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Enhancing the Retention of Phytochemicals and Organoleptic Attributes in Muscadine Grape Juice through a Combined Approach between Dense Phase CO_2 Processing and Copigmentation

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This study evaluated the phytochemical stability and organoleptic attributes of an ascorbic acid-fortified muscadine grape juice as affected by dense phase CO_2 processing (DP-CO_2) and addition of thyme polyphenolic cofactors (*Thymus vulgaris*; 1:100 anthocyanin-to-cofactor molar ratio) in efforts to prevent phytochemical losses that occur during storage of anthocyanin-containing beverages, especially in the presence of carbonyl compounds commonly produced during thermal processing and storage. DP-CO_2 processing insignificantly altered initial juice phytochemical and antioxidant content, whereas thermal pasteurization reduced anthocyanins (263 mg/L), ascorbic acid (42 mg/L), soluble phenolics (266 mg/L), and antioxidant capacity (6 μM Trolox equivalents/mL). Similar trends were observed during storage, and data showed that increasing the CO_2 level from 8 to 16% during DP-CO_2 was instrumental in reducing juice phytochemical and antioxidant degradation. Copigmentation was instrumental in retaining higher anthocyanin, soluble phenolics, and antioxidant capacity during storage without affecting initial juice aroma and flavor characteristics. Moreover, on the basis of overall likeability scores, panelists preferred copigmented juices, which had increased juice color intensity and masked the detrimental color fading that occurred during storage, especially when compared to thermally pasteurized juices. DP-CO_2 and copigmentation were effective strategies to reduce phytochemical and color deterioration that occurred in muscadine juice during storage without affecting their organoleptic attributes.

KEYWORDS: Copigmentation; dense phase CO_2 processing; anthocyanins; ascorbic acid; sensory attributes

INTRODUCTION

Anthocyanins are polyphenolic compounds that are responsible for the bright blue and red colors of many foods and act as phytochemical antioxidants with potential health-related benefits (*1*−*3*). Recent shifts in consumer preference for natural pigments have focused on applications of anthocyanins as suitable replacements for colorants used in juices and beverages. However, their relatively high cost and generally poor stability during processing and storage are factors that limit their commercial application (*1*−*6*). Any strategy or technology that may serve to alleviate these limitations and improve the quality attributes of anthocyanin-containing products is of significant importance to the food industry.

Previous investigations have demonstrated that the formation of intermolecular copigmentation complexes (copigmentation) between anthocyanins and exogenously added polyphenolic cofactors offered a protection against anthocyanin, ascorbic acid, and antioxidant capacity degradation in both model and juice systems (*7*−*10*). Moreover, previous investigations (*11*) have revealed that water-soluble polyphenolic cofactors from thyme (*Thymus vulgaris L.*) were most efficacious for stabilizing anthocyanins under the highly oxidative conditions created by activation of residual polyphenol oxidase during high-pressure processing of muscadine grape juice. However, the stabilizing effects have not been evaluated in the absence of oxidase enzymes or throughout storage. Additionally, the sensory attributes imparted by polyphenolic cofactors, at levels at which they are effective for phytochemical retention, are an important consideration affecting their use in food systems.

A promising nonthermal processing technology that may help with phytochemical stability is the continuous dense phase CO_2 process (DP-CO_2). Without heat, a reduction in the formation of carbohydrate and ascorbic acid byproducts such as carbonyl compounds is realized, which has been identified as a key factor for preventing anthocyanin degradation in fruit juices (*6*, *12*, *13*), especially those containing ascorbic acid (*7*, *14*−*16*). DP-CO_2 is a continuous pasteurization technology that uses

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pressures ≤90 MPa in combination with dissolved carbon dioxide to inactivate microorganisms and that presumably protects thermolabile phytochemical and flavor compounds. Another benefit of this processing system is the removal of dissolved oxygen, which is instrumental in preventing degradation to antioxidant phytochemicals.

This cumulative knowledge suggests that the addition of polyphenolic cofactors from thyme along with the DP-CO_2 process may decrease phytochemical and antioxidant losses that occur during the storage of juices containing anthocyanins and ascorbic acid. Therefore, this study evaluated the phytochemical stability and organoleptic attributes of a DP-CO_2 processed muscadine grape juice as affected by the addition of ascorbic acid and water-soluble polyphenolic cofactors from thyme. The poor thermal resistance and storage stability of the anthocyanins present in muscadine juice (3,5-diglucosides) make this juice a good model for studying alternative processing regimens and strategies to enhance anthocyanin stability. Results were compared to juices processed by thermal pasteurization to assess the differences between the processing methods and to observe if phytochemical degradation was increased by the formation of compounds created during thermal processing.

MATERIALS AND METHODS

Materials and Processing. Polyphenolics from dried thyme leaves (McCormick & Co., Inc., Hunt Valley, MD) were exhaustively extracted with hot water, purified using reverse phase C18 Sep-Pak Vac 20 cm^3 minicolumns (Water Corp., Milford, MA), and redissolved in 0.1 M citric acid solution as described by Del Pozo-Insfran et al. (*11*). Purified spring water (Publix, Lakeland, FL) and 100% food-grade ethanol (McCormick Distillery Co., Weston, MO) were used to purify and/or solubilize the isolated polyphenolic cofactors.

Red muscadine grapes (cv. Noble) were obtained from a local grower and hand-sorted for uniformity. Fruit was crushed and heated to 75 °C for 2 min in an open steam kettle and the juice extracted using a hydraulic basket press (Prospero's Equipment, Cort, NY). Preliminary investigations concluded that this heat treatment completely inactivated oxidase enzymes. Juice was subsequently filtered first through cheesecloth followed by vacuum filtration through a 1 cm bed of diatomaceous earth. The resultant juice was then divided into two portions for the addition of water-soluble polyphenolic cofactors from thyme at a 1:100 anthocyanin-to-cofactor molar ratio compared to a 100% juice control. The ratio corresponded to the molar concentration of total anthocyanins present in the juice and the molar concentration of total soluble phenolics present in the thyme extract in gallic acid equivalents. Copigmented and control juices were again divided into two portions, and half was fortified with ≈450 mg/L ascorbic acid as opposed to an equivalent volume of citric acid buffer as a control. The four treatments were then partitioned into equal portions for heat (HTST; 75 °C for 15 s) and DP-CO_2 pasteurization (34.5 MPa at 8 and 16% CO_2, both using a constant residence time and temperature of 6.25 min and 30 °C, respectively). The DP-CO_2 regimens were confirmed to impart >5 log reduction of aerobic microorganisms and yeast/mold according to preliminary investigations. After pasteurization, each juice treatment was divided into portions for microbial, phytochemical, and organoleptic evaluations. Samples for microbial and phytochemical analysis were immediately transferred into 20 mL screwed-cap vials and stored at 4 °C for 10 weeks, whereas samples for sensory analysis were transferred to sterile 4 L glass containers. Samples used exclusively for phytochemical assessment were dosed with sodium azide (50 mg/L) to retard microbial growth.

Physicochemical and Microbial Analyses. Individual anthocyanin 3,5-diglycosides were quantified by reverse phase HPLC using modified chromatographic conditions described by Del Pozo-Insfran et al. (*17*). 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). Mobile phases consisted of 100% acetonitrile (phase A) and water containing 10% acetic acid, 5% acetonitrile, and 1% phosphoric acid (phase B). A gradient solvent program ran phase B from 100 to 88% in 8 min, from 88 to 50% in 2 min, and held for 12 min at a flow rate of 1.8 mL/min. Anthocyanins were characterized on the basis of PDA spectral interpretation from 200 to 600 nm, comparison to authentic standards (Polyphenols Laboratories AS), and identification additionally confirmed following acid hydrolysis into their respective aglycones with 2 N HCl in 50% v/v methanol for 60 min at 90 °C.

Total ascorbic acid (the sum of L- and dehydro-ascorbic acid) was quantified by reverse phase HPLC using the conditions described by Brenes et al. (*8*). Antioxidant capacity was determined using the oxygen radical absorbance capacity (ORAC) assay with data expressed in Trolox equivalents per milliliter (μM TE/mL) as previously described (*18*). Total soluble phenolic concentrations were measured using the Folin−Ciocalteu assay (*18*) and quantified as gallic acid equivalents (GAE). Instrumental CIE color characteristics including lightness (L^*), chroma value, and hue angle were measured using a Minolta Chroma Meter CR-300 series (Minolta Co., Ltd., Osaka, Japan). pH was measured using a Thermo Orion model 720 pH-meter (Thermo Electron Corp., New Haven, CT). Total titratable acidity was determined by potentiometric titration against 0.1 N NaOH to pH 8.2 using an automatic titrator (Fisher Titrimeter II, Pittsburgh, PA) and expressed in tartaric acid equivalents. CO_2 content in the juices was determined using an Orion CO_2 electrode (Thermo Electron Corp.).

Sensory Evaluation. Flavor, aroma, and color intensity of juices with and without added polyphenolic cofactors were compared using a difference-from-control test for each of the three pasteurization treatments (DP-CO_2 at 8 and 16% CO_2, and HTST). Panelists compared the sensory attributes of the reference (no added cofactors) with those of a hidden reference and the copigmented juice. A randomized complete block design was used, and differences from control measurements were recorded on a line scale with anchors at 0 and 10 that represented "no difference" to "extremely different" in juice sensory attributes. Each panelist evaluated all three combinations of pasteurized juice. A nine-point hedonic scale was also used to compare the overall likeability of the reference and copigmented juices processed by each processing regimen.

Before sensory analysis, all juices were degassed to equalize carbon dioxide levels by placing them in a 4 L sterile glass container on a stir plate with continuous agitation for 4 h at 20 °C. Juices were then served at room temperature in randomly numbered plastic cups. A tray with a cup of water and nonsalted crackers was also given to each panelist. All sensory tests were performed at the University of Florida's taste panel facility using 60 untrained panelists (33 females, 18−29 year age range).

Statistical Analysis. Data represent the mean and standard error of juices analyzed as a 3 × 2 × 2 × 9 factorial comparing three processing conditions (DP-CO_2 at 8 or 16%, both at 34.5 MPa, or HTST), with and without thyme cofactors, with and without ascorbic acid, and evaluated at nine sampling points (unprocessed, processed, and weeks 1, 2, 3, 4, 6, 8, and 10). Linear regression, Pearson correlations, and analysis of variance were conducted using JMP software (SAS, Cary, NC), with mean separation performed using the LSD test ($P < 0.05$). All experiments were randomized and conducted in triplicate. Sensory data were recorded and analyzed using Compusense five (Compusense, Guelph, ON, Canada), and analysis of variance was conducted using Tukey's multiple-comparisons method ($P < 0.05$).

RESULTS AND DISCUSSION

This study investigated phytochemical and organoleptic changes in muscadine grape juice following DP-CO_2 pasteurization and the addition of polyphenolic cofactors from thyme as a means to reduce oxidative and themal degradation commonly associated with anthocyanin-containing juices and beverages that are commonly fortified with L-ascorbic acid. Significant differences in anthocyanins, soluble phenolics, antioxidant capacity, and organoleptic attributes were observed by processing methods, copigmentation, and ascorbic acid fortification. Thermal pasteurization was more detrimental to polyphenolics, antioxi-

Table 1. Effect of Thyme Cofactors (0 and 1:100 Anthocyanin-to-Cofactor Ratios) and Ascorbic Acid Fortification (0 and 450 mg/L) on the Total Anthocyanin, Soluble Phenolic, and Antioxidant Contents of Unprocessed and Heat (HTST; 75 °C, 15 s), and DP-CO_2 (34.5 MPa at 8 or 16% CO_2) Pasteurized Muscadine Grape Juice

		no ascorbic acid			added ascorbic acid (450 mg/L)			
treatment	cofactor ratio[a]	total anthocyanins[b] (mg/L)	soluble phenolics[c] (mg/L)	antioxidant capacity[d] (μM TE/mL)	total anthocyanins (mg/L)	soluble phenolics (mg/L)	antioxidant capacity (μM TE/mL)	total ascorbic acid (mg/L)
unprocessed	0	1110 a[e]	2121 c	23.9 a	1210 a*[f]	2579 c*	31.9 c*	441 a
	1:100	1175 a	2780 a	50.9 a	1219 a	2955 a*	59.4 a*	445 a
34.5 MPa, 8% CO_2	0	1090 a	2118 c	23.2 c	1120 a	2569 c*	31.3 c*	428 a
	1:100	1078 a	2661 b	48.0 a	1185 a*	2774 b	58.1 a*	434 a
34.5 MPa, 16% CO_2	0	1103 a	2121 c	24.7 c	1194 a*	2531 c*	35.3 c*	434 a
	1:100	1101 a	2753 a	46.4 a	1208 a*	2862 a	61.1 a*	432 a
HTST (75 °C, 15 s)	0	843 b	1754 d	17.7 d	997 c*	1968 e*	26.1 d*	399 c
	1:100	865 b	2003 c	35.9 b	1101 b*	2136 d*	50.1 b*	416 b

[a] Indicates the ratio between the molar concentration of total anthocyanins in muscadine grape juice and the molar concentration of thyme polyphenolic cofactors (expressed in gallic acid equivalents). [b] Sum of individual anthocyanin 3,5-diglucosides quantified by HPLC. [c] Expressed in gallic acid equivalents. [d] Expressed in Trolox equivalents (TE). [e] Means with similar letters within columns are not significantly different (LSD test, $P > 0.05$). [f] Means with an asterisk (*) for each response variable indicate a significant effect (LSD test, $P < 0.05$) due to the addition of ascorbic acid.

dant capacity, and organoleptic attributes as compared to the DP-CO_2 processes. Moreover, enhanced storage stability was observed for DP-CO_2 processed juices in relation to thermal pasteurization. In addition to reducing phytochemical and antioxidant losses, copigmentation increased the anthocyanin color intensity and antioxidant content of the juices and also masked the detrimental color fading that took place during storage of non-copigmented juices. L-Ascorbic acid fortification increased the initial polyphenolic and antioxidant concentrations of the juices, likely due to its reducing and antioxidant properties; however, its addition eventually resulted in decreased polyphenolic and antioxidant retention during storage.

Initial Effects of Copigmentation and Ascorbic Acid Fortification. Preliminary investigations indicated that the hyperchromic intensity (red coloration) of muscadine juice could be increased up to 5-fold by the addition of polyphenolic cofactors isolated from thyme in a 1:400 ratio. However, due to adverse bitter and astringent flavors at such high cofactor concentrations, this study evaluated juices at a 1:100 ratio on the basis of initial and informal sensory evaluations of threshold concentrations.

The anthocyanin content of the juices was not affected by the addition of the cofactors but was initially protected by the addition of L-ascorbic acid (**Table 1**) with 100 mg/L higher concentrations than the control juices, likely due to its antioxidant protection. Polyphenolic cofactor addition increased the initial content of soluble phenolics by 660 mg/L and the antioxidant capacity by 30 μM TE/mL, concentrations that were additionally increased by 175 mg/L and 9 μM TE/mL following L-ascorbic acid fortification, respectively (**Table 1**), due to the strong metal-reducing and antioxidant properties of these phytochemicals. Cofactors also increased the visual color of the juice as evidenced in a decline in hue angle from 19.2 to 10.2, which appears to the naked eye as a brighter tone of red color.

Phytochemical Changes Due to Thermal and DP-CO_2 Processing. The DP-CO_2 processes insignificantly altered juice phytochemical and antioxidant contents (**Table 1**), whereas thermal pasteurization reduced anthocyanins by 263 mg/L, soluble phenolics by 366 mg/L, L-ascorbic acid by 42 mg/L, and antioxidant capacity by 6 μmol of TE/mL.

DP-CO_2 did not affect juice pH (3.2) or titratable acidity (0.57 mequiv of tartaric acid/mL), although previous studies (*19*−*23*) have reported pH changes due to the formation of carbonic acid in aqueous systems pressurized with CO_2. The insignificant changes in juice pH observed in this study might be attributed to the low concentrations of CO_2 dissolved in the juices that resulted when this gas was stripped from the juice by vacuum during the final stage of processing. Residual CO_2 content was 6.74 and 9.75 mM for juices pressurized at 8 and 16% CO_2 levels, respectively.

Copigmentation and ascorbic acid fortification did not reduce phytochemical and antioxidant losses during thermal pasteurization, but generally helped to retain higher levels of anthocyanins, phenolics, and antioxidant capacity when compared to control treatments. Copigmented treatments contained higher soluble phenolic (249 mg/L; **Table 1**), antioxidant capacity (18 μM TE/mL), and ascorbic acid (17 mg/L) contents than control treatments, whereas ascorbic acid fortified juices presented higher anthocyanin (154 mg/L), phenolic (214 mg/L), and antioxidant (8 μM TE/mL) contents than heat-pasteurized control juices. The combined addition of ascorbic acid and thyme cofactors synergistically acted to protect phytochemicals and antioxidant levels of thermally pasteurized juices, as evidenced by the additional retention of these compounds following thermal processing.

Organoleptic Changes Due to the Addition of Thyme Polyphenolic Cofactors. For each processing treatment (DP-CO_2 or HTST), panelists compared the sensory attributes of the reference juice (no added cofactors) with those presented by the hidden reference and the copigmented juice. The hidden reference was used to determine if the consumer was in fact detecting a difference between the juices from each process and, if so, to determine the extent of organoleptic differences between the juices. Generally, the addition of polyphenolic cofactors insignificantly affected the flavor and aroma of DP-CO_2 and HTST processed muscadine grape juices (**Table 2**). The only significant difference (<1 unit on a 10-point scale) that was detected by the panelists was between the aroma of the reference and the copigmented juice processed by DP-CO_2 at 34.5 MPa and 8% CO_2; however, these two juices had similar ratings for overall likeability. Copigmented juices received higher panel scores for overall likeability than control juices processed by heat and DP-CO_2 at 16% CO_2. Juice color intensity was the only organoleptic trait for which panelists were able to detect a difference between control and copigmented juices and was attributed to the color-enhancing properties of thyme polyphenolic cofactors. The copigmented juices were consistently rated to have a more intense red color compared to their corresponding control juices. In addition, copigmentation served to mask the detrimental color changes that occurred during thermal process-

Table 2. Differences in Organoleptic Attributes (Color Intensity, Aroma, and Flavor) and Overall Likeability between Non-copigmented (Reference and Hidden Reference) and Copigmented Muscadine Grape Juice Processed by Heat (75 °C for 15 s) and DP-CO_2 (34.5 MPa at 8 or 16% CO_2) As Detected by Untrained Panelists ($n = 60$)

processing treatment	copigmentation treatment	difference in color intensity[a]	difference in aroma[a]	difference in flavor[a]	overall likeability
34.5 MPa, 8% CO_2	hidden reference	1.47^{NS} a[c]	2.07^{NS} a	3.77 ^{NS} a	6.28 a
	cofactors (1:100)[b]	5.04* b	2.98* b	4.08^{NS}a	6.05 a
34.5 MPa, 16% CO_2	hidden reference	1.13^{NS} a	1.44^{NS} a	2.53 ^{NS} a	5.65 a
	cofactors (1:100)	4.98* b	2.06^{NS} a	3.70^{NS} a	7.27 b
HTST (75 °C, 15 s)	hidden reference	1.97^{NS} a	1.67^{NS} a	2.96 ^{NS} a	3.33 a
	cofactors (1:100)	3.88* b	1.94^{NS} a	3.52^{NS} a	4.55 b

[a] Mean difference observed when compared to the given reference (non-copigmented; difference from control test). [b] Cofactor ratio indicates the ratio between the molar concentration of total anthocyanins in muscadine grape juice and the molar concentration of thyme polyphenolic cofactors (expressed in gallic acid equivalents). [c] Values with similar letters within columns of similar processing treatment (HTST or DP-CO_2) are not significantly different (Tukey's HSD, $P > 0.05$); NS and * indicate nonsignificant or significant (Tukey's HSD, $P < 0.05$) difference when compared to the given juice reference (non-copigmented), respectively.

Table 3. Effect of Thyme Cofactors (0 and 1:100 Anthocyanin-to-Cofactor Ratios) and Ascorbic Acid Fortification (0 and 450 mg/L) on First-Order Degradation Kinetic Parameters of Anthocyanins Present in Heat (HTST) or DP-CO_2 (34.5 MPa at 8 or 16% CO_2) Pasteurized Muscadine Grape Juice during Storage at 4 °C

		no ascorbic acid			added ascorbic acid (450 mg/L)		
treatment	cofactor ratio[a]	β_1[b]	$t_{1/2}$[c]	R^2	β_1	$t_{1/2}$	R^2
34.5 MPa, 8% CO_2	0	7.07 a[d]	98.1 c	0.99	17.8 c*[e]	39.0 c*	0.95
	1:100	6.49 a	107 b	0.96	12.6 b	55.0 b*	0.98
34.5 MPa, 16% CO_2	0	7.37 a	94.1 c	0.96	13.9 b*	50.0 b*	0.98
	1:100	5.50 b	126 a	0.98	10.6 a*	65.3 a*	0.98
HTST (75 °C, 15 s)	0	15.8 d	43.9 e	0.90	21.3 d*	32.5 c*	0.95
	1:100	10.3 c	67.2 d	0.99	12.7 b*	54.4 b*	0.98

[a] Indicates the ratio between the molar concentration of total anthocyanins in muscadine grape juice and the molar concentration of thyme polyphenolic cofactors (expressed in gallic acid equivalents). [b] Indicates the degradation rate (slope, β_1) of anthocyanins (days^{-1}). [c] Indicates the half-life (days) of initial anthocyanin content. [d] Means with similar letters within columns are not significantly different (LSD test, $P > 0.05$). [e] Means with an asterisk (*) for each kinetic parameter indicate a significant effect (LSD test, $P < 0.05$) due to the addition of ascorbic acid when compared to the same treatment without ascorbic acid.

Table 4. Effect of Thyme Cofactors (0 and 1:100 Anthocyanin-to-Cofactor Ratios) and Ascorbic Acid (0 and 450 mg/L) on First-Order Degradation Kinetic Parameters of Soluble Phenolics in Heat (HTST) or DP-CO_2 (34.5 MPa at 8 or 16% CO_2) Processed Muscadine Grape Juice during Storage at 4 °C

		no ascorbic acid			added ascorbic acid (450 mg/L)		
treatment	cofactor ratio[a]	β_1[b]	$t_{1/2}$[c]	R^2	β_1	$t_{1/2}$	R^2
34.5 MPa, 8% CO_2	0	25.1 c[d]	27.6 c	0.91	38.2 c*[e]	18.1 c*	0.99
	1:100	10.9 a	63.6 a	0.98	24.3 ab*	28.5 ab*	0.98
34.5 MPa, 16% CO_2	0	19.2 b	36.1 b	0.97	21.9 a	31.7 a*	0.86
	1:100	10.3 a	67.2 a	0.92	26.5 b*	26.2 b*	0.97
HTST (75 °C, 15 s)	0	36.2 d	19.1 d	0.97	52.6 d*	13.2 d	0.98
	1:100	14.5 b	47.9 b	0.87	25.0 b*	27.8 b*	0.97

[a] Indicates the ratio between the molar concentration of total anthocyanins in muscadine grape juice and the molar concentration of thyme polyphenolic cofactors (expressed in gallic acid equivalents). [b] Indicates the degradation rate (slope, β_1) of soluble phenolics (days^{-1}). [c] Indicates the half-life (days) of initial soluble phenolics content. [d] Means with similar letters within columns are not significantly different (LSD test, $P > 0.05$). [e] Means with an asterisk (*) for each kinetic parameter indicate a significant effect (LSD test, $P < 0.05$) due to the addition of ascorbic acid when compared to the same treatment without cofactors.

ing, as insignificant changes in hue values were observed for copigmented juices compared to an appreciable color fade in control juices. Although panelists did not evaluate in parallel

Table 5. Effect of Thyme Cofactors (0 and 1:100 Anthocyanin-to-Cofactor Ratios) and Ascorbic Acid Fortification (0 and 450 mg/L) on First-Order Degradation Kinetic Parameters of Antioxidant Capacity in Heat (HTST) or DP-CO_2 (34.5 MPa at 8 or 16% CO_2) Pasteurized Muscadine Grape Juice during Storage at 4 °C

		no ascorbic acid			added ascorbic acid (450 mg/L)		
treatment	cofactor ratio[a]	β_1[b]	$t_{1/2}$[c]	R^2	β_1	$t_{1/2}$	R^2
34.5 MPa, 8% CO_2	0	9.55 c[d]	72.6 c	0.93	21.2 c*[e]	32.6 d*	0.98
	1:100	8.54 b	81.1 b	0.99	7.64 a	90.7 a	0.97
34.5 MPa, 16% CO_2	0	7.37 a	94.0 a	0.97	15.4 b*	45.0 c*	0.95
	1:100	7.70 a	90.3 a	0.98	7.90 a	87.6 a	0.98
HTST (75 °C, 15 s)	0	13.0 d	53.4 d	0.94	31.6 d*	21.9 e*	0.95
	1:100	14.2 d	49.0 d	0.87	11.0 b	62.8 b	0.98

[a] Indicates the ratio between the molar concentration of total anthocyanins in muscadine grape juice and the molar concentration of thyme polyphenolic cofactors (expressed in gallic acid equivalents). [b] Indicates the degradation rate (slope, β_1) of antioxidant capacity (days^{-1}). [c] Indicates the half-life (days) of initial antioxidant capacity content. [d] Means with similar letters within columns are not significantly different (LSD test, $P > 0.05$). [e] Means with an asterisk (*) for each kinetic parameter indicate a significant effect (LSD test, $P < 0.05$) due to the addition of ascorbic acid when compared to the same treatment without ascorbic acid.

Table 6. Effect of Thyme Cofactors (0 and 1:100 Anthocyanin-to-Cofactor Ratios) on First-Order Degradation Kinetic Parameters of Total Ascorbic Acid Present in Heat (HTST) or DP-CO_2 (34.5 MPa at 8 or 16% CO_2) Pasteurized Muscadine Grape Juice during Storage at 4 °C

	without thyme cofactors			with thyme cofactors[a]		
treatment	β_1[b]	$t_{1/2}$[c]	R^2	β_1	$t_{1/2}$	R^2
34.5 MPa, 8% CO_2	57.8 b[d]	12.0 b	0.98	42.9 a*[e]	16.2 a*	0.95
34.5 MPa, 16% CO_2	46.8 a	14.8 a	0.96	43.1 a	16.1 c*	0.96
HTST (75 °C, 15 s)	152 c	4.62 c	0.83	92.5 b*	7.51 b*	0.97

[a] Indicates the ratio between the molar concentration of total anthocyanins in muscadine grape juice and the molar concentration of thyme polyphenolic cofactors (expressed in gallic acid equivalents). [b] Indicates the degradation rates (β_1) of total ascorbic acid (days^{-1}). [c] Indicates the half-life (days) of initial total ascorbic acid content (450 mg/L). [d] Means with similar letters within columns are not significantly different (LSD test, $P > 0.05$). [e] Means with an asterisk (*) for each kinetic parameter indicate a significant effect (LSD test, $P < 0.05$) due to the addition of thyme cofactors when compared to the same treatment without ascorbic acid.

the organoleptic attributes of juices processed by the different pasteurization regimes, results indicated that DP-CO_2 juices received higher panel scores for overall acceptability (>2 units) than heat-pasteurized juices, indicating a higher preference for DP-CO_2 processed juices.

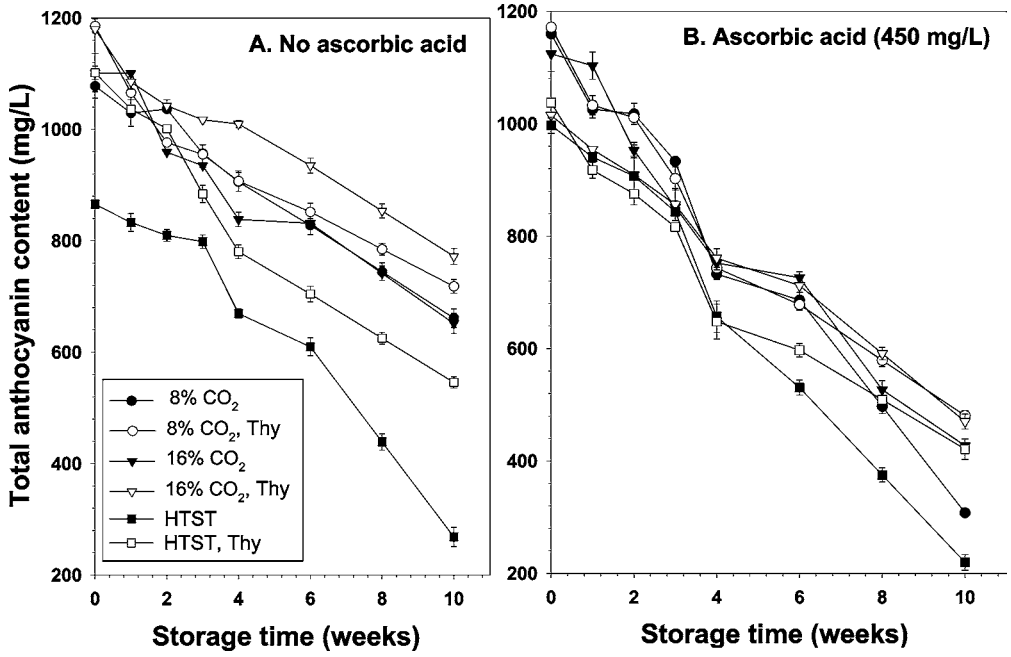

Figure 1. Total anthocyanin content of muscadine grape juice without (**A**) and with ascorbic acid (**B**; 450 mg/L) as affected by heat (HTST; 75 °C, 15 s) and DP-CO_2 (34.5 MPa at 8 or 16% CO_2) pasteurization and the addition of thyme cofactors (Thy; 0 and 1:100 anthocyanin-to-cofactor ratios). (*1*) Sum of individual anthocyanin 3,5-diglucosides quantified by HPLC.

Phytochemical Changes during Refrigerated Storage. Thermal processing and ascorbic acid fortification were the variables that most affected the polyphenolic and antioxidant capacity levels of muscadine juice throughout storage at 4 °C, whereas the DP-CO_2 processes and copigmentation helped to increase phytochemical and antioxidant retention of the juices. The effects of each independent variable (processing method, copigmentation, or ascorbic acid fortification) and its ability to alter phytochemical degradation over time were evaluated on the basis of the degradation rates calculated according to the method of Taoukis et al. (*24*). Regression analysis concluded that rates of anthocyanin, soluble phenolics, antioxidant capacity, and ascorbic acid degradation over time followed first-order degradation kinetics (**Tables 3**−**6**), in agreement with previous studies on phytochemical degradation (*3*, *8*, *14*, *17*).

Independent of CO_2 levels, DP-CO_2 juices retained 386 mg/L higher anthocyanin content (**Figure 1**) and presented a 2-fold lower anthocyanin degradation rate (**Table 3**) than thermally pasteurized juices after 10 weeks of storage at 4 °C. This marked difference was attributed to the formation of carbohydrate and/or ascorbic acid degradation byproducts during thermal processing that subsequently accelerated anthocyanin degradation during storage to yield brown, polymerized pigments that negatively affected juice quality (*6*, *12*, *13*). Comparison of kinetic parameters among treatments indicated that ascorbic acid fortification promoted anthocyanin degradation for all processing treatments, confirming the destructive interaction between these compounds in a food system (*8*, *14*−*16*); however, its addition had a more pronounced effect for DP-CO_2 juices (3.5-fold increase in degradation rates) than for heat-pasteurized juices (1.4-fold). This possibly occurred due to the slower rates of degradation initially observed for DP-CO_2 juices when compared to the significantly higher degradation rates and oxidative conditions that existed in thermally processed juices.

Addition of thyme cofactors reduced anthocyanin degradation rates by 1.5-fold (**Table 4**), extended anthocyanin half-life values from 9 to 32 days, and retained higher anthocyanin concentrations than controls from 57 to 278 mg/L after 10 weeks of storage (**Figure 1**), in a manner independent of processing method or L-ascorbic acid fortification. For noncopigmented juices processed by DP-CO_2, the CO_2 concentration had no effect on anthocyanin storage stability. However, in copigmented juices the increase from 8 to 16% inhibited anthocyanin degradation by 1.4-fold and increased anthocyanin retention by >63 mg/L by the end of storage (**Figure 1**). This trend was also observed for ascorbic acid fortified juices and likely occurred due to the protection of polyphenolic compounds present both in the juice and in the thyme extract by oxygen exclusion. Dissolved oxygen is known to significantly increase the rate and sequence of polyphenolic and/or L-ascorbic acid degradation that produce quinones or carbonyl compounds that react and accelerate anthocyanin degradation (*6*, *12*, *13*). Results also suggested that the prevention and/or reduction of compounds such as furfural formed during thermal processing and storage might be an important approach to attenuate anthocyanin degradation that can be obtained by nonthermal processing methods such as DP-CO_2. Anthocyanin losses were correlated to instrumental color evaluations ($r = 0.84$) that showed slight changes in hue values for DP-CO_2 processed juices during storage, whereas thermally processed juices showed a prominent loss of red color. As previously discussed, results from color analysis indicated that cofactor addition served to mask the detrimental color fading that occurred throughout storage.

Copigmented juices presented markedly higher concentrations of soluble phenolics and antioxidant capacity than control juices during storage (**Figures 2** and **3**), even in the presence of L-ascorbic acid, which was detected as interference in the Folin−Ciocalteu assay. Similar to results for anthocyanins, the rate of degradation for soluble phenolics and antioxidant capacity (**Tables 4** and **5**, respectively) was more pronounced for heat-pasteurized juices followed by the DP-CO_2 process at 8% CO_2 and, finally, the DP-CO_2 process at 16% CO_2. Increases in CO_2

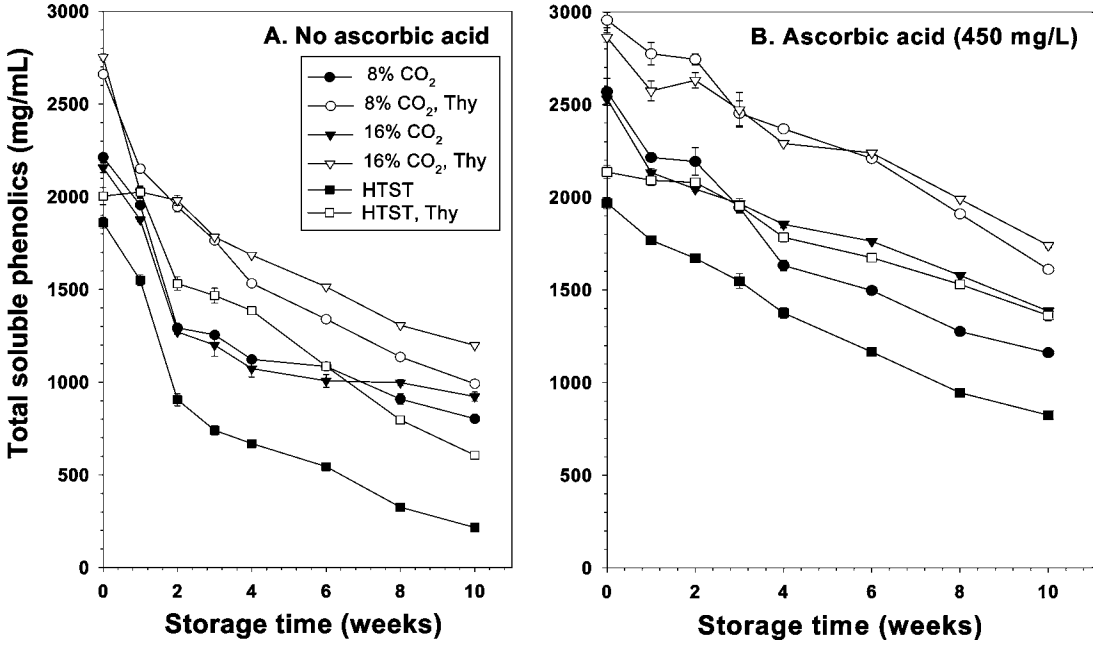

Figure 2. Levels of soluble phenolics (in gallic acid equivalents) in muscadine grape juice without (**A**) and with ascorbic acid (**B**; 450 mg/L) as affected by heat (HTST; 75 °C, 15 s) and DP-CO_2 (34.5 MPa at 8 or 16% CO_2) pasteurization and the addition of thyme cofactors (Thy; 0 and 1:100 anthocyanin-to-cofactor ratios).

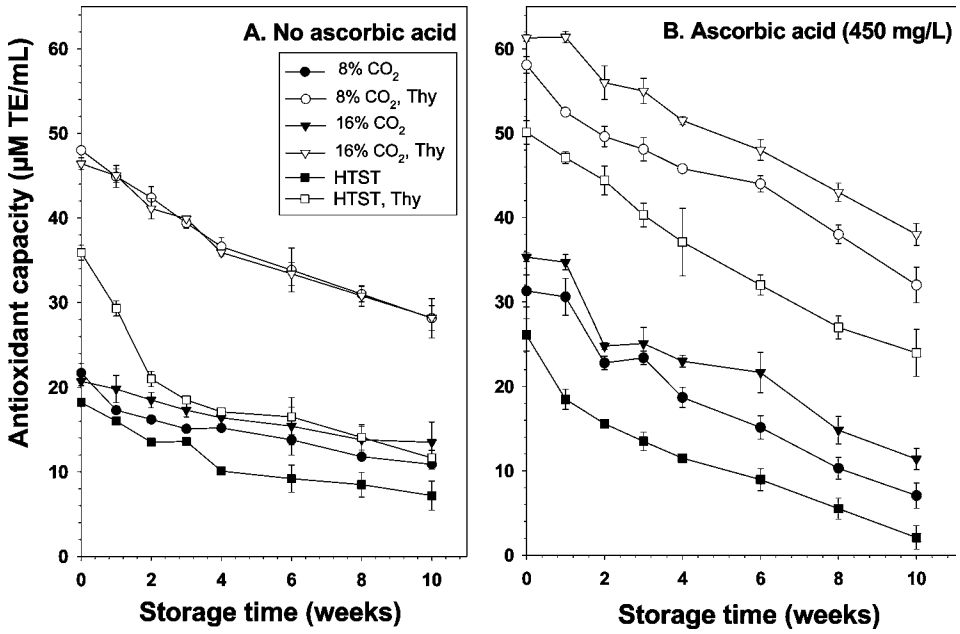

Figure 3. Antioxidant capacity (in Trolox equivalents; TE) of muscadine grape juice without (**A**) and with ascorbic acid (**B**; 450 mg/L) as affected by heat (HTST; 75 °C, 15 s) and DP-CO_2 (34.5 MPa at 8 or 16% CO_2) pasteurization and the addition of thyme cofactors (Thy; 0 and 1:100 anthocyanin-to-cofactor ratios).

concentrations from 8 to 16% during DP-CO_2 offered enhanced storage stability for antioxidant capacity, soluble phenolics, and total ascorbic acid (**Figures 2**−**4**), indicating that exclusion of dissolved oxygen was instrumental for phytochemical retention during storage. Addition of thyme cofactors reduced rates of soluble phenolic degradation by 2-fold for all treatments independent of ascorbic acid concentration (**Table 4**), a protective effect that was also observed for losses in antioxidant capacity for L-ascorbic acid fortified juices (**Table 5**). Addition of ascorbic acid generally increased the rates of soluble phenolics and antioxidant capacity degradation by 2-fold when compared to unfortified control treatments. Ascorbic acid addition also negatively affected copigmented treatments; however, juices with added cofactors showed enhanced stability compared with their respective non-copigmented counterparts as evidenced by their smaller degradation rates and higher half-life values.

Similar to trends observed for polyphenolics and antioxidant capacity, heat-pasteurized juices presented 3-fold faster rates of total ascorbic acid degradation when compared to DP-CO_2 counterparts, rates that were reduced to 2-fold by the addition of thyme cofactors (**Table 6**). Copigmentation also reduced ascorbic degradation for the juice processed at 34.5 MPa and 8% CO_2. Increasing processing CO_2 levels from 8 to 16%

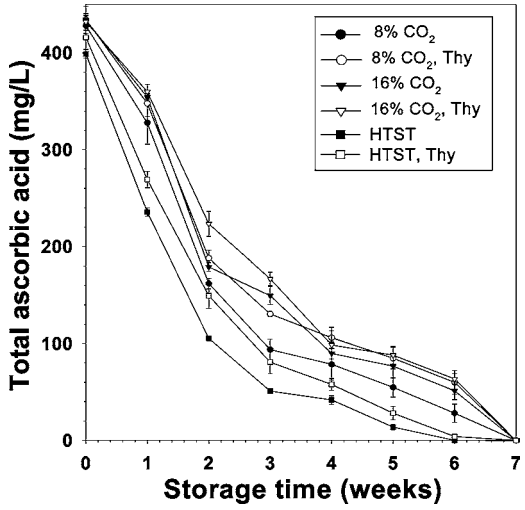

Figure 4. Total ascorbic acid (sum of L-ascorbic and dehydroascorbic acid) content of muscadine grape juice as affected by heat (HTST; 75 °C, 15 s) and DP-CO_2 (34.5 MPa at 8 or 16% CO_2) pasteurization and the addition of thyme cofactors (Thy; 0 and 1:100 anthocyanin-to- cofactor ratios).

offered enhanced storage stability for ascorbic acid, suggesting that losses of this phytonutrient can be prevented by a reduction of the oxygen content in food systems as reported by Poei-Langston and Wrolstad in anthocyanin-containing juices (*16*). These results, along with the trends observed for soluble phenolics, suggest that anthocyanin destruction occurs independently of oxygen content in the juice matrix, whereas polyphenolic and ascorbic acid degradation was likely linked to the presence of oxygen. Copigmented DP-CO_2 juices contained significantly higher ascorbic acid content than heat-pasteurized controls after 6 weeks of storage, independent of processing parameters. However, no ascorbic acid was detected in any of the juices after the seventh week of storage.

The results of this study showed that DP-CO_2 and the addition of thyme polyphenolic cofactors served to protect phytochemical and antioxidant levels in muscadine juice throughout storage without compromising the organoleptic attributes of the juice. These findings are of significant relevance for the food industry, because both strategies could be used to reduce the phytochemical and color deterioration that occur in anthocyanin-containing beverages without affecting their sensory characteristics, especially in the presence of ascorbic acid. In addition to preventing anthocyanin and ascorbic acid losses, copigmentation was shown to be an effective strategy to increase the color intensity of muscadine juice and mask the detrimental color fading that occurred during storage, parameters that along with antioxidant content are significant factors affecting consumer acceptability and preference.

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