2022 R-21 final report
Proposal Category: __X_Research ___Outreach

Proposal Status: __X_ New Proposal ___Previously funded by SRSFC; has been previously funded for ___ years

Title: **Color Stability of Extracts from Muscadine Grapes High in Malvidin 3,5-diglucoside**

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Objectives:

The objective of this proposal is to see if the color stability of muscadine juice from grapes with high amounts of malvidin 3,5-diglucoside is improved compared to that of 'Noble', a standard juice muscadine variety high in delphinidin 3,5-diglucoside.

Background and outcomes

In North Carolina, crosses were made between DVIT 2970, a *V. popenoei* selection containing 74% of total anthocyanin as malvidin 3,5-diglucoside (M35DG), and a *V. munsoniana* x *V. rotundifolia* selection, FL 17:66, that is high in total anthocyanin content (>4,000 mg/100 g dry weight). From these seedlings, nine had fruit peels consistently high (>50%) in malvidin 3,5-diglucoside and sufficiently high (300-800 mg/100g fresh weight in total anthocyanin over 2-3 years of harvests. In contrast, peels from Noble and fresh market selections had <10% of total anthocyanins as M35DG and 30-300 mg/100 g fwt.

These nine selections, plus Noble (a muscadine juice standard), one red, and five purple fresh market cultivars (all low in M35DG) were used to prepare juice to follow color stability. Three sets of 4-6 berries were used to evaluate attributes of juice color and stability and were hand crushed to recover juice. Fruit from the *V. popenoei* crosses had peels that were very tough.

Juice recovery was 20-30% across all selections (Table 1). Total anthocyanin content of the juice from *V. popenoei* crosses was generally higher than that of Noble and other cultivars (except for

the seedless JB 8-38); 19-18-31 had much less juice anthocyanin than the other numbered lines. This selection was also lowest in pH and in soluble solids content and may not have been as ripe as the other samples.

We expected to find that 19-25-31 would have the best color stability due to its high total anthocyanin content and very high malvidin 3,5-diglucoside content. However, none of the numbered lines were outstanding in indices of juice quality. Percent monomeric and polymeric color were only slightly different from named cultivars or 'Noble'. Color density and hue tended to reflect the total amount of anthocyanin. Minutes to the color half life, a test of color stability, also did not show any particular gain in time. A browning index formula of ABS420/ABS520 was also calculated for juices at pH 3.0, and for those heated after addition of peroxide. Browning indexes for all samples was <0.4 with or without heat; addition of peroxide shifted values to about 2. We also tested concord grape juice from a sealed bottle with an expiration date of 2019, a cherry concentrate that we had already found had little total monomeric anthocyanin, and frozen cherries. For these non grape samples, the browning indexes were 1.9, 1.4, and 0.7 at time 0 and % polymeric color values were 95, 86, and 31, respectively.

Table 1. Characteristics of juice derived from muscadine genotypes

Genotype	%Juice yield	SSC	Juice pH	Total anthocyanin (mg/100 g fwt) juice	%Monomeric color	% Polymeric color	Color density	Hue	minutes to half life of color
19-18-31	32.82	11.9	2.71	47.63	70.11	29.89	5.40	5.31	23.9
19-25-31	19.82	13.3	2.89	110.39	84.00	16.00	10.71	5.72	21.4
19-36-31	23.30	16.2	3.12	146.63	84.82	15.18	13.34	5.46	22.0
20-4-31	19.05	16.1	2.97	134.95	80.50	19.50	14.66	7.54	22.1
20-13-31	22.97	14.7	2.96	75.24	78.72	21.28	8.13	5.85	20.8
20-14-31	23.32	16	3.26	112.86	81.01	18.99	10.78	6.25	19.8
20-27-31	27.23	11.9	3.17	96.68	80.63	19.37	9.77	6.33	22.3
20-17-31	22.71	16.9	3.01	123.34	81.89	18.11	12.37	5.92	22.2
20-35-31	22.64	13.3	3.12	119.45	79.89	20.11	11.38	6.28	21.2
Noble	28.10	14.80	3.31	88.05	75.87	24.13	9.67	5.79	20.3
JB838 sdlss	30.61	15.2	3.17	109.63	76.55	23.45	11.90	5.43	22.9
Lane	33.39	19.9	3.49	48.84	73.17	26.83	4.73	2.59	22.7
Nesbitt	32.24	20.3	3.57	46.53	80.68	19.32	3.86	2.00	22.3
Paulk	26.67	20.1	3.36	42.83	79.38	20.62	4.39	1.99	24.1
Razzmatazz©	26.17	19.5	3.06	17.43	78.74	29.31	2.68	1.87	18.5
Supreme	34.42	20.2	3.54	44.09	83.18	16.82	3.92	1.92	22.5

Numbered lines (top 9) are multi species crosses. JB8-38 and Razzmatazz© are *V. vinifera x V. rotundifolia* crosses.

Conclusions:

We were unable to demonstrate that juice from muscadine fruit high in malvidin 3,5 diglucoside, and high in total pigment, had better color stability than the standard processing grape 'Noble', or other muscadine cultivars known to be low in malvidin 3,5 diglucoside. We will look at modifying the color stability test, using a shorter time frame or less hydrogen peroxide, or try using ascorbic acid as the oxidant. There is a possibility (although unlikely) that the anthocyanin profiles of the muscadine juices changed after addition of pH 3.0 buffer and/or heating, addition of peroxide and samples will be run on HPLC to check this.

Materials and Methods:

Frozen fruit from 2021 harvests of 'Noble' and fresh market genotypes and 9 breeding selections listed in Table 1 were thawed and heated in a water bath until pulp/juice reached 70 C (about 70 min). Samples were sieved to collect the juice portion and SSC, pH determined. Aliquots of juice were diluted at 5 to 16X with pH 3.0 citric acid buffer in order to achieve ABS520 values that were within the linear range for anthocyanin absorbance on a Shimadzu 2540 spectrophotometer (between 0.3 and 0.7 abs). The pH of buffered juices was checked to make sure all were between 3.0 and 3.05 and juice samples filtered through Whatman no.4 filter paper to clarify samples.

Monomeric and polymeric anthocyanins were determined by pH differential (pH 1.0 and pH 4.5) and sodium bisulfite methods (Giusti and Wrolstad, 2001; Talcott et al., 2003). Color density and hue/tint of diluted extracts was determined by absorbance at 420, 520, and 700 nm, where color density= $(A_{520}-A_{700}) + (A_{420}-A700)$ and hue/tint=$(A_{420}-A700)/((A_{520}-A_{700})$.

Color stability was determined using the rapid test method of Talcott et al., 2003, where loss of absorbance of diluted samples is done by bleaching samples with hydrogen peroxide and calculating rate of loss of pigment. In this method, aliquots of buffered sample are set up in pairs, and one sample has 3% hydrogen peroxide added (e.g. 166 µl of H_2O_2 per 10 ml sample). Samples were vortexed vigorously, held at 60 °C for 30 min, rapidly cooled in ice water, and absorbance at 420, 520 and 700 nm determined.

A browning index of (Abs420-Abs700)/(Abs520-Abs700) (Muche et al. 2018) was done for pH 3.0 buffered juice extracts at time 0, after heating 30 min, and after heating 30 min in the presence of hydrogen peroxide.

References

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