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# Phytochemical Stability and Color Retention of Copigmented and Processed Muscadine Grape Juice

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Muscadine (*Vitis rotundifolia*) grape juice was assessed for color and phytochemical stability as influenced by anthocyanin copigmentation with a water-soluble rosemary extract, fortification with ascorbic acid, and processing by heat or high hydrostatic pressure (HHP). The roles of polyphenolic cofactors in the presence and in the absence of ascorbic acid were assessed as a means to improve the overall processing stability of the juice. Addition of rosemary extract from 0 to 0.4% (v/v) readily formed copigment complexes with anthocyanins and resulted in concentration-dependent hyperchromic shifts from 10 to 27% that corresponded to increased antioxidant activity. The presence of ascorbic acid was generally detrimental to juice quality, especially in the presence of rosemary extract, and resulted in overall anthocyanin, ascorbic acid, and antioxidant activity losses. Although thermal and high-pressure processing methods were detrimental to juice quality, HHP resulted in greater losses after processing, likely due to action from residual oxidase enzymes. Although physicochemical attributes were enhanced by copigmentation with rosemary extract, methods to inactivate residual enzymes should be addressed prior to copigmentation to prevent degradation of anthocyanins in the presence of ascorbic acid.

**KEYWORDS: Muscadine; copigment; high pressure; ascorbic acid; anthocyanin; polyphenol oxidase**

## INTRODUCTION

Muscadine grapes (*Vitis rotundifolia*) are the predominant grape variety grown in the southern United States, with current markets existing for juice, wine, and table grapes. Native muscadines have a natural resistance to Pierce's disease (*Xylella fastidiosa*), which prevents extensive cultivation of *Vitis vinifera*, and commercial muscadine varieties have retained much of this resistance, making them a valuable crop with good potential for expansion and further development. Processing methods or treatment regimes that improve quality characteristics of muscadine grapes are therefore vital for economic growth of the crop. Phytochemical composition of muscadine grapes compares favorably to *V. vinifera* and *Vitis labrusca* in that all contain appreciable concentrations of neutral and acidic polyphenolics that act as inhibitors of oxidation (*1*, *2*). However, muscadine grapes also contain ellagic acid in free and conjugated forms (*3*−*6*), and their anthocyanins exist as 3,5-diglycosides in non-acylated forms (*7*, *8*), which is unique among grape species.

Anthocyanins are best known for their brilliant red and purple colors, and their antioxidant and antiradical capacities have been firmly established. However, combining anthocyanins and ascorbic acid was shown to be mutually destructive in the presence of oxygen (*9*, *10*) and may limit fortification efforts in anthocyanin-containing foods. No information is available on ascorbic acid fortification of muscadine grape products, but any reaction accentuating color loss would be especially detrimental to product quality due to the inherent instability of muscadine grape anthocyanins. Loss of anthocyanin color may be prevented through strict oxygen control during processing or by physical stabilization of anthocyanins through the addition of exogenous anthocyanin cofactors. Anthocyanin intermolecular copigmentation reactions are the result of associations between cofactors such as polyphenolics, metal ions, or other anthocyanins to produce weak chemical bonds with increased physicochemical attributes such as color, stability, or increased antioxidant properties (*11*). These copigment complexes form preferentially under acidic conditions and may augment visual color and/or color stability of food systems. The phytochemical stability of copigmented anthocyanins in the presence of ascorbic acid is largely unknown and may serve as a physical or chemoprotective agent during food processing.

High hydrostatic pressure (HHP) is a promising alternative to traditional thermal processing techniques for food preservation (*12*), but associated changes to a diversity of phytonutrients have not been extensively investigated. HHP eliminates the detri-

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mental effects of heat for many thermolabile compounds such as ascorbic acid (*13*−*16*), resulting in greater retention, but it may actually activate some enzymes responsible for phytochemical degradation. Making comparisons between HHP and thermal processes is one way to assess its benefits by monitoring destruction of heat-labile compounds.

The objectives of this study were to determine color, phytochemical, and antioxidant stability following processing (thermal pasteurization and HHP) in ascorbic acid-fortified muscadine grape juice as influenced by polyphenolic cofactors from rosemary (*Rosmarinus officinalis*), whose polyphenolics readily associate with anthocyanins. By monitoring phytochemical and quality characteristics of muscadine grape juice after processing, the benefits and detriments of anthocyanin cofactor addition and ascorbic acid fortification were established.

## MATERIALS AND METHODS

**Materials and Processing.** *Pasteurization and HHP Processing.* Red muscadine grapes (cv. Noble) were obtained from a local grower in central Florida and processed into juice on the day of harvest. Grapes were crushed, heated to 70 °C in an open steam kettle, and held for 60 min prior to juice extraction in a hydraulic basket press (Prospero's Equipment, Cort, NY). Juice was immediately frozen until needed, after which it was thawed and filtered through cheesecloth, followed by vacuum filtration through a 1-cm bed of diatomaceous earth. Sodium azide (50 mg/L) was added to retard microbial growth throughout the study. Stock juice was divided into four portions for copigmentation (0, 0.1, 0.2, and 0.4% v/v) with a water-soluble rosemary extract (ColorEnhance-R, 3.5% rosmarinic acid, Hauser, Bolder, CO). Juices at each cofactor concentration were then divided, and half of the samples were fortified with ca. 800 mg/L ascorbic acid as compared to an equivalent volume of water as a control. All treatments were maintained at pH 3.2 and did not change throughout the study.

Juice treatments were prepared for processing by placing 15 mL into a screw-cap glass test tube and pasteurizing in a water bath (95 °C for 15 min) as compared to nonpasteurized controls. For HHP, 8-mL portions were filled into heat-sealed plastic ampules and processed at 600 MPa for 15 min (Stansted Fluid Power, UK). Following HHP processing, ampules were emptied into screw-cap tubes for storage. All treatment combinations were stored under refrigerated conditions and analyzed within 72 h of processing.

*Ascorbic Acid Stability.* A second juice preparation was made from Noble grapes using a milder extraction regime. Fresh grapes were immediately frozen after harvest to facilitate cellular damage and placed into an open steam kettle without crushing. Grapes were heated to 60 °C and held for 30 min prior to pressing and storage as previously described. Juice was divided into three equal portions for additional processing and fortification evaluations. The first portion was subdivided, fortified with 0, 100, and 500 mg/L ascorbic acid, and then pasteurized at 95 °C for 15 min, while the second portion was initially pasteurized and then fortified. These treatments were compared to a control juice that was fortified at each ascorbic acid concentration but not pasteurized. All juices were equilibrated to 23 °C and held for 2 h, after which isolates were obtained for HPLC analysis.

**Chemical Analyses.** Physicochemical changes in muscadine juice following rosemary extract and ascorbic acid addition were evaluated as a function of processing parameters. Juices were analyzed for color characteristics on the basis of spectroscopic properties of anthocyanins and included color density, hue−tint, and anthocyanin monomeric/polymeric forms (*17*). Total soluble phenolics were measured using the Folin−Ciocalteu assay (*18*). Individual anthocyanin 3,5-diglycosides were quantified according to the HPLC conditions of Skrede et al. (*19*) using a Waters 2690 Alliance system and a 996 PDA detector. Compounds were separated on a 250- × 4.6-mm Supelcosil LC-18 column (Supelco, Bellefonte, PA) and quantified using standards of their respective 3-glucoside forms (Polyphenols Laboratories AS, Sandnes, Norway). Samples for ascorbic acid analysis were obtained by diluting juice 5-fold with 3% citric acid and passing through a preconditioned Waters $C_{18}$ Sep-Pak cartridge to remove neutral polyphenolics and quantified by reversed-phase HPLC using the chromatographic conditions of Talcott et al. (*20*) with a run time of 10 min.

Antioxidant capacity was determined using the oxygen radical absorbance capacity (ORAC) method described by Cao et al. (*21*), adapted to run on a 96-well Molecular Devices fmax fluorescent microplate reader, and compared to a standard of Trolox, a water-soluble analogue of vitamin E.

**Statistical Analysis.** Data represent the mean and standard error of three replicate juices, analyzed as a 3 × 4 × 2 factorial, comparing three processing treatments, four copigment concentrations, and the presence or absence of ascorbic acid. Multiple linear regression, analysis of variance, and Pearson correlations were conducted using JMP software (*22*) and mean separation using the LSD test ($P < 0.05$).

## RESULTS AND DISCUSSION

**Chemical Analysis.** *Effects of Rosemary Extract and Ascorbic Acid on Unpasteurized Muscadine Juice.* Muscadine grape juice color was appreciably altered by water-soluble polyphenolics in rosemary. Bathochromic and hyperchromic shifts in absorbance are commonly used as indicators of color augmentation by cofactors, and color changes were readily perceived in muscadine grape juice by the naked eye. Spectroscopic shifts under acidic conditions are known to produce large color changes due to intermolecular copigmentation (*23*). The predominance of anthocyanin 3,5-diglucosides make muscadine grapes excellent candidates for copigmentation due to their efficient cofactor association as compared to that of the corresponding 3-glucosides (*24*, *25*). The addition of rosemary extract to muscadine grape juice resulted in concentration-dependent, hyperchromic shifts at 515 nm ranging from 10 to 27%, with a corresponding 1−6 nm bathochromic shift (**Table 1**). Copigmentation also increased anthocyanin color density, a spectrophotometric measure of the overall color of an anthocyanin solution, by 38% and hue−tint (redness of the juice) values by 10% but did not decrease the anthocyanin content through polymerization reactions. Preliminary investigations determined that muscadine juice color could be increased over 120% with exogenous addition of rosemary extract, but sensory thresholds for rosemary prevent practical use at these concentrations.

Fortification of juices with ascorbic acid is a common practice in the food industry to protect against oxidation, and it provides nutritional enhancement. However, combination of anthocyanins and ascorbic acid may be mutually destructive in the presence of oxygen, which limits fortification efforts in anthocyanin-containing foods. The additions of both rosemary extract and ascorbic acid were influential in altering the total phenolic content and antioxidant capacity of muscadine juice. Addition of rosemary extract (0.1, 0.2, and 0.4%) increased total soluble phenolics linearly ($R^2 = 0.97$), with each 0.1% increasing concentrations by 185 mg/L on average, representing a ca. 10% increase in total reducing capacity of the juice. Ascorbic acid fortification also increased total phenolics by 360 mg/L on average, which represents a 22% increase over the nonfortified juice according to its detection by the Folin−Ciocalteu assay.

A linear increase in antioxidant capacity accompanied rosemary extract addition up to 0.4% ($Y = 28.93x + 30.25$, where $x$ is the % rosemary extract; $R^2 = 0.99$), which was proportional to hyperchromic shifts in absorbance ($r = 0.87$). However, ascorbic acid fortification in the presence of rosemary extract, though statistically significant, did not exhibit an appreciable increase in antioxidant capacity ($Y = 15.14x + 33.60$, where $x$ is the % rosemary extract; $R^2 = 0.99$), potentially indicating competition for radical scavenging from the diversity,

**Table 1.** Effect of Rosemary Extract (0–0.4% v/v) and Ascorbic Acid (~800 mg/L) Addition on Selected Antioxidant Compounds, Antioxidant Activity, and Color Attributes of Unprocessed (Control), Thermal Pasteurized, and High Hydrostatic Pressure Processed Muscadine Juice

| | rosemary extract (%) | no ascorbic acid | | | added ascorbic acid | | |
|---|---|---|---|---|---|---|---|
| | | unprocessed | pasteurized | HHP | unprocessed | pasteurized | HHP |
| total soluble phenolics[a] (mg/L) | 0 | 1360 d[b] | 1460 d | 1420 c | 1750 d*[c] | 1770 c* | 1660 c* |
| | 0.1 | 1570 c | 1560 c | 1620 b | 1930 c* | 2000 b* | 1850 b* |
| | 0.2 | 1750 b | 1730 b | 1680 b | 2100 b* | 2020 b* | 1890 b |
| | 0.4 | 1970 a | 1900 a | 1950 a | 2310 a* | 2200 a* | 2210 a* |
| hyperchromic shift (%) | 0 | 100 d | 94.9 d | 94.5 d | 97.3 d | 89.1 c* | 92.8 c |
| | 0.1 | 103 c | 100 c | 98.9 c | 105 c | 93.7 bc* | 101 b |
| | 0.2 | 114 b | 107 b | 105 b | 110 b* | 100 b* | 103 b |
| | 0.4 | 127 a | 116 a | 112 a | 124 a | 111 a* | 110 a |
| color density | 0 | 0.80 d | 0.80 d | 0.80 c | 0.80 d | 0.77 c* | 0.80 c |
| | 0.1 | 0.90 c | 0.90 c | 0.80 c | 0.90 c | 0.80 c* | 0.87 b* |
| | 0.2 | 1.00 b | 0.93 b | 0.90 b | 0.90 b* | 0.90 b* | 0.90 b |
| | 0.4 | 1.10 a | 1.02 a | 0.98 a | 1.08 a | 0.98 a* | 0.98 a |
| hue–tint | 0 | 0.48 d | 0.54 a | 0.49 b | 0.46 c | 0.51 b | 0.46 b |
| | 0.1 | 0.49 c | 0.57 a | 0.49 b | 0.48 b | 0.54 ab | 0.53 ab |
| | 0.2 | 0.51 b | 0.57 a | 0.50 b | 0.49 b | 0.58 a | 0.50 ab |
| | 0.4 | 0.53 a | 0.58 a | 0.54 a | 0.53 a | 0.57 ab | 0.57 a |
| monomeric anthocyanins (%) | 0 | 80.4 a | 77.3 a | 78.9 a | 80.6 a | 78.1 a | 79.9 b |
| | 0.1 | 80.5 a | 76.6 a | 79.4 a | 80.3 a | 77.0 ab | 78.3 ab |
| | 0.2 | 80.4 a | 76.8 a | 79.9 a | 80.7 a | 76.1 b | 80.8 a |
| | 0.4 | 81.0 a | 77.7 a | 79.3 a | 80.7 a | 78.1 a | 79.2 b |

[a] Expressed as gallic acid equivalents. [b] Values with similar letters within columns for each processing method are not significantly different (LSD test, $P > 0.05$) and indicate the effect of rosemary extract addition. [c] Treatments marked with an asterisk for each rosemary extract level indicate a significant effect (LSD test, $P < 0.05$) due to ascorbic acid addition when compared to the same treatment without ascorbic acid.

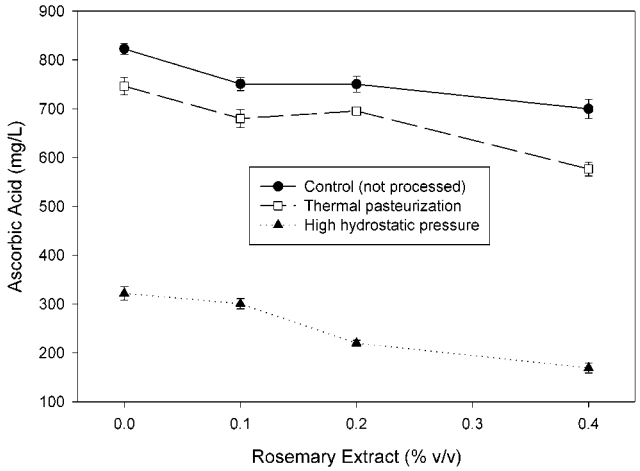


**Figure 1.** Residual ascorbic acid concentration in ascorbic acid-fortified muscadine grape juice (800 mg/L) as influenced by rosemary extract concentration, thermal, and HHP processing. Ascorbic acid was not detected in nonfortified juices. Bars represent standard error of the mean ($n = 3$).

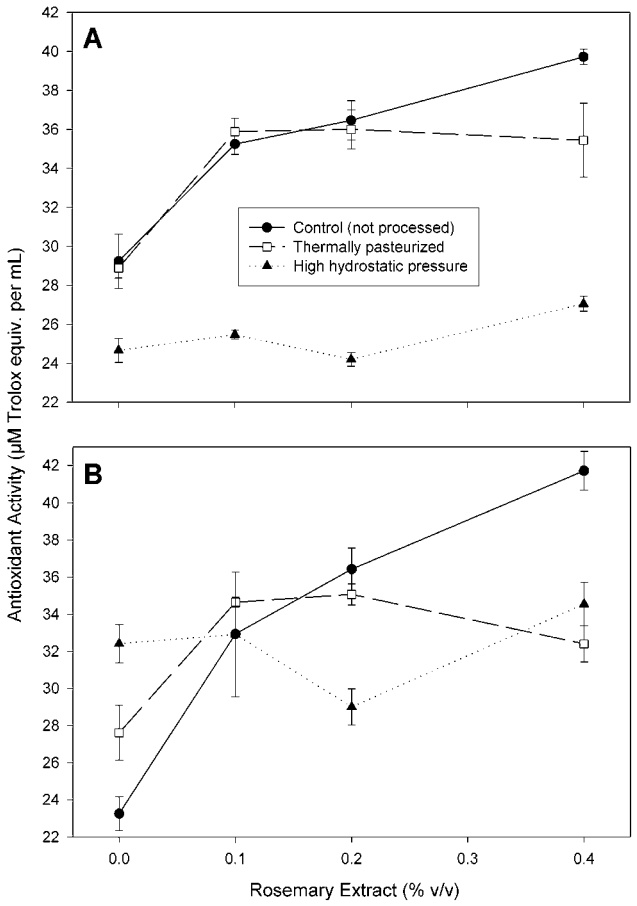


**Figure 2.** Antioxidant activity of muscadine grape juice (A) with fortification with ascorbic acid (ca. 800 mg/L) and (B) without ascorbic acid fortification and influenced by rosemary extract concentration, thermal, and HHP processing. Bars represent standard error of the mean ($n = 3$).

high concentrations, and associations between the antioxidants present (**Figures 1** and **2**). Ascorbic acid concentrations generally decreased as amounts of rosemary extract increased ($r = -0.93$), which indicated the presence of oxidase enzymes for which naturally occurring compounds either in rosemary extract (rosmarinic and caffeic acid) and/or grape juice (caffeic acid, catechin, epicatechin, procyanidins) were a substrate, resulting in co-oxidative destruction of phytonutrients (*26*, *27*). Subsequent evaluations confirmed the presence and activity of polyphenol oxidase (PPO) in muscadine grapes processed under similar conditions (data not shown). PPO was previously identified in muscadine grapes and was thought to influence browning reactions in juice but not wine due to addition of sulfites (*28*). The conditions of juice extraction were probably not severe enough to thermally inactive oxidase enzymes; therefore, their presence in unprocessed juice must be taken into consideration as a factor influencing juice quality. Anthocyanin degradation and browning in processed strawberry and blueberry products was reported as a result of indirect oxidation by

**Table 2.** Effect of Rosemary Extract (0–0.4% v/v) and Ascorbic Acid (~800 mg/L) Addition on Anthocyanin Content of Unprocessed (Control), Thermal Pasteurized, and High Hydrostatic Pressure Processed Muscadine Juice

| | | no ascorbic | | | added ascorbic | | |
|---|---|---|---|---|---|---|---|
| | rosemary extract (%) | unprocessed | pasteurized | HHP | unprocessed | pasteurized | HHP |
| delphinidin 3,5-diglucoside (mg/L) | 0 | 207 a[a] | 193 a | 178 a | 215 a | 180 a | 170 a |
| | 0.1 | 195 a | 177 ab | 171 b | 211 a | 178 a | 161 ab |
| | 0.2 | 207 a | 173 bc | 172 ab | 198 a | 177 ab | 156 bc |
| | 0.4 | 202 a | 168 c | 156 c | 206 a | 169 b | 149 c |
| cyanidin 3,5-diglucoside (mg/L) | 0 | 253 a | 251 a | 242 a | 252 a | 222 a*[b] | 219 a* |
| | 0.1 | 234 a | 234 a | 236 a | 247 a | 217 a | 211 ab* |
| | 0.2 | 253 a | 229 a | 234 a | 242 a* | 219 a | 206 b* |
| | 0.4 | 245 a | 228 a | 217 b | 245 a | 215 a | 206 b |
| petunidin 3,5-diglucoside (mg/L) | 0 | 205 a | 203 a | 186 a | 216 a | 187 a | 178 a |
| | 0.1 | 191 a | 189 ab | 184 a | 191 a | 182 ab | 168 ab |
| | 0.2 | 205 a | 186 ab | 184 a | 205 a | 181 ab | 166 b |
| | 0.4 | 205 a | 183 b | 168 b | 205 a | 178 b | 159 b |
| pelargonin 3,5-diglucoside (mg/L) | 0 | 15.0 a | 18.9 a | 14.8 a | 17.6 a* | 15.7 a* | 13.4 a |
| | 0.1 | 14.2 a | 16.6 b | 14.8 a | 18.6 a* | 14.3 a* | 12.2 a* |
| | 0.2 | 14.7 a | 16.2 b | 14.8 a | 14.8 a | 14.9 a | 12.9 a |
| | 0.4 | 16.7 a | 15.8 b | 13.1 b | 16.7 a | 14.9 a | 12.7 a |
| peonidin 3,5-diglucoside (mg/L) | 0 | 377 a | 408 a | 396 a | 410 a* | 363 a* | 363 a* |
| | 0.1 | 362 a | 378 a | 383 ab | 396 a* | 358 a | 350 ab* |
| | 0.2 | 380 a | 376 a | 379 b | 370 a* | 352 a | 337 b* |
| | 0.4 | 398 a | 371 a | 357 c | 381 a | 348 a | 336 b* |
| malvidin 3,5-diglucoside (mg/L) | 0 | 155 a | 182 a | 176 a | 180 a* | 160 a* | 158 a* |
| | 0.1 | 151 a | 168 ab | 168 a | 170 a* | 159 a | 153 ab |
| | 0.2 | 159 a | 167 ab | 167 a | 159 a | 156 a | 148 b* |
| | 0.4 | 170 a | 165 b | 159 b | 163 a | 154 a | 150 b |
| total anthocyanins[c] (mg/L) | 0 | 1210 a | 1260a | 1190 a | 1290 a | 1130 a* | 1100 a |
| | 0.1 | 1150 a | 1160 ab | 1160 a | 1260 a | 1110 ab | 1060 ab |
| | 0.2 | 1220 a | 1150 ab | 1150 a | 1170 a* | 1100 ab | 1030 b* |
| | 0.4 | 1240 a | 1130 b | 1070 a | 1210 a | 1080 b | 1010 b |

[a] Values with similar letters within columns for each processing method are not significantly different (LSD test, $P > 0.05$) and indicate the effect of rosemary extract addition. [b] Treatments marked with an asterisk for each rosemary extract level indicate a significant effect (LSD test, $P < 0.05$) due to ascorbic acid addition when compared to the same treatment without ascorbic acid. [c] Sum of individual anthocyanins quantified by HPLC.

phenolic quinones generated by PPO and peroxidase (*19*, *29*, *30*). These studies demonstrated that the rate and mechanism of anthocyanin degradation was related to their structure, with *o*-diphenolic anthocyanins rapidly oxidized via coupled oxidation reactions and non-*o*-diphenolic anthocyanins slowly degraded by *o*-quinones or secondary oxidation products (*31*). Addition of ascorbic acid served to lower hyperchromic shifts exhibited by copigmented anthocyanins by 5%, indicating a rapid color loss upon fortification prior to processing.

Total (monomeric) anthocyanins were not significantly altered by the addition of rosemary extract or ascorbic acid, despite appreciable visible color differences due to copigmentation (**Tables 1** and **2**), demonstrating limitations in colorimetric assays that do not account for copigmentation. Initial ascorbic acid addition exerted a protective effect against anthocyanin destruction caused by enzymatic activity (**Table 2**), as evident for pelargonidin, peonidin, and malvidin, indicating its antioxidant action by keeping phenolic quinones in a reduced state and stability conferred by their lack of *o*-diphenolic substitutions in the B ring. However, its protective effect was reduced as rosemary extract concentration increased, potentially due to higher quinone concentrations present. Kader et al. (*32*) also observed that ascorbic acid offered initial antioxidant protection against anthocyanin destruction in systems containing oxidative enzymes by reducing *o*-quinones to their original diphenolic state. Individual anthocyanins were characterized by HPLC on the basis of standards and PDA spectral interpretation from 200 to 600 nm and identified as 3,5-diglucosides of delphinidin, cyanidin, petunidin, pelargonidin, peonidin, and malvidin, as described by Talcott and Lee (*8*). Spectroscopic comparison to anthocyanin 3-glucosides and respective anthocyanidins, following acid hydrolysis, confirmed the diglucosidic form, based on $A_{440}/A_{max}$ ratios (<17%), as described by Hong and Wrolstad (*33*).

*Effects of Processing.* Samples having undergone two processing techniques (thermal pasteurization and HHP) were compared to a nonprocessed control following rosemary extract and/or ascorbic acid addition to muscadine grape juice (**Tables 1** and **2**). Direct comparison of thermal and HHP techniques is challenging since many physicochemical changes are temperature dependent. Both processes are capable of producing a commercially sterile product, especially under highly acidic conditions (*34*, *35*), but HHP is generally used only for shelf life extension. For comparison purposes, each process time was held constant at 15 min.

Physicochemical differences were observed in muscadine grape juice as affected by processing methods and the addition of rosemary extract and/or ascorbic acid. In general, HHP was more detrimental to anthocyanins, ascorbic acid, and color characteristics as compared with thermally pasteurized and control juices due to oxidase enzymes that were active during HHP processing. Potential mechanisms for destruction include the role of PPO and/or autoxidative mechanisms resulting in co-oxidation of anthocyanins and ascorbic acid, despite the potent antioxidant properties of rosemary polyphenolics (*36*). Mutual destruction of anthocyanins in the presence of ascorbic acid also has been documented in model systems (*10*), with rates of destruction proportional to dissolved oxygen in the system. Dissolved oxygen may be linked to enhanced oxidation under high-pressure conditions due to the Le Chatelier principle, where reaction rates change under decreased volume (*37*). However, pressure-induced oxidation was not suspected in the

current study, since additional investigations of juice containing rosemary extract and ascorbic acid in the absence of dissolved oxygen were conducted. These data revealed that neither deaeration nor antioxidants from rosemary extract could completely prevent ascorbic acid loss in muscadine grape juice (data not shown). Poei-Langston and Wrolstad (*10*) observed destruction of anthocyanins and ascorbic acid in nitrogen-sparged systems and proposed a potential condensation mechanism for their mutual destruction that did not involve oxygen. Phytochemical degradation, especially loss of ascorbic acid, in muscadine juice may be due to a similar complexation mechanism with anthocyanins or flavonoids, from autoxidation reactions, or may have been catalyzed by the presence of oxidase enzymes during or after HHP processing, as has been demonstrated in several fruit systems (*38*−*40*).

Only small differences in total soluble phenolics were observed following processing in the presence of rosemary extract and ascorbic acid (<3%), indicating that overall reducing capacity was unaffected by heat or pressure (**Table 1**), similar to the retention previously observed during storage of muscadine wines and juices (*8*). However, ascorbic acid concentration was significantly affected by processing method, with losses of 9.2% after thermal pasteurization compared to 63.1% after HHP (**Figure 1**). Copigmentation by rosemary extract did not prevent ascorbic acid destruction and actually enhanced losses as use rate increased. Since rosemary is known to contain a variety of PPO substrates, such as cinnamic acid derivatives, oxidation of these compounds may have enhanced destruction of ascorbic acid. Additionally, hyperchromic shifts in absorbance were reduced following processing by 5.8−13.3% compared to controls, depending on rosemary extract concentration, indicating that color-degrading reactions were affected by processing, ascorbic acid, and rosemary polyphenolics. Color density also decreased by processing treatments in a similar manner; however, changes in hue−tint and percent monomeric anthocyanins were unaffected by ascorbic acid addition and only reflected color degradation, as affected by processing regimes.

Anthocyanins also underwent losses during processing in the presence of added rosemary extract or ascorbic acid (**Table 2**). Without added ascorbic acid, total anthocyanin decreases were equal among all treatments (3−5%), but in the presence of ascorbic acid anthocyanin losses were influenced by processing and resulted in 12.4 and 18.1% loss during pasteurization and HHP, respectively, at 0.4% rosemary extract. Formation of hydrogen peroxide from ascorbic acid oxidation may contribute to anthocyanin degradation and formation of polymeric anthocyanins; furthermore, these peroxides may have activated residual peroxidase, which further degraded both ascorbic acid and anthocyanins (*41*). Addition of rosemary extract cofactors did not prevent anthocyanin losses, despite their color-enhancing and antioxidant properties, but it accentuated anthocyanin destruction in the presence of ascorbic acid. Individual anthocyanins followed a similar trend, with the greatest losses occurring at 0.4% rosemary extract in the presence of ascorbic acid. Losses ranged from 12 to 15% following thermal pasteurization for delphinidin, cyanidin, petunidin, and pelargonidin, compared to 15−25% for HHP. Peonidin and malvidin were more stable in the presence of rosemary extract and ascorbic acid, probably due to their structural features. The presence of *o*-dihydroxy groups has been shown to cause decreased stability under enzymatic oxidation (*42*), and differences among individual anthocyanins present in the juice and their ability to form copigment complexes also influenced stability, since anthocyanins containing *o*-dihydroxy groups generally experienced greater losses following processing. The greatest retention was observed for malvidin, which is well known for its stability (*43*, *44*) and generally resisted degradation in the presence of ascorbic acid and rosemary extract.

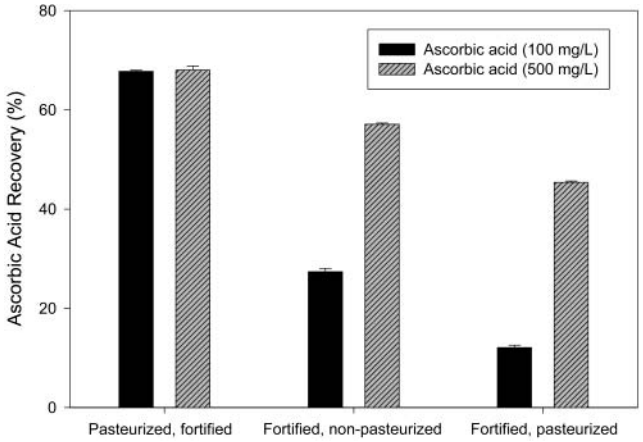


**Figure 3.** Ascorbic acid recovery in muscadine grape juice as influenced by fortification prior to and after pasteurization and by fortification without pasteurization at initial concentrations of 100 and 500 mg/L. Bars represent standard error of the mean ($n = 3$).

Antioxidant capacity significantly changed as a function of processing method, addition of rosemary extract, and/or ascorbic acid (**Figure 2**). The linear response in antioxidant capacity prior to processing with increasing rosemary extract concentration was not observed after processing, potentially reflecting activity of PPO or other oxidases. In the absence of ascorbic acid, processing was detrimental to antioxidant properties in relation to the control. However, the antioxidant capacities of thermally pasteurized juices containing ascorbic acid were not different from those of unprocessed controls, except at 0.4% rosemary extract, which again reflected the quinone-reducing ability of ascorbic acid. HHP caused a 25% overall decrease in antioxidant capacity, indicating dynamic changes under pressure influenced by anthocyanins and ascorbic acid that was likely accentuated by residual PPO. For high pressure to be a viable option for fresh muscadine grape juice, issues surrounding removal, inactivation, or inhibition of native oxidase enzymes are critical for quality retention.

*Enzyme Action on Ascorbic Acid.* Subsequent studies with ascorbic acid-fortified muscadine grape juice, obtained following a mild hot-press regime, provided additional evidence for action of oxidase enzymes such as PPO in contributing to quality deterioration (**Figure 3**). Pasteurization to denature residual enzymes, followed by fortification, gave >68% retention of ascorbic acid, which reflected nonenzymatic, concentration-independent destruction of ascorbic acid in the presence of anthocyanins. In nonpasteurized juices, PPO was suspected in co-oxidative reactions that caused 73 and 43% ascorbic acid loss at 100 and 500 mg/L ascorbic acid fortification, respectively. Greater retention at higher ascorbic acid concentrations was an indication of enzyme suppression keeping substrates in a reduced form. Additionally, the combination of enzyme action prior to pasteurization and degradation due to thermal pasteurization resulted in ascorbic acid losses exceeding 88% when fortified at 100 mg/L. These extreme ascorbic acid losses highlight the importance of enzyme inactivation prior to ascorbic acid fortification of muscadine grape juice for nutrient and quality retention. Processing and treatment parameters that allow for ascorbic acid fortification without phytochemical and quality

deterioration will be critical to future development and market expansion of muscadine grape products.

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