Title: Evaluating Winemaking Potential for University of Arkansas Wine Grape Cultivars and Selections

Final Report

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Objectives:

- 1. Evaluate impact of winemaking methods on University of Arkansas Fruit Breeding Program wine grape cultivars and selections
- 2. Determine physiochemical attributes of juice, must, and wines from the University of Arkansas Fruit Breeding Program wine grape cultivars and selections
- 3. Disseminate information about winemaking potential of University of Arkansas Fruit Breeding Program wine grape cultivars and host a workshop for commercial grape growers and winemakers to evaluate the wines

Justification and Description:

The Fruit Breeding Program at the University of Arkansas was founded in 1964 by Dr. James N. Moore and has progressed with continued work by Dr. John Clark. The program has released over 50 fruit cultivars including blackberries, table grapes, muscadines, peaches, strawberries, and blueberries.

There are very few wine grape breeding programs in the United States, and each of those programs typically breed wine grapes suited for their region. Since the 1980's, the Fruit Breeding Program has been breeding wine grapes targeted for the Mid-South region of the United States. Evaluation of the winemaking potential of selections from the breeding program provides critical data for the release of wine grapes. Within the University of Arkansas System, the Fruit Breeding Program as worked collaboratively with the Food Science Department to evaluate the production of wines from the grape selections that have commercial potential for the southern region of the United States.

In 2016, the University of Arkansas System Division of Agriculture released their first two wine grapes, 'Opportunity' and 'Enchantment'. 'Opportunity' is a white wine grape, while 'Enchantment' is red. These cultivars will be best utilized by Mid-South wine-grape and wine industry and will expand options for wine grape production in this region. 'Opportunity' wine has a fruit-forward aroma with good body and green apple flavor. 'Enchantment' wine is similar to 'Petit Syrah' in color and mouthfeel. Since there are not many red grape cultivars that produce well in this region, 'Enchantment' can be a positive addition to the Mid-South grape and wine industry due to the versatility for wine production. Both cultivars have good adaptation and consistent productivity in Arkansas and produce quality wines.

Grape breeding is a decades-long process since grapes do not produce fruit for 2-3 years after planting. In addition, wine grape plants can be evaluated for productivity in the field, but a substantial amount of fruit is needed for wine production, which limits evaluation of winemaking techniques (skin contact time, tannin additions, types of yeast, etc.). Although the University of Arkansas Fruit breeding program has discontinued wine grape breeding, there are two advanced selections with potential for release, one an early ripening muscat selection (A-2359), the other an offspring of 'Gewürztraminer' (A-2574). There is still limited knowledge on the winemaking potential for the newly released cultivars and these two advanced selections. By getting grapes from both the Fruit Research Station and commercial growers who are evaluating the grapes, a larger quantity of fruit will be available for wine production.

The proposed work for the 2018 includes the following components:

<u>PI Threlfall, Co-PI Clark, Co-PI Howard, and Co-PI Mayfield will evaluate winemaking</u> potential for University of Arkansas wine grape cultivars and selections. Two wine grape cultivars and two wine grape advanced selections will be evaluated. Grapes will be harvested from the Fruit Research Station, Clarksville, AR and other commercial growers. After harvest, the grapes will be transported to the Food Science Department, Fayetteville, AR for wine production and evaluation of physiochemical attributes. A workshop with the wine industry personnel will be held at the Food Science Department to evaluate the wines.

The following is the proposed methods of each objective for the project:

Objective 1. Methods for wine production

The wine grape cultivars 'Opportunity' and 'Enchantment' and two advanced selections will be evaluated. Grapes will be harvested from the Fruit Research Station, Clarksville, AR and other commercial growers.

Red Wine Production

About 100 kg of 'Enchantment' grapes will be hand harvested and placed into storage at 2°C for approximately 36 hr. Grapes will be room temperature (21°C) for processing. Grapes will be weighed, then crushed and destemmed. The must (seeds, skins, and juice) will be separated into two equal lots (100 kg each) and placed in 60-L plastic containers with food-grade polyethylene liners for fermentation. Initial juice composition will be analyzed. Prefermentation treatments (no tannins and tannins added) will be applied. The musts will be inoculated with D254 yeast (0.26 g/L) and Fermaid yeast nutrient (0.26 g/L) (Lallemand, Montreal, Canada). The bags will be partially sealed with tape to allow carbon dioxide to escape during fermentation. During fermentation, the must cap will be punched down twice daily through the bag without exposing the must to air. The grapes will be fermented at 15°C on the skins until dryness (0° Brix). The must will be pressed in a 70-L Enrossi bladder-type press at 4 bar pressure (Enoagricol Rossi, Calzolaro, Italy), and the wine will be collected into glass carboys with fermentation locks. The wine will be racked three times to clarify and remove spent yeast cells. After completion of the fermentation, sulfur dioxide will be added as potassium metabisulfite. The wine will be further divided into two treatments of oak (no oak and oak added) and remain on the oak for a few weeks. The wine will be filtered using a 0.45 µm filter, then bottled into 375-mL bottles and stored at 15°C. Note: Wine will be made from grapes harvested from the Fruit Research Station in Clarksville, AR and the commercial grower, but treatments and replications might vary if the quantity of fruit is limited.

White Wine Production

About 50 kg of 'Opportunity' grapes and the two advanced selections (A-2359 and A-2574) will be hand harvested and placed into storage at 2°C for approximately 36 hr. Grapes will be room temperature (21°C) for processing. Grapes will be weighed, then crushed and destemmed. The must (seeds, skins. and juice) will be pressed in a 70-L Enrossi bladder-type press at 4 bar pressure (Enoagricol Rossi, Calzolaro, Italy), and the juice will be collected into glass carboys and cold settled overnight. The juice will be racked into two equal lots into glass carboys. Initial juice composition will be analyzed. The juice will be fermented in glass carboys with fermentation locks. Two different yeast types (EC1118 and GRE23) will be used for fermentation. The juice will be inoculated yeast (0.26 g/L) and Fermaid yeast nutrient (0.26 g/L) (Lallemand, Montreal, Canada). The wine will be racked three times to clarify and remove spent yeast cells. After completion of the fermentation, sulfur dioxide will be added as potassium metabisulfite. The wines will cold stabilize for 2 months at 2°C. The wine will be further divided into two treatments of sugar addition (no sugar and sugar added) prior to bottling. The amount of sugar added with be determined after bench trials, and sorbate will be added to prevent refermentation. The wine will be filtered using a 0.45 µm filter, then bottled into 375-mL bottles and stored at 15°C. Note: Wine will be made from grapes harvested from the Fruit Research Station in Clarksville, AR and the commercial grower, but treatments and replications might vary if the quantity of fruit is limited.

Objective 2. Methods for physiochemical analysis

Juice, must, and wine samples will be taken during the winemaking process for physiochemical analysis (composition, volatile compounds, nutraceuticals and color) in triplicate. Juice and must samples will be frozen (-20°C) and the wine samples will be stored at 15°C until analysis. The physiochemical attributes of samples will be evaluated at room temperature (24°C).

Composition analysis

Soluble solids, pH, and titratable acidity. Soluble solids, pH, and titratable acidity will be done on juice samples and pH and titratable acidity will be done on must and wine samples. Titratable acidity and pH will be measured with an automated titrimeter and expressed as g/L tartaric acid. Total soluble solids (expressed as %) of the sample will be measured using a refractometer. **Sugar and acid analysis.** Organic acids and sugars will be determined using HPLC. Glucose, fructose, and citric, tartaric, malic, succinic and lactic acids of the just, must, and wine will be measured using procedures described in Walker et al. 2003. The HPLC will be equipped with a Bio-Rad HPLC Organic Acid Analysis Aminex HPX-87H ion exclusion column (300 x 7.8 mm) and a Bio-Rad HPLC column (150 x 7.8 mm) in series. A Bio-Rad Micro-Guard Cation-H refill cartridge (30 x 4.5 mm) will be used as guard column. The peaks will be quantified using external standard calibration based on peak height estimation with baseline integration.

Volatile analysis

Volatile analysis. The volatile aroma compound profile of juice, must, and wine will be determined using solid-phase micro-extraction-gas chromatography (SPME-GC) and SPME-GC-MS. For SPME extraction of volatile compounds, 1 mL of juice will be placed in a 20 mL glass headspace vial with a small glass coated magnetic stir bar and the vial will be sealed with a PFTE lined silicone septa. The headspace volatiles will be extracted for 30 min at 65°C onto a Supelco 2 cm stable flex 85 μ m CAR/PDMS fiber. The SPME fiber will be manually placed into the injection port of a Varian CP-3800 gas chromatograph. Volatiles will be desorbed from the fiber and separated with a fused silica capillary column Agilent HP-5 column (30 m length, 0.25 mm id, 1 μ m film thickness). The oven temperature will be held at 28°C for 5 min and then ramped to 250°C at a rate of 8°C/min (total run time 32.75 min). To identify chromatogram peaks, the same procedure will be performed using a GC-MS. Mass spectral data will be done in scan mode with a mass scan range from 20 to 300 *m/z*. Volatile substances will be identified by comparison of their

mass spectra with the Wiley7NIST0.5 mass spectral library and relevant literature data (Kraujalyté et al 2012).

Nutraceutical analysis

Ellagitannins and flavonols. The juice or wine will be passed through 0.45 μ m PTFE syringe filters prior to High Pressure Liquid Chromatography (HPLC) analysis. The ellagitannins will be analyzed on a Waters Alliance HPLC system equipped with a Waters model 996 photodiode array detector and Millenium version 3.2 software. Separation will be performed using a Phenomenex Aqua 5 μ m C18 (250 x 4.6 mm) column with a binary gradient of 2% acetic acid for mobile phase A and 0.5% acetic acid in water/acetonitrile (1:1 v/v) for mobile phase B at a flow rate of 1.0 mL/min. A linear gradient will be run from 10 to 55% B (0-50 min), from 55 to 100% B (50-60 min), and from 100 to 10% B (60-65 min). The ellagitannins and flavonols will be identified on the basis of comparison of HPLC retention times to our previous HPLC results obtained using identical HPLC conditions and LC-MS methods. The ellagitannin peaks will be quantified at 255 nm as ellagic acid equivalents using external calibration curves of ellagic acid, with results expressed as milligram ellagic acid equivalents per 100 mL. The flavonols will be quantified at 360 nm as rutin with results expressed as rutin equivalents per 100 mL.

Anthocyanins. The anthocyanin analysis by HPLC will be performed based on previous methods with a 250×4.6 mm Symmetry C₁₈ column. The mobile phase will consist of a binary gradient of 5% formic acid (A) and 100% methanol (B). The flow rate will be 1.0 mL/min with a linear gradient from 2 to 60% B over 60 min. The anthocyanin peaks will be quantified at 510 nm using a photodiode array detector. Individual anthocyanins will be quantified as milligrams of cyanidin 3-glucoside equivalents per 100 mL. Total monomeric anthocyanins will be expressed as the sum of all individual anthocyanin concentrations.

Total phenolics. Total phenolics will be measured using the Folin-Ciocalteu assay (Slinkard and Singleton, 1977) with a gallic acid standard and a consistent standard curve based on serial dilutions. Absorbencies will be measured at 760 nm, and results will be expressed as mg of gallic acid equivalents (GAE) per 100 mL.

Total condensed tannin. The DMAC assay will be used to measure procyanidins with a procyanidin standard and a calibration curve. Samples will be diluted and DMAC standard will be added. Absorbance readings will be recorded using a spectrophotometer at 640 nm (Prior et. al., 2010).

Color analysis

L*a*b, hue and chroma values. The juice or wine color will be measured with a Colorgard system/05 with Unicam Helios Beta UV-vis spectrophotometer; standardized with DI water to CIE Lab transmission values L=100, a=0, b=0; L*a*b values measured to determine darkness (L*), green/red (a*), and blue/yellow (b*) characteristics.

Color density. The juice or wine color density will be measured with an 8452A photodiode array spectrophotometer (Hewlett-Packard, Palo Alto, CA) will be used to measure color density at 420 nm and 510 nm.

Polymeric color. The juice or wine polymeric color will be measured using potassium metabisulfite added to bleach monomeric anthocyanins; anthocyanin-tannin complexes remain unbleached; absorbancies of samples taken at 420, 510, and 700 nm using a spectrophotometer (Guisti and Wrolstad, 2001).

Objective 3. Methods for disseminating information and hosting a workshop

Provide the grape and wine industry with information on wine production methods and physiochemical attributes of University of Arkansas Fruit Breeding Program wine grape cultivars and selections and host a commercial grape grower and winemaker workshop to evaluate the wines.

Disseminate information

Provide the grape and wine industry with information on wine production methods and unique attributes of University of Arkansas Fruit Breeding Program cultivars and selections. Present findings at the Arkansas Association of Grape Growers annual conference and other conferences in the southern region.

Host workshop

Host a workshop at the University of Arkansas Food Science Department. Commercial grape growers and winemakers in Arkansas and surrounding regions will be invited to get industry feedback on the potential for the University of Arkansas Fruit Breeding Program wine grape cultivars and selections.

Results

Wine grapes in Arkansas were harvested in August 2018 and used for wine production which takes 6-9 months. The 2018 vintage will be bottled May-June 2019. The reviewer of this proposal prior to funding commented "*Lots of work for a small amount of \$…One year may not be enough to have a complete understanding of wine quality. Needed work given limited wine grape cultivar development for the region*". Winemaking is indeed a cost and time intensive project. The funds from this project had multiple impacts for the University of Arkansas Grape and Wine Program. These funds 1) supported research for two dissertation chapters for a graduate student on Physicochemical Properties of Wines Produced from Grapes Grown in the State of Arkansas, 2) enabled the evaluation of wines from University of Arkansas grape cultivars and selections from 2015 and 2017; 3) provided the ability to show the potential for wine grape cultivars for the southern region. The plan will be to have physiochemical and sensory data on the wine from the 2015, 2017, and 2018 vintages.

The following are the results for each objective of the project:

Objective 1. Wine production

One red cultivar ('Enchantment'), one white cultivar ('Opportunity'), and two white advanced selections (A-2359 and A-2574) were harvested from the Fruit Research Station, Clarksville, AR in August 2018. Red and white wine production data from 2015 and 2017 will be compared to the following to evaluate the quality at harvest and winemaking potential.

Red Wine Production

About 127 kg of 'Enchantment' grapes were harvested on August 8, 2018. Pre-fermentation treatments (no tannins and tannins added) were applied. The grapes were used for the production of about 83 L of wine. After completion of the fermentation, sulfur dioxide was added as potassium metabisulfite. *Note: The wines are being stored at 15°C. In February 2019, the wine will be further divided into two treatments of oak (no oak and oak added) and remain on the oak for a few weeks and then bottled in May-June 2019.*

White Wine Production

About 60, 56, and 65 kg of A-2359, A-2574, and 'Opportunity' grapes were harvested August 7-8, 2018. A-2359 produced about 36 L of juice for wine production, A-2574 produced about 34 L of juice for wine production, and 'Opportunity' produced about 42 L of juice for wine production. *Note: These wines will being cold stabilized at 2°C until February 2018. The wines will be further divided into two treatments of sugar addition (no sugar and sugar added) prior to bottling in May-June 2019.*

Objective 2. Physiochemical analysis

For the 2018 vintage, the initial juice and must soluble solids, pH and titratable acidity was done at harvest. 'Enchantment' had a soluble solids of 17.3%, pH of 3.10, and a titratable acidity of 0.69%, and the soluble solids of the must was adjusted to 21% prior to fermentation. A-2359 had a soluble solids of 16.4%, pH of 3.41, and a titratable acidity of 0.62%, and the soluble solids of the juice were adjusted to 21%. A-2574 had a soluble solids of 20.5%, pH of 3.29, and a titratable acidity of 0.78%. 'Opportunity' had a soluble solids of 16.4%, pH of 3.47, and a titratable acidity of 0.72% and the soluble solids of the juice were adjusted to 21%. The wines will be analyzed for physiochemical analysis (composition, volatile compounds, nutraceuticals and color) at bottling in May-June 2019 along with wines from 2015 and 2017.

For the 2017 vintage of 'Enchantment', the changes in physicochemical composition, aroma profiles from wine production techniques were evaluated. This objective was to evaluate the effects of tannin and oak addition on the basic composition, nutraceutical compounds, color, and volatile aroma profile of wines produced from Enchantment grapes grown in Arkansas after one year of bottle aging. This experimente was repeated in 2018, and analysis will occur in 2019 after bottling. Not only will this evaluate the impact on aroma profiles, phenolic compounds, and color from addition of tannin and oak during winemaking, but also the stability of the color of Enchantment wine. The initial data for 2017 'Enchantment' wine is presented in Tables 2-4.

Objective 3. Disseminating information and hosting a workshop

This project has provided the grape and wine industry an increased awareness of University of Arkansas Fruit Breeding Program wine grape cultivars and selections. A commercial grape grower and winemaker workshop was hosted to evaluate the wines from 2015 and 2017. *Note: After the wine from 2018 is bottled, another wine sensory evaluation will be done on wines from 2015, 2017, and 2018.*

Disseminate information

This project provided the grape and wine industry with information on wine production methods and unique attributes of University of Arkansas Fruit Breeding Program cultivars and selections. The results from this research have been used for posters and presentation at conferences for the Arkansas Association of Grape Growers, American Society of Enology and Viticulture (ASEV), ASEV-Eastern Section, and Southern Region American Society for Horticultural Sciences. The content will also be presented in other conferences in 2019 and 2020.

Posters 2018

Mayfield, S.E., R.T. Threlfall, L.R. Howard, J.R. Clark, and N.B. Stebbins. 2018. Evaluation of Anthocyanins in Arkansas-Grown Enchantment Grapes and Wine. American Society for Enology and Viticulture-Eastern Section 43th Annual Conference. July 9-11, King of Prussia, PA.

Mayfield, S.E., R.T. Threlfall, L.R. Howard, N.B. Stebbins, and J.R. Clark. 2018. Identification of anthocyanins in Enchantment grapes and during wine production. 69th Annual American Society for Enology and Viticulture Conference, June 18-21, Monterey, CA. (1st place winner)

Invited Presentation 2018

Threlfall, R.T. 2018. Arkansas Uncorked. Midwest Grape and Wine Industry Institute. Iowa State University. October 31, 2018, Ames, IA

Presentation 2019

Threlfall, R.T., S.E. Mayfield, J.R. Clark, and M.L. Worthington. 2019. Evaluating Winemaking Potential for University of Arkansas Wine Grape Cultivars and Selections. Southern Region-American Society for Horticulture Science Annual Meeting. February 3-5, Birmingham, AL.

Host workshop

The University of Arkansas System Division of Agriculture Food Science Department (Fayetteville, AR) hosted a tasting of wines produced from the University of Arkansas System breeding lines. The wine tasting was held in conjunction with an Alcohol and Tobacco Tax and Trade Bureau (TTB) Wine Compliance Seminar *(sponsored by the Arkansas Association of Grape Growers)* on May 23, 2018. There were 45 registrants at the seminar from the grape and wine industries in Arkansas, Oklahoma, and Missouri. The wine tasting was held after the seminar, and 26 people evaluated the Arkansas wines from 2015 and 2017. The attendees that tasted the wine have had years of experience with wine evaluation. The results of the evaluation are presented in Figure 1. The wines were evaluated for overall impression, overall appearance, overall aroma, and overall flavor on a 9-point verbal hedonic scale (1-dislike extremely, 5=neither like nor dislike, and 9=like extremely. These wines were all scored 6-8 (like slightly to like very much). Most importantly, the commercial grape growers and winemakers provided positive feedback on the potential for the University of Arkansas Fruit Breeding Program wine grape cultivars and selections. **The grape and wine industry also expressed interest in growing the grapes or selling the wine produced from these grapes**.

Conclusion

Within the University of Arkansas System Division of Agriculture, the Horticulture Department Fruit Breeding Program as worked collaboratively with the Food Science Department for over 30 years to evaluate the production of wines from the grape selections that have commercial potential for the southern region of the United States. This project has enabled the continued investigation of the wine making potential for the new cultivars 'Enchantment' and 'Opportunity' and the two potential advanced breeding selections, A-2359 and A-2574.

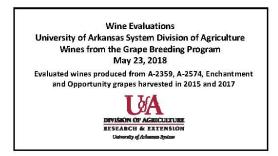
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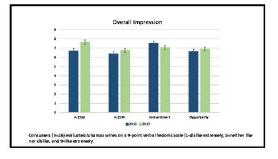
Evaluation of the winemaking potential of wine grape selections from the University of Arkansas System Division of Agriculture breeding program provided not only critical data for the release of the wine grapes, but also demonstrated to potential for these grapes to the commercial grape and wine industries in the southern region.

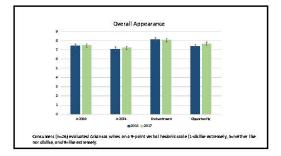
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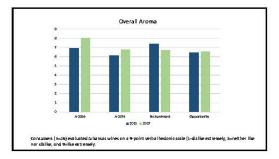
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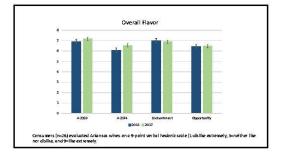
Figure 1. Expert sensory panel evaluations of wines (A-2359, A-2574, 'Enchantment' and 'Opportunity' from 2015 and 2017 vintages) from the University of Arkansas grape breeding program.

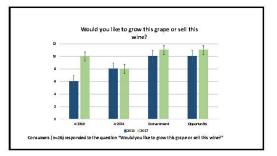












Genotype	Harvest date	Soluble solids (%)	рН	Titratable acidity (% tartaric)
A-2359	August 19, 2015	18.2	3.48	0.52
	August 17, 2017	14.9	3.056	0.73
	August 8, 2018	16.4	3.41	0.62
A-2574	August 19, 2015	19.5	3.45	0.58
	August 17, 2017	16.5	3.01	0.63
	August 7, 2018	20.5	3.29	0.78
Enchantment	August 25, 2015	19.3	3.60	0.83
	August 17, 2016	14.7	3.50	0.82
	August 8, 2018	17.3	3.10	0.69
Opportunity	August 11, 2015	16.6	3.63	0.39
	August 17, 2017	14.0	2.98	0.80
	August 8, 2018	16.4	3.47	0.72

Table 1. Harvest (2015, 2017 and 2018) composition of wine grape genotypes (A-2359, A-2574, 'Enchantment' and'Opportunity') from the University of Arkansas grapes breeding program.

			pН	TA (%)	Glucose	Fructose	Glycerol	Ethanol	Citric	Tartaric	Pyruvic	Malic	Succinic	Lactic	Acetic
Tannin Level	Oak Level	Month	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
No tannin	American oak	0	3.44	0.62	35.61	145.6	67.42	10.72	380.1	486.8	14.89	392.5	374.5	22.59	25.71
		2	3.48	0.62	28.22	128.4	66.63	11.89	355.6	413.1	11.33	361.1	403.4	26.13	26.06
		4	3.48	0.66	33.80	137.5	63.79	11.63	389.8	590.6	0.00	534.5	434.6	22.43	22.96
	French oak	0	3.44	0.62	46.20	161.0	63.84	9.81	379.7	479.8	14.85	388.1	371.4	21.58	21.29
		2	3.47	0.62	47.85	164.9	66.80	11.90	404.4	516.9	14.63	426.0	415.9	21.39	21.96
		4	3.47	0.66	47.59	163.7	65.69	11.94	381.0	589.0	6.69	478.0	438.2	17.36	21.70
	No oak	0	3.44	0.61	17.85	118.5	64.64	10.50	247.3	278.3	5.72	232.1	355.5	21.33	40.01
		2	3.49	0.63	39.60	147.9	63.08	11.42	292.6	328.0	4.59	334.9	453.8	28.87	29.59
		4	3.49	0.67	44.19	155.8	66.87	12.14	378.5	575.4	0.00	499.8	415.8	22.58	24.28
Tannin	American oak	0	3.39	0.62	38.40	215.7	63.19	10.20	300.0	367.0	9.61	273.4	350.4	17.47	27.95
		2	3.43	0.63	44.94	229.0	68.12	12.25	307.6	349.4	8.27	281.9	373.2	21.38	34.82
		4	3.43	0.66	45.60	239.4	64.67	11.94	191.3	556.7	0.00	390.5	338.2	8.33	28.09
	French oak	0	3.39	0.62	46.36	240.7	65.95	10.06	234.1	272.4	4.18	180.5	308.0	15.77	26.70
		2	3.43	0.63	52.73	258.1	62.99	11.58	345.2	419.6	10.57	334.2	364.4	20.11	30.85
		4	3.43	0.66	51.99	257.7	63.23	11.45	196.9	567.9	0.00	399.8	336.0	5.98	26.60
	No oak	0	3.39	0.62	49.21	251.6	68.34	11.12	243.5	282.8	5.70	211.7	337.9	16.00	26.08
		2	3.44	0.63	49.87	251.0	67.43	12.07	304.2	345.0	8.54	290.4	383.5	14.14	25.63
		4	3.43	0.66	48.32	248.8	65.63	11.88	274.4	562.0	6.59	379.1	359.4	9.86	25.95

Table 2. Composition of 'Enchantment'	wine with different tannin	and oak addition durir	g storage at 15°C (2017).

Table 3. Color analysis of 'Enchantmen't wine with different tannin and oak addition during storage during storag	e at 15°C
(2017).	

			Total condensed tannin	L*	a*	b*	x	У	z	Abs (420 nm)	Abs (520 nm)	Color density	% Polymeric color
Tannin Level	Oak Level	Month	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
No tannin	American oak	0	38.06	1.04	1.07	0.26	0.14	0.11	0.10	10.05	15.30	19.36	42.49
		2	37.39	0.83	1.33	-0.19	0.12	0.09	0.11	9.22	15.00	21.20	47.57
		4	32.00	1.42	1.30	0.43	0.18	0.16	0.15	14.66	16.23	22.65	45.08
	French oak	0	36.62	0.95	1.67	0.50	0.14	0.11	0.09	10.25	15.69	19.18	42.14
		2	38.68	0.71	1.80	-0.19	0.12	0.08	0.11	8.43	14.48	20.96	46.41
		4	40.33	1.19	1.76	0.21	0.17	0.13	0.13	11.39	13.78	19.62	49.14
	No oak	0	36.61	0.95	1.32	0.35	0.14	0.12	0.09	10.70	16.14	19.73	42.40
		2	38.93	0.93	1.72	0.04	0.14	0.10	0.10	8.50	14.18	20.53	46.06
		4	36.29	1.40	1.44	0.22	0.18	0.15	0.15	10.44	13.11	19.45	56.70
Tannin	American oak	0	32.83	0.87	0.97	0.42	0.12	0.10	0.08	17.37	22.38	17.31	44.87
		2	32.27	0.76	1.57	-0.00	0.11	0.08	0.09	8.35	14.86	21.31	44.22
		4	32.41	0.86	1.81	0.36	0.14	0.09	0.08	8.48	12.14	17.69	61.66
	French oak	0	33.11	1.60	1.65	0.42	0.22	0.18	0.17	17.32	22.22	17.80	40.95
		2	31.44	0.81	1.37	0.20	0.12	0.09	0.09	8.82	15.33	21.98	43.58
		4	32.75	0.78	1.62	0.26	0.12	0.09	0.07	9.12	12.63	18.42	72.92
	No oak	0	35.22	0.57	1.78	0.47	0.11	0.06	0.04	12.40	17.94	18.32	40.74
		2	37.88	0.80	1.97	0.15	0.14	0.09	0.08	8.04	14.42	20.71	44.93
		4	38.04	1.27	1.86	0.12	0.18	0.14	0.14	11.18	14.35	19.86	82.36

Table 4. High Performance Liquid Chromatogram (HPLC) analysis of the anthocyanins in 'Enchantment wine with different tannin and oak addition during storage at 15°C (2017).

* "Dpd" = delphinidin, "cyd" = cyanidin, "ptd" = petunidin, "pnd" = peonidin, "mvd" = malvidin, "glc" = glucoside, "acy" = anthocyanin

			Dpd-3-glc	Cyd-3-glc	Ptd-3-glc	Pnd-3-glc	Mvd-3-glc	Total ACY
Tannin Level	Oak Level	Month	Mean	Mean	Mean	Mean	Mean	Mean
No tannin	American oak	0	12.33	1.54	16.99	10.17	38.02	94.12
		2	6.19	0.49	9.00	5.06	22.36	49.58
		4	6.67	0.64	9.88	5.25	22.63	51.42
	French oak	0	12.27	1.45	16.98	9.72	37.65	89.87
		2	7.16	0.64	10.37	5.45	24.70	55.22
		4	7.64	0.70	10.91	5.71	24.64	56.86
	No oak	0	11.59	1.34	16.27	9.30	36.79	86.13
		2	7.42	0.63	10.72	5.85	25.12	56.85
		4	7.58	0.80	11.01	5.72	24.60	56.72
Tannin	American oak	0	11.48	1.20	14.89	9.30	32.76	80.86
		2	6.72	0.51	9.18	6.01	22.24	51.17
		4	7.30	0.64	9.73	5.92	21.22	51.92
	French oak	0	10.90	1.34	14.10	9.02	30.31	75.99
		2	6.50	0.51	8.90	5.48	20.85	48.71
		4	6.85	0.56	8.84	5.20	19.50	47.60
	No oak	0	14.05	1.61	17.96	11.76	38.55	100.38
		2	7.96	0.55	10.59	6.89	24.70	59.27
		4	8.78	0.87	11.37	6.44	24.58	60.34