JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 11325–11332 11325
DOI:10.1021/jf102223f

Effects of Concentration Prior to Cold-Stabilization on Anthocyanin Stability in Concord Grape Juice

Kristin S. Alongi, Olga I. Padilla-Zakour, and Gavin L. Sacks*

New York State Agricultural Experiment Station, Department of Food Science and Technology, Cornell University, 630 West North Street, Geneva, New York 14456, United States

The color of Concord grape juice produced by concentration before cold-stabilization and detartration (direct-to-concentrate, DTC) was compared to juice produced via cold-stabilization prior to concentration (standard concentrate, SC) and evaluated by several metrics. Using the Boulton copigmentation assay, the majority of the absorbance at 520 nm in bottled SC juice (72%) was due to monomeric anthocyanins. Following reconstitution, DTC juice had a 63% greater absorbance at 520 nm than SC juice. A significant loss of anthocyanins was observed using a paired *t* test during cold-stabilization of single-strength juice during SC processing (mean loss: 79 mg/L as cyanidin-3-glucoside, 23% of total anthocyanins), while no significant loss of anthocyanins or change in other color metrics was observed during cold-stabilization of DTC concentrate. The concentration of anthocyanins in the SC bitartrate crystals was 0.80% w/w compared to 0.13% w/w in the DTC bitartrate crystals. Between DTC and SC, no difference in copigmentation was observed in cold-stabilized concentrate or reconstituted juice, indicating that the increased stability of anthocyanins could not be credited to greater copigmentation in DTC during detartration. HPLC analyses indicated that anthocyanin species with higher pK_h and thus proportionally greater flavylium ion concentration at juice pH are preferentially lost during SC processing. The proportional changes in color metrics during shelf life stability testing (0−16 weeks, 2−30 °C) were not significantly different between SC and DTC juices.

KEYWORDS: Concord; grape concentrate; potassium bitartrate; anthocyanin-3-glucoside

INTRODUCTION

In the US, the primary cultivar used for purple grape juice is Concord (*Vitis labruscana* Bailey cv. 'Concord'). Concord juice is typically produced by the hot press method in the Eastern United States and the hot break method in Washington State (*1*). In the hot press method, grapes are heated to 60 °C before enzyme addition. In hot break, they are initially heated to temperatures >75 °C and cooled to 60 °C, and then undergo depectinization (*1*).

Grapes are uniquely high in tartaric acid, and fresh grape juice will precipitate potassium bitartrate crystals during cold storage. To prevent this bitartrate instability from occurring in bottled juice, a cold-stabilization is usually performed on single-strength juice, which can cause a loss of anthocyanin pigments (*1*). In Concord grape juice, a 20−40% loss of anthocyanins was reported to occur following detartration (*2*). Losses have also been observed during cold-stabilization in wine production, and bitartrate crystals from Carignan wines are reported to contain 0.2−0.3% w/w anthocyanins on a dry weight basis (*3*). Since bitartrate crystals from grape juice are typically smaller and less pure than those from wine, comparable or greater amounts of anthocyanin loss would be expected during cold-stabilization of grape juice (*4*).

*Corresponding author. E-mail: gls9@cornell.edu.

The mechanism for the loss of anthocyanins during detartration is not well understood. During cold storage, anthocyanins adhere to the surface of a growing bitartrate crystal and are lost from solution (*5*,*6*). Occlusion of anthocyanins within the crystal lattice does not appear to occur. The attractive forces responsible for this adsorbance are variously proposed to be ionic, hydrogen-bonding, or charge-transfer in nature (*7*,*8*).

The pigments in grape juice may exist in several forms, which for simplicity have been categorized by previous authors as one of three pigment classes: monomeric anthocyanins, polymeric pigments, and copigmented complexes (*9*). The stability of each of these classes during cold-stabilization is unknown. Monomeric or "free" anthocyanins are anthocyanidin glucosides. The molar absorptivity of monomeric anthocyanins is highly pH dependent, resulting in a range of colors from red to colorless with increasing pH, and are readily bleachable by bisulfite (*10*). Polymeric pigments represent the fraction of color that is not bleached by bisulfite, and are formed via covalent reactions of anthocyanins with other juice components (*11*). Copigmented complexes in juices are formed through noncovalent interactions of anthocyanins with other compounds, such as flavonols (*9*), or other anthocyanins ("self-association") (*12*). Such complexes play a prominent role in the color of young wines (*9*).

Traditional juice processing methods (standard concentration, SC) involve a concentration step following cold-stabilization (*13*).

Published on Web 10/07/2010

Table 1. Sample Points throughout Processing of Grape Juice Standard Concentrate Hot Press and Hot Break (PSC, BSC) and Direct to Concentrate (DTC)[a]

sample point	PSC and BSC	DTC
1	juice after first enzyme treatment and heating (before screw press)	juice after first enzyme treatment and heating (before screw press)
2	juice before cold storage	not applicable
3	juice before concentration	juice before concentration
4	concentrate before storage	concentrate before storage
5	concentrate after storage	concentrate after storage
6	reconstituted juice	reconstituted juice

[a] $n = 4$ at each time point.

Alternatively, the order of these two steps can be switched such that concentration precedes cold storage ("direct-to-concentrate", DTC), and detartration is performed on the concentrate. Anecdotally, DTC production has been reported to improve anthocyanins' stability compared to SC practices, but the impact of this practice has not been characterized in the literature. Assuming anecdotal accounts were correct, we hypothesized that monitoring changes in the contributions of monomeric anthocyanins, polymeric pigments, and copigmented complexes to Concord juice color throughout processing and storage could provide insight into the mechanism behind differences in anthocyanin stability between DTC and SC. In this study, we analyzed pigmentation in juices produced via hot press DTC in comparison to hot press and hot break juice processed by SC methods.

MATERIALS AND METHODS

Grapes. All Concord grapes were hand harvested from a single nearby vineyard (Penn Yan, NY) and received at the New York State Agricultural Experiment Station (Geneva, NY) in the fall of 2009. The grapes were grown using the standard cultivar practices of the region. Prior to processing, grapes were stored at 2 °C in plastic crates (relative humidity = 75%) for no more than 7 days. Grapes varied in maturity from 14 to 16 °Brix, measured using a Leica Auto Abbe refractometer (Buffalo, NY).

Sample Collection. Samples for the juice were collected at six time points throughout processing, outlined in **Table 1**. Bitartrates were collected after cold storage, time point 3 for hot break standard concentration (BSC) and hot press standard concentration (PSC) and time point 5 for direct-to-concentrate methods (DTC).

Standard Concentrate Hot Break (BSC) and Hot Press (PSC) Processing. PSC and BSC processing was performed on the grapes in 230 kg batches, according to industry standard practices (*1*). A schematic summarizing the processing steps is shown in **Figure 1**. Two replicates of standard concentrate processing with both hot break and hot press treatments were performed. On October 15, 2009, the first replicates of hot break standard concentrate and hot press standard concentrate were conducted. The second replicates of each were performed a week later on October 22, 2009.

Both hot break and hot press standard processes began with destemming and crushing grapes in a Mori (Florence, Italy) Eno 20 destemmer−crusher. The hot break grapes were then heated to 82 °C in a steam-jacketed kettle and subsequently cooled to 60 °C. Adex G depectinizing enzyme (DSM, Parsippany, NJ) was then added at 0.03 mL per kg of grapes along with Pressanier-J paper as a press-aid at 7.5 g per kg of grapes (supplied by Welch Foods Inc., Westfield, NY) during agitation. The must was then held at 60 °C for 30 min. The hot press standard concentrate processing followed the same protocol but was initially heated to 60 °C, not 82 °C. Depectinizing enzyme and paper press aid were added when 60 °C was reached.

After the 60 °C hold, both hot break and hot press standard concentrate juices were pressed in a Buffalo Hammer Mill press (Buffalo, NY) and then pasteurized at 85 °C for 1 min in a MicroThermics (Raleigh, NC) tubular pasteurizer. A clarifying enzyme, K201 (DSM, Parsippany, NJ), was then added at 150 mg/L, and the juices were stored at 2 °C for 2 weeks.

Following cold storage, the juice was siphoned off of the bitartrates and the turbidity was measured on a HACH 2100P turbidimeter (Loveland, CO) to ensure that the juice was under 100 NTU. Juices were concentrated with a Unipektin AG falling film two-effect evaporator at 50−55 °C and −0.9 atm (Zürich, Switzerland) to 59 °Brix. Following concentration, juice was stored at 2 °C for two weeks. After storage, the hot break and hot press standard concentrates were reconstituted with water to 16 °Brix and then hot filled (MicroThermics tubular pasteurizer, Raleigh, NC) at 85 °C with a 1 min hold prior to filling and 1 min hold in the bottle before cooling. Juice was packed into 240 mL Ball PET bottles (Broomfield, CO) for use in shelf life studies.

Direct-to-Concentrate (DTC) Processing. DTC processing is summarized in the schematic shown in **Figure 1**. Two replicates were performed on October 19 and 26, 2009, at the New York State Experiment Station (Geneva, NY). Grapes were processed in approximately 230 kg batches. DTC processing was similar to PSC processing, with the only variation in processing occurring following pressing. A second pectinase enzyme treatment, K201 (DSM, Parsippany, NJ) was then added at 300 mg/L to 57 °C juice. The second enzyme treatment required 1 h until a negative pectin level by alcohol test was observed. The juice was then put through a plate and frame filter with Celite 503 diatomaceous earth (DE) and concentrated with a Unipektin AG falling film 2 effect evaporator (Zürich, Switzerland) to 59 °Brix. The concentrate was stored at 2 °C for two weeks.

After cold-storage and detartration, DTC concentrate was reconstituted to 16 °Brix with water, and hot filled (MicroThermics tubular pasteurizer, Raleigh, NC) at 85 °C with a 1 min hold in the machine and 1 min hold in the bottle before cooling. Juice was packed into Ball PET bottles (Broomfield, CO), which were then used for shelf life studies.

Color Analyses. The total color intensity was measured as the absorbance at 520 nm and determined on a Pharmacia LKB Novaspec II spectrometer (Uppsala, Sweden) using a 1.0 mm path length cuvette for juice and a 0.25 mm path length cuvette for concentrate (Aline, Inc. Specvette Redondo Beach, CA) to give a reading in the linear range of the spectrometer.

A modified version of the Levengood−Boulton assay (*14*) was used to measure the absorbance at 520 nm due to copigmentation, polymeric pigment, and monomeric anthocyanins. The modification was that assays were conducted at the pH of the sample rather than adjusting all samples to pH 3.6 as suggested by Boulton so that the contribution of copigmentation to anthocyanin stability during processing could be quantified. However, the pH of all juices at the end of processing was identical, pH = 2.9. The pH was taken prior to the analysis (Cole-Parmer Accumet basic pH meter, Vernon Hills, IL). Model solutions of the juice and concentrate were made with corresponding levels of glucose (Sigma Aldrich, Milwaukee, WI), fructose (Sigma Aldrich, Milwaukee, WI), and tartaric acid (Fisher Scientific, Fair Lawn, NJ), and the pH of the model solution was adjusted with NaOH (Fischer Scientific, Fair Lawn, NJ) to the pH of the individual sample . Absorbance at 520 nm was determined on a Barnstead Turner spectrophotometer (Fischer Scientific, Fair Lawn, NJ).

The pH differential method (*15*) was used to determine several metrics, including total anthocyanins (mg/L as cyanidin-3-glucoside), color intensity, polymeric pigment, and the percentage of polymeric pigment in the juices. Potassium metabisulfite bleaching was used to determine the percentage of polymeric pigment.

There was significant variability in color intensity (absorbance at 520 nm) among DTC and PSC treatment replicates following crushing, since the grapes were harvested at different maturities for each replicate. To account for this, all absorbance values were normalized to the initial time point 1 to facilitate statistical comparisons between DTC and PSC treatments:

$$\text{normalized absorbance at time point } N \text{ (Norm-AU)} = \frac{\text{Abs 520 at time point } N}{\text{Abs 520 at time point 1}}$$

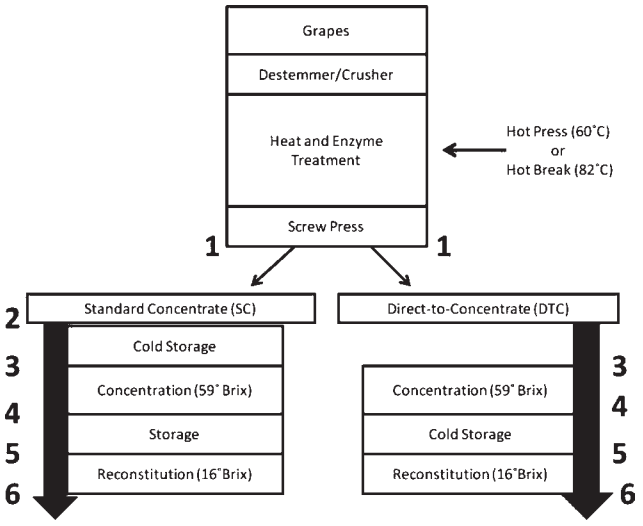

Figure 1. Methods of Concord grape juice production with variations in heat treatments (hot press or hot break) and concentrate processing (standard concentration or direct-to-concentrate). Numbers correspond to sampling points, described in detail in **Table 1**.

Time point 1, the sample after heat treatment, was used as the denominator because it occurred prior to the divergence of DTC and PSC processing strategies. This normalization approach was not used for comparison of PSC and BSC treatments because these treatments diverged at the first processing step. All color analyses were performed in analytical duplicates.

Anthocyanins in the final, reconstituted PSC and DTC juices were also evaluated on a HP 1100 HPLC system (Agilent, Santa Clara, CA) by a previously described method (*16*). Briefly, juices were filtered through a 0.2 μm filter and 20 μL was injected directly onto a C_{18} reverse phase column (250 mm × 4.6 mm i.d., 5 μm particle size). Solvent A was water/phosphoric acid (99.5/0.5; v/v), and solvent B was acetonitrile/water/phosphoric acid (50/49.5/0.5; v/v/v). Following injection with 100% A and a 2 min hold, B was ramped from 0% to 100% over 40 min. Column eluent was monitored by a diode array detector, and the signal at 520 nm used for peak detection and quantification. Delphinidin-3-glucoside, cyanidin-3-glucoside, malvidin-3-glucoside, and delphinidin-3-*p*-coumaryl-glucoside (gift from Dr. Justine Vanden Heuvel, originally from Dr. Geza Hrazdina, Cornell University) were used for identification.

Color Stability Analysis. Shelf life studies of bottled juices were performed at three different temperatures: 30 °C, 18 °C, and 2 °C. Samples were taken at 0, 2, 9, and 16 weeks. Samples were centrifuged on an Eppendorf microcentrifuge 5417 C at 14000 rpm for 15 min to remove turbidity at time points 9 and 16 weeks. Color was assessed using the previously described methods: color intensity (absorbance at 520 nm), modified Levengood−Boulton assay (*14*) and pH differential method (*15*).

Anthocyanin Content and Light Microscopic Analysis of Bitratrate Crystals. The bitartrate crystals from PSC and DTC processing were analyzed for total anthocyanin concentration. The bitartrate crystals from cold storage were dissolved in 0.1 N HCl, as described in (*3*) and the solution assessed by pH differential method. DTC crystals were also washed with ethanol. The amount of anthocyanins was reported on a w/w % basis of the bitartrate crystal. Light microscopy was performed on a MEIJI Techno microscope (Saitama, Japan) with phase contrast. A 100× magnification was used on bitartrate crystals from PSC processing and 400× magnification from bitartrate crystals from DTC processing.

Statistical Analysis. All processes were performed in duplicate, with two additional analytical replicates for each sample point. Means and standard error were calculated using Microsoft Excel software (Redmond, WA). Data treated with analysis of variance (ANOVA) using JMP 8.0 (SAS Inst. Inc., Cary, NC) and means were compared with Tukey−Kramer HSD at a 95% confidence interval.

RESULTS AND DISCUSSION

Color Composition of Concord Grape Juice. The contribution of monomeric anthocyanins, copigmented complexes, and polymeric pigments to overall Abs 520 in the final juice produced from hot press standard concentrate methods (PSC) was calculated using the Boulton copigmentation assay (*14*) and is shown in **Figure 2**. The total absorption of the juice at 520 nm was 16.4 AU, with the majority (11.8 AU, 72%) assigned to the monomeric anthocyanin fraction. Hong and Wrolstad similarly reported that the majority of Abs 520 in Concord grape colorant was due to monomeric anthocyanins in their 1990 publication (*17*), although copigmentation was not considered.

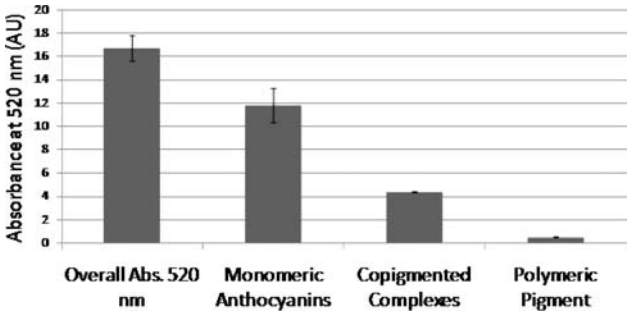

Figure 2. Color profile of Concord grape reconstituted juice produced from the standard concentrate hot press (PSC) method: overall absorbance along with the color contribution due to monomeric anthocyanins, copigmented complexes, and polymeric pigment, measured by Boulton copigmentation assay. Error bars represent one standard error.

Copigmention contributed to 26% of the Abs 520 of standard PSC juice. It is not clear if this copigmented color is primarily due to $\pi-\pi$ stacking with other small molecules vs self-association. The Boulton copigmentation assay only measures the increase in Abs 520 compared to that predicted from Beer's law and does not provide further chemical information about the copigmented species. The contribution of copigmentation to PSC juice in our work was comparable to results from earlier work on Muscadine, which indicated that the removal of natural cofactors from Muscadine grape juice resulted in a loss of about 25% of the overall color intensity (*18*). The contribution of copigmentation was also within the range previously reported in young red wines, 8−46% (*19*−*21*).

Polymeric pigments contributed little to the overall color intensity of the final reconstituted juice (0.5 AU at 520 nm). Concord grape juice is relatively low in tannin, and the final PSC juice was relatively young, which likely explains the limited role of polymeric pigments. The low contribution of polymeric pigment is in concordance with previous reports on Concord grape extract (*17*).

Effect of Heat Treatments on Concord Grape Juice Color. To determine the effect of hot break vs hot press heat treatments on Concord grape juice color, we observed the overall absorption at 520 nm of hot press standard concentrate (PSC) and hot break standard concentrate (BSC) throughout processing (**Figure 3**). Final reconstituted grape juice from PSC and BSC had Abs 520 of 16.7 and 17.7 AU, respectively. There were no significant differences between the hot press and hot break treatments at any time point during processing, in concordance with previous work by our group on New York State grown Concord (*22*).

Effect of Concentrate Parameters on Concord Grape Juice Color and Bitartrate Crystal Composition. A comparison of Abs 520 of the juice during cold storage (juice before cold storage to juice before concentration) and the final reconstituted juice of PSC, DTC, and BSC is shown in **Figure 4**. There was significant variability in grape color among treatment replicates, since the grapes were harvested at different maturities for each replicate. To account for this variability, all absorbance values were

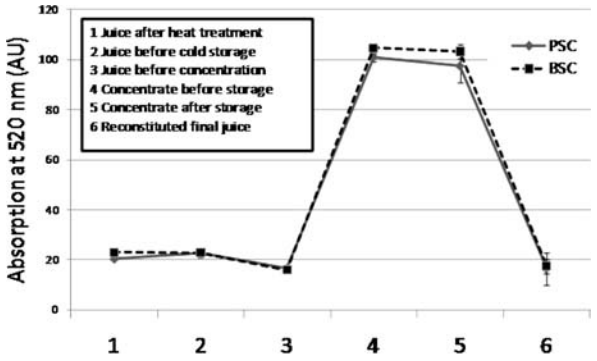

Figure 3. Absorption at 520 nm of Concord grape juice standard concentrate from hot press (PSC) and standard concentrate from hot break (BSC) throughout processing. Error bars represent one standard error. $n = 4$.

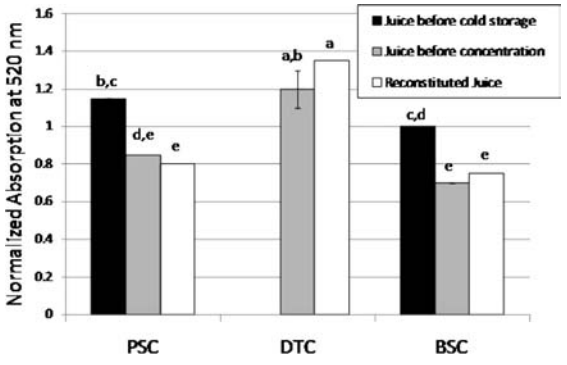

Figure 4. Comparison of single-strength Concord grape juice color from hot press direct-to-concentrate (DTC), standard concentrate from hot press (PSC), and standard concentrate from hot break (BSC) at different processing stages. Values are reported as the absorption at 520 nm at each step, normalized to the juice after heat treatment (Norm-AU) as described in the text. Error bars represent one standard error. Columns not connected by the same letter are significantly different, p value <0.05. $n = 4$.

normalized with respect to their color after depectinization (time point 1), as described in Materials and Methods, and reported as normalized absorption units, Norm-AU.

In the standard concentrate methods, BSC and PSC, the final reconstituted juices had normalized absorbancies of 0.8 Norm-AU, or a 20% decrease in color in the final juice compared to the initial juice following depectinization (**Figure 4**). The decrease in color in the final juice was attributable solely to the cold-storage step, with no significant change in color observed in the intermediate steps, i.e. concentration, concentrate storage, and reconstitution. A comparable loss in Abs 520 during cold-stabilization and detartration has been previously reported (*2*).

The normalized absorbance of the DTC juice following reconstitution (1.35 Norm-AU) was not significantly different from the normalized absorbance prior to concentration and cold storage. The color of the reconstituted DTC juice was also significantly higher than the color in both PSC and BSC juices. The absorbance of DTC final juice was 63% greater than that of PSC, confirming anecdotal evidence that DTC produces juices with enhanced color intensity in comparison to traditional SC methods.

The DTC and SC methods differed in three respects. In DTC, the second pectinase treatment, plate and frame filter step, and concentration occur prior to cold storage. This second depectinatization prior to concentration was necessary to prevent fouling of the concentrator. The timing of the second pectinase enzyme treatment and additional filtering step did not appear to

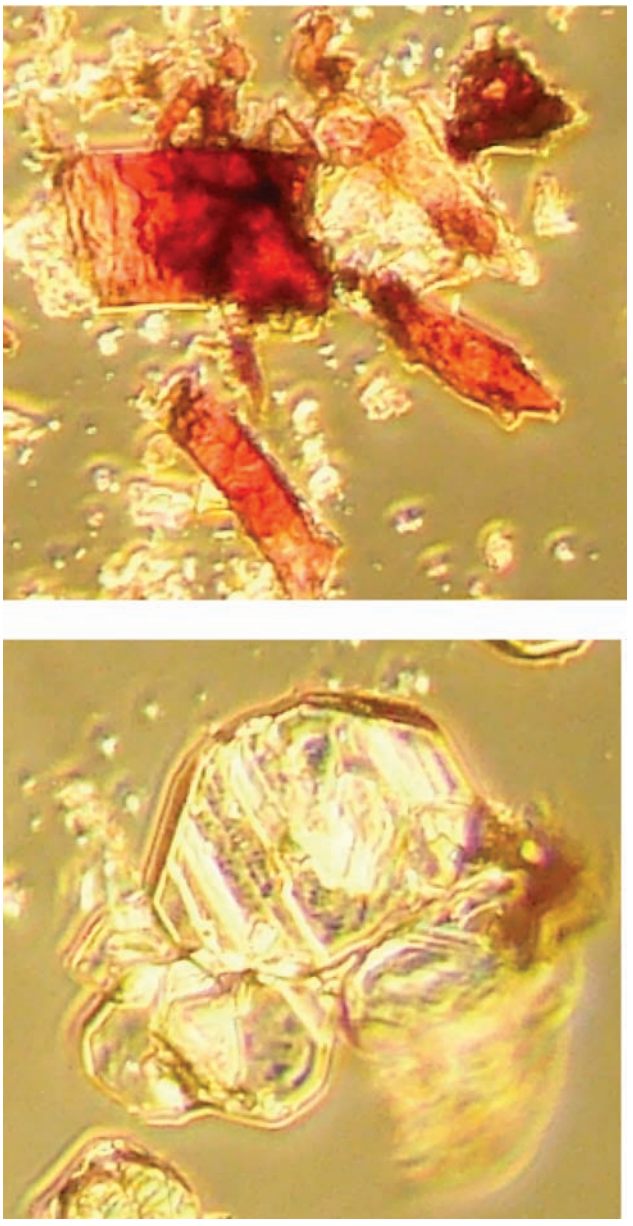

Figure 5. Light microscopy images using phase contrast of Concord grape juice bitartrate crystals from (top) PSC processing, 100× magnification, and (bottom) DTC processing, 400× magnification.

be critical; DTC juice sampled after these steps but prior to concentration, then cold stabilized as single strength, showed a similar decrease in Abs 520 to SC juice (data not shown). Therefore, the differences between SC and DTC methods could be assigned solely to differences in anthocyanin loss occurring during cold-stabilization of single strength vs cold-stabilization of concentrate.

The bitartrate crystals formed by DTC and SC processing were visibly different (**Figure 5**). Crystals formed during cold-storage of SC juices were approximately 3−4× larger than the DTC crystals, more irregularly shaped, and purplish-black, with the color likely due to coprecipitation of anthocyanins with the crystals. Anthocyanins reportedly adhere to the bitartrate crystal surface during crystal growth (*5*, *6*), and bitartrate crystals sampled from wine during cold-storage are reported to contain 0.2−0.3% w/w anthocyanins (*3*). By comparison, the DTC crystals were smaller and colorless, see **Figure 5**. DTC crystals also suggest more isotropic growth than those of SC.

Table 2. Observed and Estimated Anthocyanin Losses during Cold-Stabilization of Juice from Hot Press Standard Concentrate (PSC) and Anthocyanin Content of Respective Bitartrate Crystals[a]

anthocyanins in bitartrate crystals (% w/w basis)	0.8
estimated bitartrate loss (g/L)	8.03
estimated anthocyanin change with bitartrate crystals (mg/L)	−64
observed change in anthocyanins during detartration	−79 ± 15
observed change in anthocyanins during detartration (%)	−23 ± 4

[a] Estimated anthocyanin losses were calculated from the estimated bitartrate loss multiplied by the anthocyanin content of the crystals. Anthocyanin concentrations are calculated as cyanidin-3-glucoside equivalents.

There was no significant decrease in the concentration of total anthocyanins (mg/L as cyanidin-3-glucoside by pH differential) during detartration of the DTC concentrate. In contrast, during each replicate of SC, there was a significant loss (mean = 79 ± 15 mg/L). To determine if the difference in anthocyanin loss between the DTC and SC methods could be explained by coprecipitation with bitartrate crystals, we analyzed the composition of the bitartrate crystals collected from each method. The crystals were dissolved in 0.1 N HCl and anthocyanins quantified by the pH differential method. The concentration of anthocyanins in PSC crystals was 0.8% w/w. By comparison, the anthocyanin concentration of bitartrate crystals from the DTC method was 0.13% w/w. The concentration of potassium bitartrate lost during PSC and DTC cold storage was estimated from the difference in titratable acidity between the non-detartrated juice and final juice. Similar decreases in titratable acidity, 3.2 g/L as tartaric acid, were observed in PSC and DTC, resulting in similar estimated potassium bitartrate losses of 8.03 g/L. Assuming the sampled crystals contained negligible concentrations of other impurities, the estimated anthocyanin loss due to coprecipitation can be calculated (**Table 2**). The calculations outlined in **Table 2** illustrate that the majority of observed anthocyanin loss in PSC (79 mg/L) can be accounted for by anthocyanins coprecipitating with bitartrate crystals (64 mg/L). Additionally, this latter number may be underestimated due to potential impurities in the crystal, as has been reported in wine (*3*).

Changes in Copigmentation during Processing. During PSC, we observed a significant overall decrease in color intensity ($p < 0.05$) during the detartration of single strength juice, as outlined in **Figure 4**. The normalized absorbance decreased from 1.1 Norm-AU before cold storage to 0.8 Norm-AU after the two week cold-stabilization. There was no significant change in absorbance when DTC concentrate underwent this detartration step. To better characterize the differences between the processes, we evaluated changes in copigmented complexes during DTC and PSC processing (**Figure 6**). Polymeric pigment was not considered due to its low contribution to Abs 520.

Copigmentation has been reported to enhance anthocyanin stability in aqueous solutions (*8*, *23*). Since the degree of copigmentation is dependent on both the anthocyanin and cofactor concentration, i.e. second order, we expected a proportionally greater contribution of copigmentation to Abs 520 in concentrate compared to juice. We initially hypothesized that the DTC process would result in reduced loss of color intensity because copigmentation would increase the anthocyanin stability or solubility and prevent anthocyanin coprecipitation with bitartrate crystals. This hypothesis appears to be incorrect. **Figure 6**, bottom, illustrates that for both DTC and PSC the normalized absorbance due to copigmentation decreases by 50% in the final reconstituted juice as compared to initial juice. This loss is consistent with color analyses of wine during aging which show that copigmentation decreases as a function of time (*24*, *25*). The 50% drop in Abs 520 due to copigmentation following cold-stabilization of PSC is greater than the 20% loss in total Abs 520

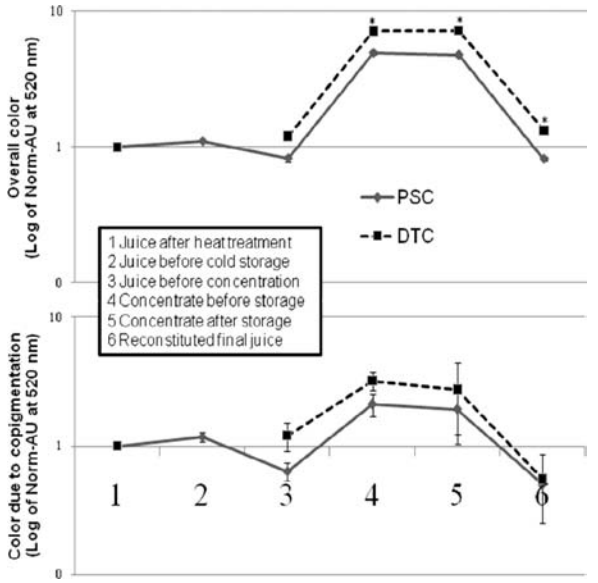

Figure 6. Changes in the total absorbance (520 nm) and copigmented complexes of direct-to-concentrate (DTC) and hot press standard concentrate (PSC) Concord grape juice throughout processing, reported in the log of the normalized absorbance at 520 nm. Error bars represent one standard error. * symbolizes significantly (p value <0.05) different values between DTC and PSC.

(**Figure 6**, top), possibly because of the simultaneous coprecipitation of cofactors like flavonols and hydroxycinnamic acids along with anthocyanins (*3*). Since copigmentation in both SC and DTC following concentration and in the final, reconstituted juices is not significantly different, copigmentation does not directly or indirectly account for the enhanced color intensity observed in DTC.

Interestingly, we observe only a 3−4-fold increase in the amount of copigmentation in concentrate compared to the initial single strength juice. Because copigmentation is second order, we had expected to see an approximately [(59 °Brix)/(16 °Brix)]^2 = 13.5-fold increase in copigmentation during the concentration stage. Copigmentation effects are reported to diminish at lower pH (*26*), and the proportionally lower contribution of copigmentation than expected may be due to the lower pH of concentrate compared to the pH of juice (2.5 vs 3.1).

As previously discussed, there was no significant decrease in the concentration of total anthocyanins (mg/L as cyanidin-3-glucoside) during detartration of the DTC concentrate but a significant loss during each PSC replicate. Similarly, based on the Boulton assay, we observed significantly higher absorbance due to monomeric anthocyanins in DTC final juice, 2.1 ± 0.7 Norm-AU, as compared to the PSC/BSC treatments, 1.0 ± 0.1 Norm-AU. "Monomeric anthocyanins" and "anthocyanins by pH differential" measure similar components, except that the former will be dependent on the pH of the juice, which changes during processing. In summary, there is a reduction in coprecipitation of monomeric anthocyanins with bitartrates during DTC processing, but this phenomenon is not mediated by copigmentation.

HPLC Analysis of Anthocyanins in Finished Juices. To better understand the mechanism behind monomeric anthocyanin loss in PSC but not DTC during cold storage, anthocyanins in PSC and DTC reconstituted juices were analyzed by HPLC, see **Figure 7** and **Table 3**. Delphinidin, malvidin, and cyanidin 3-glucosides were identified by comparison with authentic standards, and eluted in the range 18−25 min. The *p*-coumaryl derivative of delphinidin-3-glucoside (t_R = 36.4 min) was also identified

by comparison to an authentic standard. Several other peaks were tentatively identified based on a combination of factors. Peak retention order was compared to assignments reported in two other recent studies of Concord juice anthocyanins (*27*, *28*), and along with absorbance maxima were used to distinguish peonidin-3-glucoside, λ_{max} = 526 nm, and petunidin-3-glucoside, λ_{max} = 518 nm (*29*). Peaks eluting just after the delphinidin-3-(6-*p*-coumaryl)-glucoside peak (t_R = 36−38 min) were tentatively identified as other anthocyanin-3-coumarylglucosides based on similarity in retention time and the presence of a shoulder at 310−320 nm (*29*). Coumarylated species are the most common acylated anthocyanins in Concord grapes (*30*), and the contribution to total peak area is second only to the anthocyanin-3-glucosides. Peaks eluting prior to the anthocyanin-3-glucosides at t_R = 15−17 min were tentatively identified as anthocyanin-3,5-diglucosides based on previous references and an Abs 440/Abs Max ratio <0.2 (*29*). Peaks eluting after the monoglucosides at t_R = 27−30 min were tentatively identified as anthocyanin-3-(6-acetyl)-glucosides based on their elution just after anthocyanin-3-glucosides (*28*), the lack of a 310−320 nm shoulder, and an Abs 440/Abs Max ratio >0.25 (*29*).

Significantly smaller peaks were observed for several anthocyanins in the PSC juice in comparison to the DTC juice. The largest decreases were observed for the region tentatively identified as anthocyanin-3-(6-*p*-coumaryl)-glucosides (34−42%), with

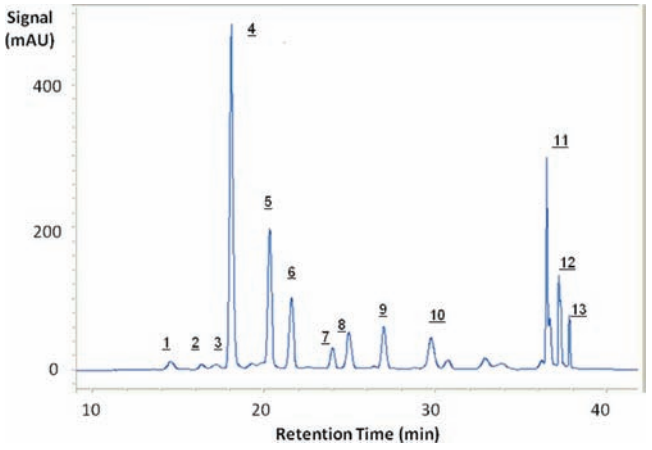

Figure 7. HPLC chromatogram of standard concentrate reconstituted Concord grape juice at 520 nm. Peak numbers correspond to the peaks listed in **Table 3**.

modest decreases also observed for anthocyanin-3-glucosides (3−21%), indicating that these species were preferentially lost during cold storage. Peaks tentatively identified as diglucosides, acetylated monoglucosides and coumarylated diglucosides showed negligible differences, and in some cases were slightly higher in the PSC juice. Interestingly, delphinidin-3-glucoside showed the smallest decrease of the five anthocyanin-3-glucosides during detartration, even though it is widely reported to be most rapidly hydrolyzed during storage in juicelike conditions (*31*).

These results are comparable to those of Vernhet et al., who showed that coumarylated species are more likely to be lost from solution than monomeric anthocyanins during detartration. In this previous work, coumaric acid derivatives represented a higher percentage of the total anthocyanins in bitartrate crystals than in their corresponding wines (*3*). The authors attempted to explain the preferential loss of coumarylated anthocyanins as due to lower solubility of these compounds in comparison to anthocyanin-3-glucosides. This hypothesis would also explain why diglucosides only experienced negligible losses. However, it is not clear with this explanation why DTC should yield no significant coprecipitation of anthocyanins with bitartrate crystals. Concentration results in a decrease in pH and an increase in the flavylium ion form, as described below, which is expected to increase solubility. The pH of our single-strength Concord juice (3.1), however, is already well below the pK_h of coumarylated anthocyanins (~4.0), so no large change in solubility is expected.

An alternative explanation for differential losses among species is that the stability of an anthocyanin species during detartration is related to its pK_h. The pK_h value of the monoglucosides decrease with electron withdrawing substitutes at the 3′ and 5′ positions of the B-ring, with the order OH > OCH_3 > H. Based on these principles and published pK_h values, we observed that anthocyanin-3-glucosides with higher literature pK_h values (*31*−*33*) had a larger percent decrease in PSC reconstituted juice: delphinidin-3-glucoside (3% decrease, pK_h = 2.36), petunidin-3-glucoside (13%, predicted 2.36 < pK_h < 2.6), malvidin-3-glucoside (15%, pK_h = 2.6), cyanidin-3-glucoside (15%, pK_h = 3.01), peonidin (21%, predicted pK_h > 3.01). Additionally, coumarylated anthocyanin-3-glucosides, which reportedly have higher pK_h values (*34*), were lost to a greater extent (34−42%) than other anthocyanin species in the juice. Conversely, 3, 5-diglucosides are reported to have lower pK_h values than monoglucosides, which may explain their negligible losses (*34*).

Our alternative hypothesis, in which the likelihood of coprecipitation is correlated positively with a higher pK_h values,

Table 3. Retention Time, Areas, and Peak Assignments from Standard Concentrate and Direct-to-Concentrate Juices by HPLC Analysis[a]

		average area			
peak	t_R (min)	SC juice	DTC juice	% decrease	assignment
1	15.4	290	280	−3*	*3,5-diglucoside*
2	16.4	166	180	9*	*3,5-diglucoside*
3	17.1	205	203	−1	*3,5-diglucoside*
4	18.1	7430	7673	3*	delphinidin-3-glucoside
5	20.3	3063	3528	15*	cyanidin-3-glucoside
6	21.6	1694	1921	13*	*petunidin-3-glucoside*
7	24.0	477	579	21*	*peonidin-3-glucoside*
8	24.9	1005	1153	15*	malvidin-3-glucoside
9	27	974	839	−14*	*acetylated 3-glucoside*
10	29.7	1135	1184	4*	*acetylated 3-glucoside*
11	36.4	3170	4247	34*	delphinidin-3-(6-*p*-coumaryl)- glucoside
12	37.2	1469	2091	42*	*coumarylated 3-glucoside*
13	37.8	534	713	34*	*coumarylated 3-glucoside*

[a] Percent decrease of SC compared to DTC is reported. * indicates that decrease was significant, p <0.05. Assignments are based on external standards (normal font) or tentatively identified (italicized) based on evaluation of UV/vis spectral features and relative retention times reported in other sources, as described in the text.

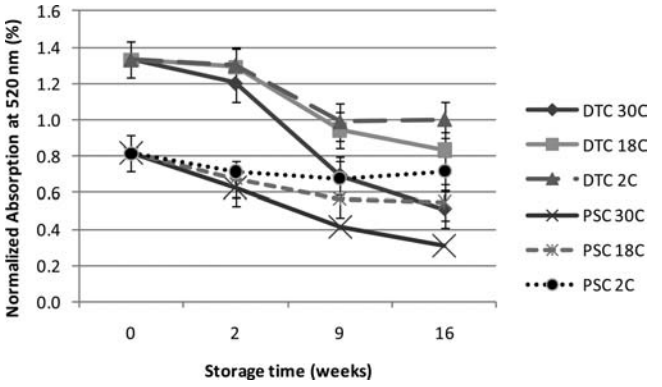

Figure 8. Shelf life study of Concord grape juice reconstituted to 16 °Brix from DTC and PSC concentrates at 30 °C, 18 °C, and 2 °C for 16 weeks. Normalized absorbance at 520 nm is reported. Error bars represent one standard deviation.

suggests that the flavylium ion is more likely to coprecipitate with bitartrate crystals, as based on the K_h equilibrium:

$$K_h = [\text{BH-OH}][H^+]/[AH^+][H_2O]$$

$$B = \text{the carbinol base}, \qquad AH^+ = \text{flavylium ion}$$

This suggests that interactions occurring during coprecipitation are between the flavylium form of the anthocyanins and the deprotonated sites of the bitartrate crystals, although as previously mentioned coprecipitation does not involve incorporation of the anthocyanin into the crystal lattice (*7*). Higher pH will result in a more negative surface charge on the bitartrate crystals (*35*), which would in turn increase the likelihood of coprecipitation with flavylium forms. The pH of single strength Concord juice from SC was 3.1 prior to cold-stabilization, while the pH of the DTC concentrate was 2.5. Celotti et al. (*35*) suggest that at pH = 2.5 there is a neutral surface charge on the bitartrate crystal, as opposed to a negative overall charge at pH = 3.1. While lower pH should also increase the flavylium ion concentration of all anthocyanin species, this may be less important than the availability of negatively charged bitartrate sites. Finally, we would also expect that DTC should have higher ionic strength during detartration, and thus a shorter Debye length for charged bitartrate crystals, which may further reduce the likelihood of coprecipitation.

Color Stability in DTC and SC during Shelf Life Studies. The overall color (absorption at 520 nm) of DTC and PSC final juices at 30 °C, 18 °C, and 2 °C was analyzed for stability during storage, reference **Figure 8**. Abs 520 of all samples decreased over time. All DTC juices had consistently greater 520 nm absorbencies than the PSC juices at the same temperature. DTC juices also had higher turbidity, and all samples were centrifuged after the 9 week time point. The reason for increased turbidity with DTC samples was unknown.

The percent loss in color intensity after 16 weeks storage is shown in **Table 4**. The decrease in absorbance was not significantly different between PSC and DTC processing for any storage temperature. The DTC juices have a significantly greater absorbance at 520 nm at all time points, indicating that the increased color intensity associated with DTC processing will still be present throughout juice storage.

In conclusion, direct to concentrate (DTC) methods yielded greater overall absorbance at 520 nm in final Concord juice, as compared to traditional hot press (PSC) and hot break (BSC) processing methods. The discrepancy is linked to the preferential loss of monomeric anthocyanins during the cold storage and detartration of single strength juice in standard concentrate methods, while no anthocyanin losses were observed during cold-stabilization of concentrate. We hypothesize that this difference is due to the lower pH of concentrate, which raises the surface charge of the bitartrate crystals, preventing anthocyanin adherence. If our hypothesis is correct, we expect that the fraction of anthocyanins that coprecipitate with potassium bitartrate will be dependent on pH, ionic strength, and water activity, a hypothesis which could be validated with model systems. Finally, these findings may have implications to the wine industry for red wines undergoing cold-stabilization, as it may be possible to modify wine properties to minimize losses during cold-stabilization. However, the different properties of wines, including higher tannins and presence of ethanol and other fermentation products, may result in different outcomes than those observed with Concord juice.

Table 4. Percentage of Color Loss during Storage for DTC and PSC Reconstituted Concord Grape Juice ($n = 4$) at 30°C, 18°C, and 2°C[a]

	percentage decrease in color following 16 weeks of storage	
temp (°C)	PSC (%)	DTC (%)
30	61.6 ± 8.3	61.7 ± 7.6
18	32.9 ± 16	37.5 ± 13
2	13.5 ± 11	24.9 ± 14

[a] Calculated by comparing the normalized absorption (520 nm) at the final storage time point of 16 weeks to the initial absorption at the start of the shelf life study.

ACKNOWLEDGMENT

We acknowledge Herb Cooley and Thomas Gibson for their assistance with juice production and Nick Jackowetz and David Manns for assistance with analysis.

LITERATURE CITED

(1) Morris, J.; Striegler, K. R. Grape juice: factors that influence quality, processing technology, and economics. In *Processing fruits science and technology*, 2nd ed.; Barrett, D. M., Somogyi, L. P., Ramaswamy, H. S., Eds.; CRC Press: Boca Raton, 2005.

(2) Ingalsbe, D. W.; Neubert, A. M.; Carter, G. H. Concord grape pigments. *J. Agric. Food Chem.* **1963**, *11* (3), 263.

(3) Vernhet, A.; Dupre, K.; Boulange-Petermann, L.; Cheynier, V.; Pellerin, P.; Moutounet, M. Composition of tartrate precipitates deposited on stainless steel tanks during the cold-stabilization of wines. Part II. Red wines. *Am. J. Enol. Vitic.* **1999**, *50* (4), 398−403.

(4) McClellan, M. R.; Race, E. J. Grape juice processing. In *Production and packaging of non-carbonated fruit juices and fruit beverages*; Hicks, D., Ed.; Blackie; Van Nostrand Reinhold: Glasgow; New York, 1990.

(5) Balakian, S.; Berg, H. W. Role of polyphenols in behavior of potassium bitartrate in red wines. *Am. J. Enol. Vitic.* **1968**, *19* (2), 91.

(6) Correagorospe, I.; Polo, M. C.; Hernandez, T. Characterization of the proteic and the phenolic fraction in tartaric sediments from wines. *Food Chem.* **1991**, *41* (2), 135−146.

(7) Rodriguezclemente, R.; Correagorospe, I. Structural, morphological, and kinetic aspects of potassium hydrogen tartrate precipitation from wines and ethanolic solutions. *Am. J. Enol. Vitic.* **1988**, *39* (2), 169−179.

(8) Talcott, S. T.; Peele, J. E.; Brenes, C. H. Red clover isoflavonoids as anthocyanin color enhancing agents in muscadine wine and juice. *Food Res. Int.* **2005**, *38* (10), 1205−1212.

(9) Boulton, R. The copigmentation of anthocyanins and its role in the color of red wine: A critical review. *Am. J. Enol. Vitic.* **2001**, *52* (2), 67−87.

(10) Mazza, G.; Miniati, E. *Anthocyanins in fruits, vegetables, and grains*; CRC Press: Boca Raton, 1993.

(11) Monagas, M.; Bartolomé, B. Anthocyanins and Anthocyanin-Derived Compounds. In *Wine chemistry and biochemistry*; Moreno-Arribas, M. V., Polo, M. C., Eds.; Springer: New York; London, 2009; p 439.
(12) Scheffeldt, P.; Hrazdina, G. Co-pigmentation of anthocyanins under physiological conditions. *J. Food Sci.* **1978**, *43* (2), 517−520.
(13) Bates, R. P.; Morris, J. R.; Crandall, P. G. Grape juice. In *Principles and practices of small- and medium-scale fruit juice processing*; Anonymous, Ed.; University of Florida: 2001; Vol. 146, p 135.
(14) Levengood, J.; Boulton, R. The variation in the color due to copigmentation in young Cabernet Sauvignon wines. In *Red Wine Color: Revealing the Mysteries*; Waterhouse, A. L., Kennedy, J. A., Eds.; 2004; Vol. 886, pp 35−52.
(15) Lee, J.; Durst, R. W.; Wrolstad, R. E. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *J. AOAC Int*. **2005**, *88* (5), 1269−1278.
(16) Bonerz, D. P. M.; Nikfardjam, M. S. P.; Creasy, G. L. A new RP-HPLC method for analysis of polyphenols, anthocyanins, and indole-3-acetic acid in wine. *Am. J. Enol. Vitic*. **2008**, *59* (1), 106−109.
(17) Hong, V.; Wrolstad, R. E. Characterization of anthocyanin-containing colorants and fruit juices by HPLC photodiode array detection. *J. Agric. Food Chem*. **1990**, *38* (3), 698−708.
(18) Talcott, S. T.; Lee, J. H. Ellagic acid and flavonoid antioxidant content of muscadine wine and juice. *J. Agric. Food Chem*. **2002**, *50* (11), 3186−3192.
(19) Jensen, J. S.; Demiray, S.; EgebO, M.; Meyer, A. S. Prediction of wine color attributes from the phenolic profiles of red grapes (Vitis vinifera). *J. Agric. Food Chem*. **2008**, *56* (3), 1105−1115.
(20) Main, G. L.; Morris, J. R. Impact of pruning methods on yield components and juice and wine composition of Cynthiana grapes. *Am. J. Enol. Vitic*. **2008**, *59* (2), 179−187.
(21) Versari, A.; Boulton, R. B.; Parpinello, G. P. A comparison of analytical methods for measuring the color components of red wines. *Food Chem*. **2008**, *106* (1), 397−402.
(22) Iyer, M. M.; Sacks, G. L.; Padilla-Zakour, O. I. Impact of harvesting and processing conditions on green leaf volatile development and phenolics in concord grape juice. *J. Food Sci.* **2010**, *75* (3), C297−C304.
(23) Talcott, S. T.; Brenes, C. H.; Pires, D. M.; Del Pozo-Insfran, D. Phytochemical stability and color retention of copigmented and processed muscadine grape juice. *J. Agric. Food Chem*. **2003**, *51* (4), 957−963.
(24) Harbertson, J. F.; Spayd, S. Measuring phenolics in the winery. *Am. J. Enol. Vitic*. **2006**, *57* (3), 280−288.
(25) Somers, T. C. Polymeric nature of wine pigments. *Phytochemistry* **1971**, *10* (9), 2175.
(26) Asen, S.; Stewart, R. N.; Norris, K. H. Copigmentation of anthocyanins in plant-tissues and its effect on color. *Phytochemistry* **1972**, *11* (3), 1139.
(27) McCallum, J. L.; Yang, R.; Young, J. C.; Strommer, J. N.; Tsao, R. Improved high performance liquid chromatographic separation of anthocyanin compounds from grapes using a novel mixed-mode ion-exchange reversed-phase column. *J. Chromatogr., A* **2007**, *1148* (1), 38−45.
(28) Wang, H. B.; Race, E. J.; Shrikhande, A. J. Characterization of anthocyanins in grape juices by ion trap liquid chromatography-mass spectrometry. *J. Agric. Food Chem*. **2003**, *51* (7), 1839−1844.
(29) Durst, R.; Wrolstad, R. E., Separation and characterization of anthocyanins by HPLC. In *Handbook of food analytical chemistry*; Wrolstad, R. E., Ed.; J. Wiley: Hoboken, NJ, 2005.
(30) Hrazdina, G. Anthocyanin composition of Concord grapes. *Food Sci. Technol*. **1975**, 8.
(31) Figueiredo, P.; Elhabiri, M.; Toki, K.; Saito, N.; Dangles, O.; Brouillard, R. New aspects of anthocyanin complexation. Intramolecular copigmentation as a means for colour loss? *Phytochemistry* **1996**, *41* (1), 301−308.
(32) Mazza, G.; Brouillard, R. Recent developments in the stabilization of anthocyanins in food-products. *Food Chem*. **1987**, *25* (3), 207−225.
(33) Stintzing, F. C.; Stintzing, A. S.; Carle, R.; Frei, B.; Wrolstad, R. E. Color and antioxidant properties of cyanidin-based anthocyanin pigments. *J. Agric. Food Chem*. **2002**, *50* (21), 6172−6181.
(34) Wrolstad, R. E. Symposium 12: Interaction of natural colors with other ingredients - Anthocyanin pigments - Bioactivity and coloring properties. *J. Food Sci*. **2004**, *69* (5), C419−C421.
(35) Celotti, E.; Bornia, L.; Zoccolan, E. Evaluation of the electrical properties of some products used in the tartaric stabilization of wines. *Am. J. Enol. Vitic*. **1999**, *50* (3), 343−350.

Received for review June 9, 2010. Revised manuscript received September 16, 2010. Accepted September 22, 2010. This study was funded by the New York Wine and Grape Foundation and Welch Foods, Inc. K.S.A. also received support from the Cornell University College of Agricultural and Life Sciences.