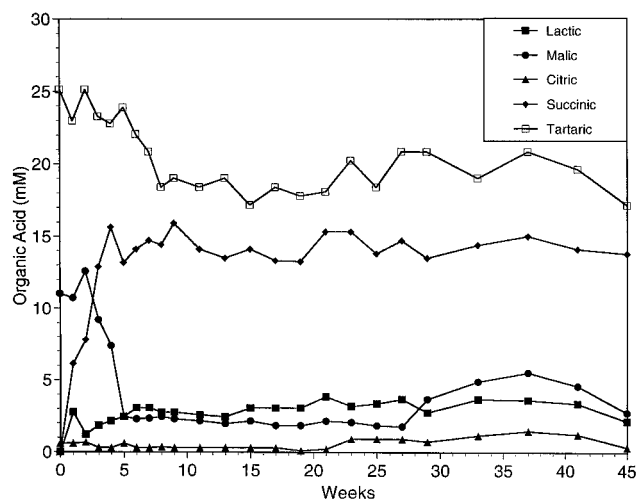
The objective of this study was to determine the nature of organic acids present in fermenting muscadine wines, follow the progress of these acids after active fermentation, and identify the acid(s) responsible for the characteristic increase in total acidity that accompanies muscadine wine fermentation.

## MATERIALS AND METHODS

**Sample Preparation.** Noble muscadine grapes were harvested from the research vineyard at Florida A&M University at optimum ripeness (pH 3.10; °Brix = 15.8; titratable acidity as tartaric acid = 5.8 g/L). The grapes were divided into three equal batches and fermented using *Saccharomyces cerevisiae* (Montrachet strain) as previously described (Lamikanra, 1987). Malolactic fermentation was not induced, and wines were stored at 18 °C until samples were taken. Alcohol was removed from samples (100 mL) using a rotary evaporator at 30 °C until 25 mL of the wine had been evaporated. The dealcoholized wine was then frozen in liquid nitrogen and lyophilized immediately. Dry samples were stirred in acetic acid solution (pH 2.5; 20 mL) to dissolve the organic acids (Wicks *et al.*, 1983). The mixture was centrifuged at 5000g, and the supernatant was filtered through a 0.45 µm membrane filter. The supernatant was lyophilized and redissolved in the same volume of H<sub>2</sub>SO<sub>4</sub> (0.1 M). The solution was used for HPLC analysis.

**Analysis of Organic Acids.** Organic acids were determined on an ISCO HPLC system interfaced with an HP 3394A integrator. The system was equipped with an Interaction Ion 300 250 mm × 4 mm i.d. column and an LDC Spectromonitor II (LDS Division of Milton Roy, Riviera Beach, FL) UV monitor. The acids were monitored at 213 nm. Isocratic elution was performed using a 0.1 M H<sub>2</sub>SO<sub>4</sub> 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.

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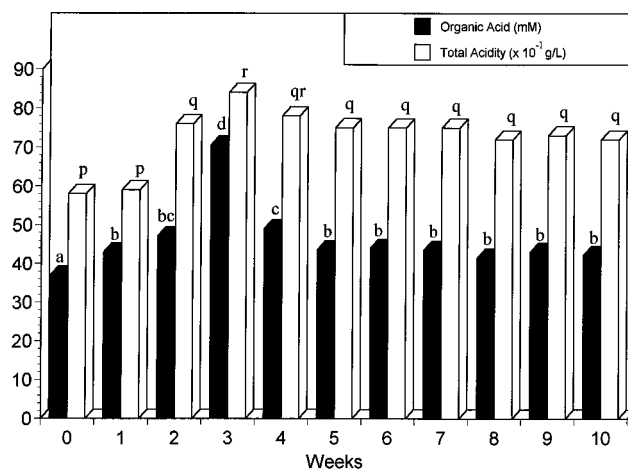


**Figure 1.** Organic acid content of Noble muscadine wine during active fermentation and aging.

## RESULTS AND DISCUSSION

Organic acids identified were succinic, citric, malic, lactic, and tartaric acids (Figure 1). At the onset of fermentation, tartaric and malic acids were the predominant acids, with concentrations of 25 and 11 mM, respectively. The most noticeable trends in the acid contents were the loss of tartaric and malic acids and the increase in succinic acid with time. Tartaric acid content decreased slowly, and after the eighth week, the rate of decrease in the concentration of the acid decreased. By the end of 8 weeks, the tartaric acid level appeared to have stabilized at about 17 mM. Loss of malic acid was more rapid during fermentation. By the end of the fermentation period, the malic acid content had decreased by over 70% of its original value, but later increased slightly after 27 weeks. Loss of malic and tartaric acids in wines could result from numerous possible reactions such as the microorganism-initiated and/or enzyme-catalyzed reactions, salt precipitation, and oxidation–reduction reactions (Amerine and Joslyn, 1970; Amerine *et al.*, 1979). An interesting observation was the amount of increase in succinic acid that occurred during fermentation. This acid increased from an almost negligible amount at the onset of fermentation to 15 mM at the end of this period. The relative ratios of tartaric acid to malic, succinic, lactic, and citric acids were 1:0.39, 1:0.07, 1:0.00, and 1:0.01, respectively. After 7 months, the ratios had changed to 1:0.23, 1:0.77, 1:0.21, and 1:0.21, respectively.

Total acidity increased from 05.8 to 9.0 g/L during fermentation. A gradual decrease in the total acidity occurred after this period, stabilizing at about 7.5 g/L after 10 weeks. The increase in total acidity observed during fermentation appears to be the result of the increase in succinic acid, which was high enough to offset the decrease in tartaric and malic acids. This is evident from the comparison of a plot of the combined acid concentrations with the corresponding wine total acidity values (Figure 2). The effect of the decrease in tartaric and malic acids normally accounts for the decrease in total acidity of non-muscadine wines during vinification. Succinic acid is relatively resistant to bacterial attack that could result in the partial loss of other acids such as tartaric and malic acids. This stability should reduce the tendency for the total acid level to drop significantly in the presence of relatively high levels of succinic acid. The expected range of succinic acid level in wines as a percentage of weight of



**Figure 2.** Total organic acid and titratable acidity. Means were separated using Duncan's multiple range test. Bars without the same letters (a–d for organic acid and p–r for total acidity) are significantly different ( $P > 0.05$ ,  $n = 3$ ).

alcohol produced normally varies from 0.68 to 2.25, with most of the succinic acid being produced at the early stages of fermentation. It is believed to be primarily formed from the glyoxylate and is usually a product of yeast growth (Thoukis *et al.*, 1965). It has also been suggested that 10–12% of sugars are diverted to yeast biomass during fermentation, of which 0.3% could be succinic acid (Ough, 1992). The calculated weight of succinic acid relative to that of ethanol in this study (1.9%) falls within the expected range. Production of succinic acid proceeded until all of the fermentable sugars had been exhausted.

Succinic acid is usually the predominant nonvolatile organic acid produced during fermentation, accounting for over 90% of acids produced. Although the same trend was observed, the molar ratios of tartaric/succinic (1:0.77) and malic/succinic (1:3) for the muscadine wine are higher than the expected range of 1:0.08 to 1:0.37 for tartaric/succinic and 1:0.17 to 1:1.3 for malic/succinic acid ratios, respectively, on the basis of previous reports for nonmuscadine wines (Amerine *et al.*, 1979; Thoukis *et al.*, 1965; Shimazu and Watanabe, 1981). Previous studies (Andersen and Broadbeck, 1989; Lamikanra *et al.*, 1995) have indicated the relative ease of production of succinic acid and its derivatives by muscadine grapes and plants. The presence of this acid at such high levels relative to other organic acids in muscadine wines confirms these earlier findings and is inconsistent with similar reports for non-muscadine varieties. Its concentration in the finished wine (0.18 g/100 mL) is also much higher than the threshold of 0.0034–0.0035 g/100 mL reported for the acid (Amerine *et al.*, 1979) and would be expected to impart its salt–bitter acid character to muscadine wines (Thoukis *et al.*, 1965).

Succinic acid has the highest rate of esterification when compared with the other common organic acids in table wines (Amerine and Joslyn, 1970). The high content of the acid in muscadine wine is thus consistent with the high levels of succinic acid esters that were found in muscadine wines (Lamikanra *et al.*, 1996). In the same study, it was also reported that methyl succinate contributed significantly to the muscadine wine aroma.

The variation in the relative contents of malic and lactic acids with time does not suggest a significant level of malolactic fermentation of malic to lactic acid. Although malic acid is less resistant to microbial attack

than succinic and tartaric acids, the loss of over 70% of this acid during fermentation is considerably greater than the expected range of 10–30% loss (Amerine *et al.*, 1979). The possible interconversion of succinic and malic acids in muscadine grapes was suggested in an earlier study in which the loss of succinic acid was observed to occur concurrently with an increase in malic acid content as muscadine grapes matured (Lamikanra *et al.*, 1995). A reversed trend appeared to have occurred during fermentation as shown by this study. Flanzy *et al.* (1967) suggested that the conversion of malic to succinic acid during carbonic maceration is the main source of succinic acid in anaerobic fermentation. The production and metabolism of malic and succinic acids are closely related in that they both proceed by way of oxalacetic acid intermediates. Research is needed to determine why they appear to be more readily converted from one to the other in the *V. rotundifolia* grapes relative to other grape species.

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