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Microbial Stability, Phytochemical Retention, and Organoleptic Attributes of Dense Phase CO_2 Processed Muscadine Grape Juice

DAVID DEL POZO-INSFRAN, MURAT O. BALABAN, AND STEPHEN T. TALCOTT*

Department of Food Science and Human Nutrition, University of Florida, P.O. Box 110370, Gainesville, Florida 32611-0370

Dense phase CO_2 processing (DP-CO_2) is a promising alternative to thermal pasteurization potentially inactivating microorganisms without affecting food phytochemicals or organoleptic characteristics. To demonstrate these effects, studies were conducted by changing processing pressure and CO_2 concentration in relation to microbial destruction. Subsequent storage stability (10 weeks at 4 °C) of muscadine grape juice processed by DP-CO_2 (34.5 MPa at 8% or 16% CO_2) was evaluated and compared to a heat-pasteurized juice (75 °C, 15 s). Thermal pasteurization decreased anthocyanins (16%), soluble phenolics (26%), and antioxidant capacity (10%) whereas no changes were observed for both DP-CO_2 juices. DP-CO_2 juices also retained higher anthocyanins (335 mg/L), polyphenolics (473 mg/L), and antioxidant capacity (10.9 μmol of Trolox equivalents/mL) than thermally pasteurized juices at the end of storage. Insignificant differences in sensory attributes (color, flavor, aroma, and overall likeability) were observed between unprocessed and DP-CO_2 juices, while significant differences were observed between unprocessed and heat-pasteurized juices. Panelists preferred DP-CO_2 over heat-pasteurized juices throughout the first 6 weeks of storage, whereby the growth of yeast and mold adversely affected the juice aroma. Comparable microbial counts were observed between DP-CO_2 and thermally pasteurized juices during the first 5 weeks of storage. DP-CO_2 protected phytochemicals in muscadine juice during processing and storage without compromising microbial stability or sensory attributes over 5 weeks of storage.

KEYWORDS: Dense phase CO_2 processing; anthocyanins; polyphenolics; sensory attributes; microbial inactivation

INTRODUCTION

Dense phase CO_2 processing (DP-CO_2) is a continuous, nonthermal processing system for liquid foods that utilizes pressure (<90 MPa) in combination with carbon dioxide (CO_2) to destroy microorganisms as a means of food preservation. Numerous studies have investigated the efficacy of pressurized CO_2 and supercritical CO_2 (SC-CO_2) to inactivate microorganisms and enzymes in batch or semicontinuous systems (*1*−*8*). However, information relating to deleterious changes in color and phytochemicals during processing and storage is generally lacking, especially for continuous processing systems (*9*, *10*). Of particular importance to our research group is the prevention of deleterious changes in color and phytochemicals during processing and storage of muscadine grape products. DP-CO_2 is a novel processing technology that could potentially reduce microbial loads without negatively affecting phytochemicals or sensory characteristics of muscadine grape products and other liquid foods. To prove the effectiveness of DP-CO_2 processing as a novel food processing technology, the microbial destruction, phytochemical stability, and sensory attributes of DP-CO_2 processed muscadine grape juice was compared to a thermally pasteurized juice (HTST; 75 °C, 15 s). Treatments were additionally evaluated following storage for 10 weeks at 4 °C. Results of this study demonstrated differences between microbial, phytochemical, and sensory attributes of juices processed by DP-CO_2 and thermal processing, parameters that are of significant importance to assess the benefits offered by novel processing technologies.

MATERIALS AND METHODS

Materials. Muscadine grapes (*Vitis rotundifolia* cv. Noble) were obtained from a local grower in central Florida and held frozen (−20 °C) until needed. Fruit was rapidly thawed by placing them under running tap water and hand-sorted for uniformity of ripeness. Grapes were then crushed and heated to 75 °C for 2 min in an open steam kettle to facilitate juice extraction, and the juice was subsequently extracted using a hydraulic basket press (Prospero's Equipment, Cort, NY). Preliminary investigations demonstrated that this juice extraction method was sufficient to inactivate oxidase enzymes. The juice was immediately filtered through cheesecloth followed by vacuum filtration through a 1 cm bed of diatomaceous earth.

* To whom correspondence should be addressed. Tel: (352) 392-1991. Fax: (352) 392-9467. E-mail: sttalcott@ifas.ufl.edu.

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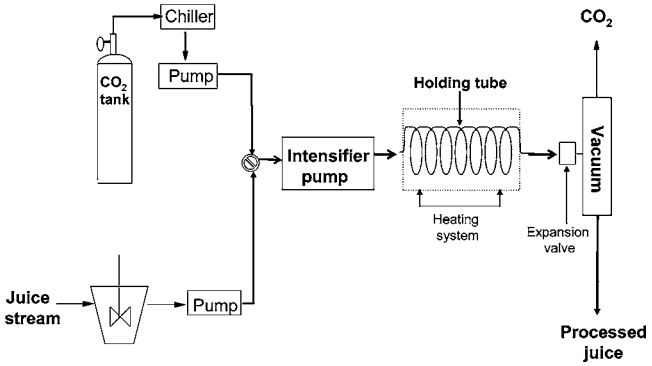

Figure 1. Schematic diagram of the DP-CO_2 processing equipment.

Processing Equipment. The DP-CO_2 system was constructed by APV (Chicago, IL) for Praxair (Chicago, IL) and provided as a gift to the University of Florida (Gainesville, FL). The equipment is capable of continuously treating liquid foods with CO_2 at pressures up to 69 MPa. The system mixes cooled, pressurized liquid CO_2 with a juice feed pressurized by its own pump (**Figure 1**). The mixture is then pressurized by a reciprocating intensifier pump and subsequently fed to a holding tube (79.2 m, 0.635 cm i.d.) for the specified residence time, which is modified by changing the flow rate of the mixture. An external heater and insulation electronically control the temperature of the system, and upon exiting the holding tube the juice is depressurized by passing through a back-pressure valve and finally collected into a holding tank.

For thermal processing, juice was pumped by a peristaltic pump (Cole Parmer, Chicago, IL) through a stainless steel tube (6.3 m, 0.457 cm i.d.) into a temperature-controlled water bath (Hart Scientific, American Fork, UT) where it was calibrated to hold the juice at 75 °C for 15 s. The juice was then passed through a cooling stainless steel tube (12 m, 0.457 cm i.d.) and chilled to 10 °C whereby it was collected into sterile glass containers.

Microbial Inactivation Study. Preliminary investigations were conducted to determine the DP-CO_2 parameters that could achieve a required >5 log reduction of aerobic microorganisms and yeasts/molds using response surface methodology with parallels to the potential destruction of pathogenic organisms. Microbial counts (yeast and molds and total aerobic microorganisms) were used as the dependent variables in the experimental design that was conducted in duplicate. Each study required 11 experiments with 4 factorial points, 4 star points, and 3 center points for replication. A high initial microbial load in the juice (8.1×10^6 CFU/mL of yeasts/molds, 1×10^5 CFU/mL of total aerobic microorganisms) was required and obtained by incubating the filtered juice for 4 days at 21 °C. Juice was then subjected to DP-CO_2 using different pressures (1.2−40.2 MPa) and CO_2 levels (0−15.7%) using a constant residence time (6.25 min) and temperature (30 °C). Microbial inactivation was evaluated immediately after processing.

Microbial counts were made from triplicate samples of each processing treatment serially diluted (1×10^{-1} to 1×10^{-6}) in duplicate by mixing 1 mL of each juice with 9 mL of sterile Butterfield's buffer. Total plate counts were determined on aerobic count plates and yeast/mold plates (3M Petrifilm Microbiology Products, St. Paul, MN) by plating 0.1 mL of the dilutions onto the agar in triplicate and enumerated after 48 h at 35 °C and 72 h at 24 °C, respectively, according to the manufacturers' guidelines. Experimental data were analyzed by regression analysis using JMP software (SAS, Cary, NC), fit to quadratic polynomial equations, and results were used to select two DP-CO_2 conditions for assessment of phytochemical stability and sensory evaluation: (i) D-1 (34.5 MPa, 8% CO_2) and (ii) D-2 (34.5 MPa, 16% CO_2).

Scanning electron microscopy was used to investigate changes in yeast microstructure due to DP-CO_2. Yeast cells present in the grape juice before and after processing were treated according to the conditions described by Park et al. (*1*) before being observed in the scanning electron microscope (Hitachi S-4000, Pleasanton, CA).

Phytochemical and Microbial Stability Study. Muscadine grape juice was divided into three equal portions for subsequent processing by two DP-CO_2 conditions (34.5 MPa at 8% or 16% CO_2) and thermal pasteurization at 75 °C for 15 s. After processing, each juice was again divided into three proportions for assessment of microbial, phytochemical, and sensory characteristics. Samples for microbial and phytochemical analysis were immediately transferred into sterile 20 mL screwed cap vials and stored at 4 °C for up to 10 weeks, whereas samples for sensory analysis were transferred to sterile 4 L glass containers. Sodium azide (50 mg/L) was added to the samples used for phytochemical analysis in order 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. (*11*). 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 and 88% to 50% in 2 min and then held for 12 min at a flow rate of 1.8 mL/min. Anthocyanins were characterized on the basis of UV−vis spectral interpretation from 200 to 600 nm and comparison to authentic standards (Polyphenols Laboratories AS, Sandnes, Norway), and identification was additionally confirmed following acid hydrolysis into their respective aglycons with 2 N HCl in 50% (v/v) methanol for 60 min at 90 °C.

Antioxidant capacity was determined using the oxygen radical absorbance capacity (ORAC) assay with data expressed in Trolox equivalents per milliliter (μmol of TE/mL) (*12*). Total soluble phenolic levels were measured using the Folin−Ciocalteu assay (*12*) and quantified as gallic acid equivalents. 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 a Orion CO_2 electrode (Thermo Electron Corp., New Haven, CT). Microbial counts throughout storage were determined as previously described.

Sensory Evaluation. Flavor, aroma, and color intensity of fresh and processed juices were compared using a difference from control test. A randomized complete block design was used, and difference from control measurements were recorded on a line scale with anchors at 0 and 10 that represented "no difference" to "extremely different" in sensory attributes. Panelists compared the sensory attributes of the reference (fresh/unprocessed juice) with those presented by the hidden reference (fresh juice) and the thermally or DP-CO_2 processed juices. A 9-point hedonic scale was also conducted in order to compare the overall likeability of fresh (hidden reference) and processed juices.

Before sensory analysis, all juices (fresh and DP-CO_2 and thermally processed) were degassed in order to equalize carbonation levels by placing them in a 4 L sterile glass container on a hot plate with continuous stirring for 4 h at 20 °C. Juices were then served on a tray at room temperature in randomly numbered plastic cups with the reference sample placed at the center of the tray. A cup of deionized water and nonsalted crackers were also provided to the panelists between evaluations. All sensory tests were performed at the University of Florida's taste panel facility using 60 untrained panelists (31 females, 95% in the 18−44 age range).

Statistical Analysis. Data represent the mean and standard error of juices analyzed as a 3 × 9 factorial comparing three processing conditions (DP-CO_2 at 8% or 16%, both at 34.5 MPa, or thermally pasteurized) evaluated at nine sampling points (unprocessed, processed, week 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, Ontario, Canada), and analysis of variance was conducted by using the Tukey's multiple comparisons method at the 5% significance level.

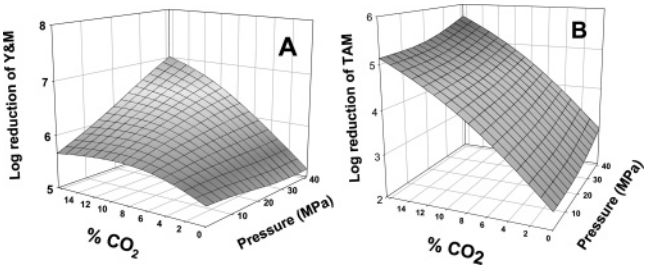

Figure 2. Inactivation of yeast/molds [Y&M (**A**)] and total aerobic microorganisms [TAM (**B**)] after DP-CO_2 pasteurization of muscadine grape juice as influenced by processing pressure (0–40 MPa) and CO_2 content (0–15.7%).

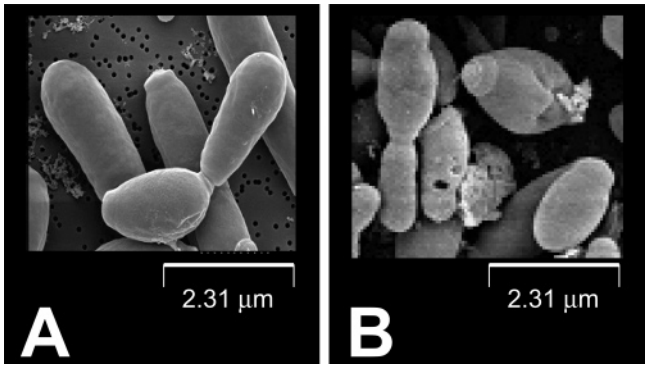

Figure 3. Scanning electron micrographs of naturally occurring yeast cells in muscadine juice before (**A**) and after DP-CO_2 at 34.5 MPa and 16% CO_2 (**B**).

RESULTS AND DISCUSSION

Microbial Inactivation Study. The effects of DP-CO_2 at various processing pressures (0−40 MPa) and CO_2 levels (0−18%) on yeast/mold and total aerobic microorganism destruction can be observed in **Figure 2**. Results showed that although processing pressure was a significant factor affecting microbial inactivation, CO_2 content was the processing parameter that had the major influence in microbial log reduction. This trend has also been observed in previous studies (*1*, *3*, *7*, *9*, *10*) which reported that microbial inactivation by DP-CO_2 and SC-CO_2 is governed essentially by the transfer rate and the penetration of carbon dioxide into cells, the effectiveness of which can be improved by increasing pressure, decreasing the pH of the suspension, and increasing the processing temperature. Studies investigating CO_2 systems under pressure and supercritical CO_2 batch systems have reported that microbial inactivation is also highly dependent on other processing parameters such as residence time and number of pulse cycles as well as the composition of the food medium (*1*−*6*). Results also demonstrated that, under identical processing conditions, yeast/molds were destroyed at significantly higher rates than aerobic microorganisms. Moreover, the synergistic effect between pressure and CO_2 was only observed for the inactivation of yeast/molds. Microbial inactivation is highly dependent on the type of microorganisms present in the food matrix due to distinct microbial cell microstructure and the diffusion of CO_2 into the microbial cell (*1*, *3*, *7*, *8*). For instance, vegetative cells, including yeasts and molds, are pressure and CO_2 sensitive, whereas bacterial spores are more pressure resistant and thus need higher pressures for complete inactivation. Park et al. (*1*) showed that a combined treatment of carbonation and HHP at 500 MPa yielded an 8 log reduction of *Staphylococcus aureus*, *Fusarium oxysporum*, and *Fusarium sporotrichioides* while only a 4 log reduction was obtained for *Bacillus subtilis*. Overall, microbial reduction is attributed to the fact that CO_2 solubility increases with increments of processing pressure (*1*, *2*) which consequently affects the diffusion of CO_2 into the microbial cell as well as the explosive decompression that occurs during DP-CO_2 processing. Results of this optimization study were used to determine those DP-CO_2 conditions that achieved >5 log reduction of aerobic microorganisms and yeast/molds that set the processing conditions of 34.5 MPa with 8% CO_2 (D-1) and 16% CO_2 (D-2).

Micrographic observations aided in elucidating the mechanism of yeast destruction and concluded that explosive decompression of the microbial cell along with changes in cell membrane structure occurred during DP-CO_2 (**Figure 3**). Conversely, heat-pasteurized yeast cells still appeared round and pert but with slightly textured surfaces (data not shown). Results also indicated that the number of decompressed/injured cells was directly related to increments in processing CO_2 levels. Previous investigations have also demonstrated that microbial destruction by pressurized CO_2 systems was based on gas dissolution inside a microbial cell that when rapidly decompressed to atmospheric pressure caused fatal damage to cell functioning and explosive decompression of the cell (*1*, *2*, *6*, *8*). Other theories concerning bacterial death by CO_2 pressurization have indicated that the depressurization leads to leakage of cellular components and changes in the cell membrane permeability which is responsible for cell damage and eventual microbial death (*1*, *3*, *6*). Related studies have shown that removal of essential intracellular substances such as phospholipids and hydrophobic compounds from cells or cell membranes plays important roles as mechanisms of microbial inactivation (*4*, *6*, *8*). Additionally, DP-CO_2 can alter biological systems by causing protein denaturation, lipid phase changes, and rupture of membranes inside the microbial cell (*1*, *6*, *8*).

Phytochemical and Microbial Stability. Differences in phytochemical and antioxidant levels were observed in muscadine grape juice as affected by processing methods and storage. Thermal pasteurization was found to be more detrimental to anthocyanins, soluble phenolics, and antioxidant capacity as compared with DP-CO_2 and unprocessed juices. Moreover, enhanced oxidative stability and retention of antioxidant compounds were observed for DP-CO_2 processed juices throughout storage. However, microbial stability of the latter was only comparable to heat-pasteurized juices for the first 5 weeks of storage.

Thermal pasteurization decreased total anthocyanins by 16%, total soluble phenolics by 26%, and antioxidant capacity by 10% whereas no significant changes were observed for either DP-CO_2 processes (**Table 1**). Individually quantified anthocyanins (data not shown) followed a similar trend with greater losses occurring for *o*-dihydroxy-substituted anthocyanins (delphinidin and cyanidin) with respect to the methoxylated anthocyanins (peonidin and malvidin) as previously observed in muscadine grape juice (*13*). Losses ranged from 8% to 16% following thermal pasteurization for delphinidin, cyanidin, and petunidin, while peonidin and malvidin remained stable (<4% losses). Anthocyanin degradation during processing and storage was highly correlated to total soluble phenolics ($r = 0.86$) and antioxidant capacity ($r = 0.82$). Insignificant changes in juice pH (3.2) or titratable acidity (0.56 mequiv of tartaric acid/mL) were observed between treatments after processing or storage. Initial CO_2 content was 6.70 and 9.81 mM for juices pressurized at 8% and 16% CO_2 levels, respectively.

Trends for polyphenolic and antioxidant changes during storage were similar to those observed after processing where

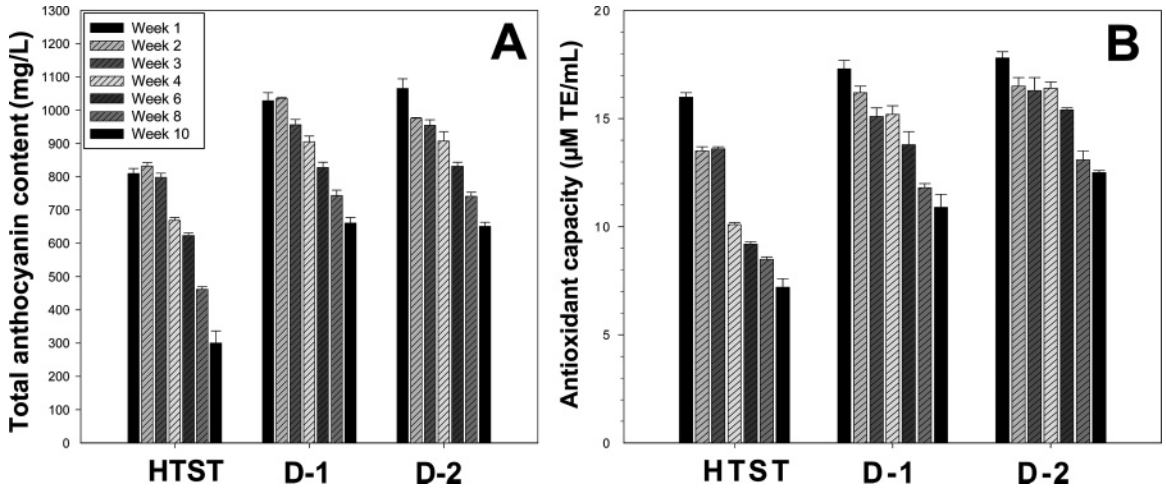

Figure 4. Total anthocyanin content of heat (HTST; 75 °C, 15 s) and DP-CO_2 pasteurized (D-1, 34.5 MPa, 8% CO_2; D-2, 34.5 MPa, 16% CO_2) muscadine juice during refrigerated storage (4 °C).

Table 1. Effect of Heat (75 °C for 15 s) or DP-CO_2 (D-1, 34.5 MPa, 8% CO_2; D-2, 34.5 MPa, 16% CO_2) Pasteurization on the Total Anthocyanin, Soluble Phenolic, and Antioxidant Content of Unprocessed Muscadine Grape Juice

treatment	total anthocyanin (mg/L)	soluble phenolic (mg/L)	antioxidant capacity (μmol of TE/mL)
unprocessed	1105 a[a]	2211 a	22.1 a
DP-1 (34.5 MPa, 8% CO_2)	1077 a	2213 a	20.7 a
DP-2 (34.5 MPa, 16% CO_2)	1102 a	2157 b	21.7 a
HTST (75 °C, 15 s)	866 b	1859 c	18.2 b

[a] Values with similar letters within columns are not significantly different (LSD test, $P > 0.05$).

DP-CO_2 processed juices presented reduced oxidative degradation when compared to thermally pasteurized juices, especially for anthocyanins. Regression analysis concluded that polyphenolic and antioxidant losses throughout storage followed first-order degradation kinetics, in accordance with other anthocyanin-containing juices (*11*, *14*, *15*), and that greater losses in polyphenolics and antioxidant capacity (1.4-fold) were observed for thermally pasteurized juices when compared to both DP-CO_2 processes. Independent of CO_2 concentration, the DP-CO_2 juices retained higher total anthocyanins and antioxidant capacity (335 mg/L and 10.9 μmol of Trolox equivalents/mL respectively; **Figure 4**), and 473 mg/L higher total soluble phenolics (**Figure 5**) than thermally pasteurized juices after 10 weeks of storage at 4 °C. Increased anthocyanin and polyphenolic degradation presented by heat-pasteurized juices presumably occurred due to formation of byproducts from carbohydrate and organic acid degradation during thermal processing and storage such as furfurals and other carbonyl compounds that can form condensation products with anthocyanins and polyphenolics. Previous studies have shown that the formation and occurrence of these compounds, accelerated by heat and acid, promote polyphenolic degradation to yield brown or polymerized pigments that negatively impact juice quality (*16*−*19*). Comparable degradation rates were observed among individual anthocyanins (38%) present in both DP-CO_2 juices, while again ortho-dihydroxylated anthocyanins present in heat-pasteurized juices showed higher degradation rates than their methoxylated

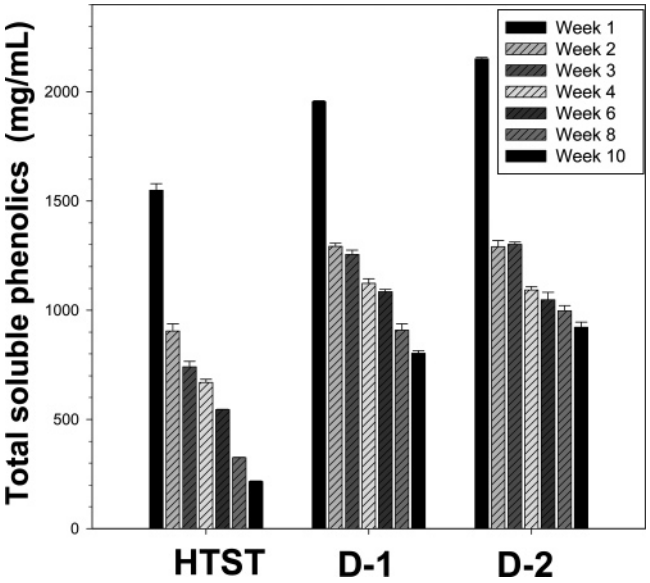

Figure 5. Total soluble phenolic content of heat (HTST; 75 °C, 15 s) and DP-CO_2 pasteurized (D-1, 34.5 MPa, 8% CO_2; D-2, 34.5 MPa, 16% CO_2) muscadine juice during refrigerated storage (1–10 weeks at 4 °C).

counterparts. Delphinidin and cyanidin showed the greatest losses (69%) after thermal processing followed by petunidin (48%), peonidin (45%), and lastly malvidin (24%). These results are in accordance to earlier reports that investigated the stability of different anthocyanins that demonstrated malvidin as the most stable anthocyanin (*13*, *21*−*23*). However, in actual food systems, the relative stability of an anthocyanin is likely a function of its matrix, structural features, and the combined conditions of processing and storage (*12*−*19*).

The concentration of CO_2 utilized during DP-CO_2 processing insignificantly affected anthocyanin stability during storage, while increasing CO_2 from 8% to 16% offered enhanced storage stability for antioxidant capacity and total soluble phenolics (**Figures 4B** and **5**, respectively). These results may suggest that anthocyanin destruction occurs independently of oxygen content in the juice matrix, while polyphenolic degradation is directly linked to the presence of oxygen. Poei-Langston and Wrolstad (*20*) also observed the destruction of anthocyanins in nitrogen-sparged systems and proposed a condensation mechanism for their destruction that did not involve oxygen. Model

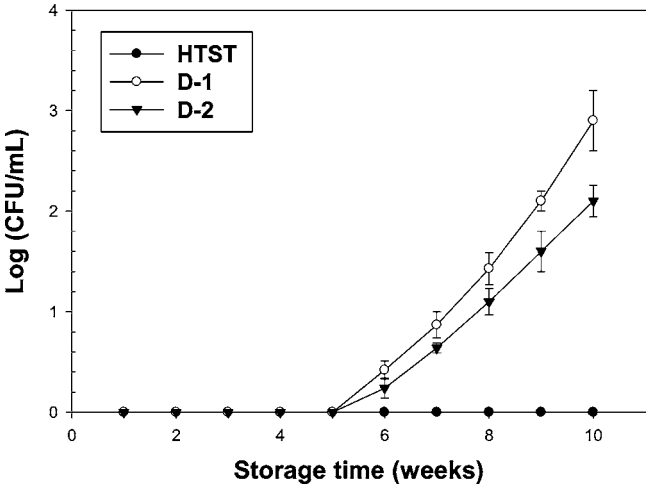

Figure 6. Yeast/mold counts of heat (75 °C, 15 s) and DP-CO_2 pasteurized (D-1, 34.5 MPa, 8% CO_2; D-2, 34.5 MPa, 16% CO_2) muscadine juice during refrigerated storage (4 °C).

systems containing anthocyanins and ascorbic acid have also demonstrated the destruction of these phytochemicals under both aerobic and anaerobic conditions (*15*, *19*−*23*), and therefore exclusion of oxygen during processing would not be sufficient to prevent anthocyanin degradation. However, the prevention and/or reduction of furfurals formation during processing and storage might be an important approach to attenuate anthocyanin degradation. Consequently, DP-CO_2 could be used as a strategy to reduce the degradation of these phytochemical compounds.

Similar microbial stability was observed between DP-CO_2 and thermally pasteurized juices during the first 5 weeks of storage at 4 °C; however, significant differences were observed subsequently through 10 weeks (**Figure 6**). Yeast/mold counts for both DP-CO_2 juices continuously increased throughout subsequent storage whereas no changes were observed for heat-pasteurized juices. Increasing the processing CO_2 content from 8% to 16% served to delay microbial growth after the 6th week of storage, an effect that might be attributed to oxygen stripping by CO_2 from the juices and/or the inactivation of microbial spores during processing. Previous investigations have shown that a combined approach between pressure, temperature, and CO_2 is needed to completely inactivate bacterial spores (*3*−*5*). For example, Enomoto et al. (*24*) examined the lethal effect of DP-CO_2 on spore cells of *Bacillus megaterium* and observed that the bactericidal effect of CO_2 was found to be enhanced with increasing temperature and treatment time. Similarly, Kamihara et al. (*25*) and Haas et al. (*26*) observed that temperatures above 70 °C were needed to inactivate endospores of *B. subtilis*, *Bacillus stearothermophilus*, and *Clostridium sporogenes* 3679. Changes in microbial counts were readily perceived in informal sensory evaluations by the presence of gas formation and the appearance of a yeast-like aroma that increased throughout storage. Independently of pasteurization techniques, insignificant changes in the number of total aerobic microorganisms were observed during storage (data not shown).

Sensory Evaluation. Insignificant differences in flavor, aroma, and color intensity were observed between the reference (fresh/unprocessed), the hidden reference, and both DP-CO_2 processed juices (**Table 2**). However, significant differences in flavor and aroma were detected by panelists ($P < 0.012$) between the reference and the heat-pasteurized juice. Although not specifically quantified, the formation of cooked and burnt flavors is often associated with off-flavor development in heat-pasteurized juices (*16*). Panelists ranked both DP-CO_2 juices higher in overall likeability than the heat-pasteurized juice, whereas no difference was observed between DP-CO_2 juices and the unprocessed juice. Panel scores for overall likeability were in average 6.05 for the hidden control and both DP-CO_2 juices compared to 4.02 for the heat-pasteurized juice (a higher number indicates a higher preference for the juice). As previously discussed, results from informal evaluations suggested that color intensity, aroma, and flavor of DP-CO_2 processed juices were comparable to control juices throughout the first 6 weeks of storage, after which the presence of a yeasty aroma negatively impacted their sensory attributes.

Table 2. Differences in Organoleptic Attributes (Color Intensity, Aroma, Flavor) and Overall Likeability between Fresh (Reference and Hidden Reference), Heat (75 °C for 15 s), and DP-CO_2 (D-1, 34.5 MPa, 8% CO_2; D-2, 34.5 MPa, 16% CO_2) Processed Muscadine Grape Juice Detected by Untrained Panelists ($n = 60$)

	difference in			
	color intensity[a]	aroma[a]	flavor[a]	overall likeability
hidden reference	1.48^{NS} a[b]	2.38^{NS} a	2.46^{NS} a	6.23 a
DP-1 (34.5 MPa, 8% CO_2)	2.00^{NS} a	2.74^{NS} a	4.32^{NS} a	5.98 a
DP-2 (34.5 MPa, 16% CO_2)	1.67^{NS} a	2.20^{NS} a	3.92^{NS} a	5.95 a
HTST (75 °C, 15 s)	1.78^{NS} a	5.11* b	8.47* b	4.02 b

[a] Difference observed when compared to given reference (difference from control test). [b] Values with similar letters within columns are not significantly different (Tukey's HSD, $P > 0.05$). ^{NS} or *: nonsignificant or significant (Tukey's HSD, $P < 0.05$) difference when compared to the given reference, respectively.

DP-CO_2 served to protect polyphenolic and antioxidant levels throughout processing and storage and did not compromise sensory attributes of the juices until 5 weeks of refrigerated storage. However, microbial stability of DP-CO_2 juices was only comparable to heat-pasteurized counterparts for the first 5 weeks of storage. This technology was proven to be a feasible pasteurization technique especially for juices containing heat-labile phytochemical, antioxidant, and flavor compounds.

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