Pennsylvania Wine Market & Research Promotion Program

Final Report

A financial status report and a project performance report will be required on a semi-annual basis. October and April reports are due. A final report may serve as the last semi-annual report due 30 days after completion of the contract. Grantees shall monitor performance to ensure that time schedules are being met and projected goals by time periods are being accomplished. Please submit reports to: RA-AGCommodities@pa.gov.

SECTION 1 – SUMMARY INFORMATION

Date of Report: September 20, 2021

Contract/PO#: PO-63019424 Fiscal Year: 2020-2021 Round of Grant: (i.e. Round 1, Round 2, etc) 4

Title of Paper: Final Report for "Improving Tannin Extraction in Wines Made from Red Interspecific Hybrid (Vitis ssp.)"

Organization: The Pennsylvania State University

Project Coordinator: Dr. Helene Hopfer

Organization Address: Department of Food Science, 218 Food Science Bldg.

City/State/Zip: University Park, PA 16802

Business Phone: 814-863-5572 Cell Phone: NA

Email: hopfer@psu.edu

Progress Report: ☐ October ☐ April ☒ Final

Area of Focus: ☒ Research ☐ Marketing

SECTION 2 –OBJECTIVES | TIMELINES | OUTCOMES | BUDGET

(A comparison of actual accomplishments to the objectives for that period?)

This project is a systematic investigation of the utility of pre-fermentation enzyme treatments to increase polyphenol extraction and color stability in red hybrid wines.
Recent findings (Springer et al., 2016; Springer & Sacks, 2014) showed that tannin-binding proteins and, to a lesser extent pectin, present in higher concentrations in hybrid grapes relative to *V. vinifera* grapes are responsible for low tannin concentrations in finished wines made from red hybrid grapes. If those tannin binding components could be enzymatically degraded in the pre-fermentation period in a way that renders them less able to sequester condensed tannin, this would provide PA winemakers with a relatively easy and cost-effective tool to markedly improve red hybrid wine quality.

Objective 1: To increase final concentrations of protein-precipitable tannin in wines made at bench-scale from red hybrid grapes through the use of exogeneous proteolytic and pectinolytic enzymes during the prefermentation maceration period. **Objective 2**: To evaluate the effect of cap management practices, specifically pre-fermentation cold maceration and continuous must agitation, on protein extraction and resulting proteolytic and pectinolytic enzyme activity. **Objective 3**: Based on the optimized parameters and conditions from the bench-scale trials (Objectives 1 and 2), conduct a pilot-scale winemaking trial to both corroborate those results found at bench-scale and to produce research wine in sufficient quantity for evaluation by winemakers at extension events, presentations and workshops (Year 2).
As an exploratory subobjective, we will also ferment protease-treated musts in a continuously agitated fermenter which we hypothesize will result in increased final tannin concentrations in wine beyond what can be achieved through the use of enzymes alone.

Based on the results reported in the last progress report, fermentation trials with pre-fermentative enzyme additions for pectinase, cellulase and papain were replicated in an additional red hybrid grape cultivar, Chambourcin. Similar to the first set of experiments, grapes sourced from Penn State's Horticultural farm and one commercial grower have been fermented in replicate. Treatments included an untreated control, and pre-fermentation treatments with cellulase, pectinase, papain, and combinations of enzymes (all two-way and three-way combinations). Two different level of enzymes were added – a high level and a low level, based on recommendations from the enzyme suppliers to gain insight into potential concentration dependencies. Samples were monitored for progression of fermentation (i.e., time to reach dryness), and finished wines were characterized by Folin-Ciocalteu assay for total phenolic content (TPC) in gallic acid equivalents (GAE), to compare to previous results.
In addition, the high-throughput Adams-Harbertson assay (Harbertson et al. 2003; Harbertson et al., 2015) was developed to determine tannin concentration, iron-reactive phenolics, and polymeric pigments of the treated wines. Data analysis is on-going, but first results are shown for the TPC analysis:

- Pre-fermentation treatments of Chambourcin grapes all led to higher total phenolic content (TPC) in the finished wines compared to the untreated control, however, the effect differed depending on the enzyme (**Figure 1**).
- Pectinase treatments led to highest TPC contents in the finished wine, which were significantly above the control levels.
- Different enzyme addition rates led to mixed results: in general, a dose effect was found for most enzyme treatments, except for the 3-enzyme combination and the cellulase+papain treatments. For pectinase and cellulase a higher enzyme dosage led to a higher total phenolic content in the finished wines, while for all other treatments, the reverse effect was observed.

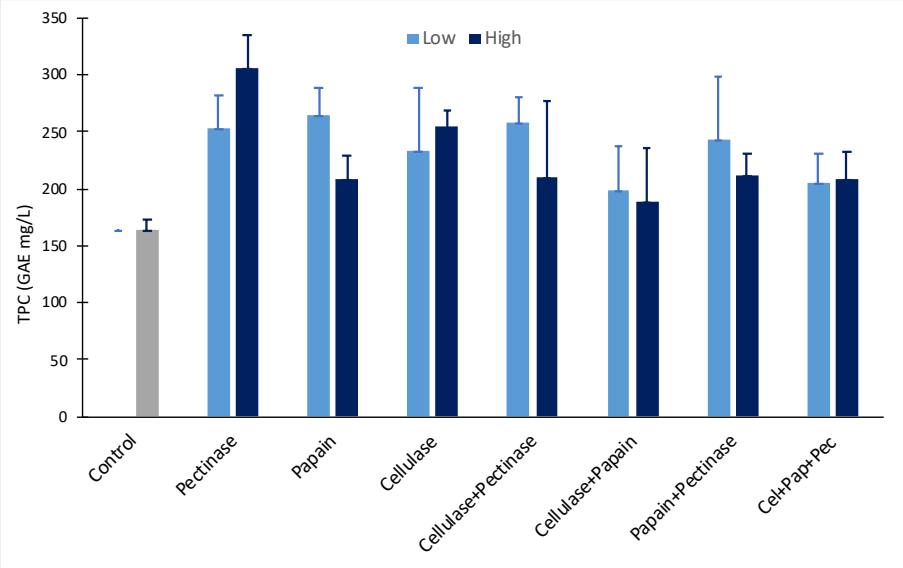

Figure 1. *Total phenolic content (TPC) in gallic acid equivalent (GAE) concentrations of experimental Chambourcin wines that were treated with different enzymes prior to fermentation at two different addition rates. Shown are averages of biological replicates (n=2-4) with standard deviation error bars.*

Overall, these results validate that pre-fermentation enzyme treatments increase total phenolic content in different red hybrid wines, with the greatest increases for pectinase, cellulase, and combinations of these enzymes.

These findings were replicated in another red hybrid variety, Noiret. Similarly to the Chambourcin results, the different pre-fermentation enzyme treatments had different effects on tannin retention in the finished Noiret wines (**Table 1**). The single enzyme pectinase, papain, and cellulase treatments as well as the combined treatment all led to tannin concentrations that were significantly below the tannin levels of the untreated control. The combination of papain and cellulase showed the highest tannin and anthocyanin concentrations, which indicates that there may be a benefit to adding enzymes at different stages. This was illustrated by the combined treatment, where the papain, a proteolytic enzyme, was added after maceration.

Table 1. *Effects of pre-fermentation enzyme treatment on tannins, anthocyanins, small and large polymeric pigments as assessed with the Adams-Harbertson assay in Noiret wines. Values in columns that share the same letter are not significantly different from each other as assessed by Tukey post-hoc comparison.*

Treatment	Tannin [mg/L CE]	Anthocyanin [mg/L M3GE]
Control	676^{ABC}	$\mathbf{713^{A}}$
Pectinase	621^{CD}	591^{BCD}
Papain	619^{BCD}	635^{BC}
Cellulase	621^{CD}	619^{BC}
Pectinase & Papain	732^{AB}	595^{BCD}
Pectinase & Cellulase	658^{BCD}	580^{CD}
Papain & Cellulase	$\mathbf{749^{A}}$	$\mathbf{658^{AB}}$
Combined	574^{D}	548^{DE}
Combined, Late Papain Addition	$\mathbf{741^{A}}$	526^{DE}
Combined, Late Addition	701^{ABC}	493^{E}

In addition to the work described above we also investigated the role that various plant material and macromolecules play in tannin precipitation, as current research supports that cell wall material is involved in binding tannins (Beaver et al., 2020; Osete-Alcaraz et al., 2020). We used a high-tannin *V. viniferia* wine (Cabernet Sauvignon) as a model for these studies. In our first experiments, Cabernet Sauvignon was dosed with 10% (w/v) ground cell wall material from two different Arabidopsis cultivars, differing in cell wall composition. The tannin retention significantly decreased in wines containing added cell wall material relative to the control, as observed by the total iron reactive phenolics; similar results were found at a 1% dosing rate as well (**Table 2**).

We then studied the role of individual macromolecules on tannin retention, specifically pectin (both a low methoxyl and high methoxyl content pectin), cellulose and maltodextrin. When comparing the iron reactive phenolics, no statistically significant difference was observed at 1% dosing. However, when protein precipitation was used to isolate the tannins in the treated wines, wine dosed with maltodextrin had significantly lower tannins compared to the wines dosed with pectin or cellulose (**Table 3**). These surprising results point towards different effects of plant cell wall macromolecules on wine tannins, which in turn would inform which pre-fermentation enzyme treatments would be most effective in increasing tannin retention in red hybrid wines.

Table 2. *Iron Reactive Phenolics (IRP) in Cabernet Sauvignon with 1% added Arabidopsis cell wall material.*

Treatment	IRP (mg/L CE)
control	1301 ± 66 a[a]
Columbia (wild type)	1213 ± 18 b
PGX1-AT (mutant)	1191 ± 23 b

[a] Different letters correspond to statistical difference at $\alpha = 0.05$

Table 3. *Polyphenol Content of Cabernet Sauvignon with added macromolecules (1%)*

Treatment	Tannin (mg/L CE)	IRP (mg/L CE)
control	385 ± 40 a [1]	1053 ± 35 a
pectin HM[2]	343 ± 12 a	960 ± 67 a
pectin LMA[3]	377 ± 7 a	954 ± 50 a
maltodextrin	193 ± 10 b	980 ± 39 a
cellulose	-[4]	967 ± 70 a

[1] Different normal text letters within the same column are significantly different at $\alpha = 0.05$.
[2] High methoxyl content.
[3] Low methoxyl content.
[4] Gel formation interfered with tannin measurement.

Objective 2: After trialing a commercial fermentor that allows for continuous agitation (GoFermentor) in a white wine-winemaking project, the exploration of using this continuously agitated fermenter in conjunction with enzyme-treated musts was stopped for several reasons: (i) the GoFermentor system decreases exposure to oxygen during fermentation, which is beneficial in white wine-making, but not in most red winemaking protocols; (ii) although the punchdown function works, the pressing at the end of fermentation is highly inefficient due to the less-than-ideal set-up of the system; (iii) the bags in which the fermentation takes place are prone to puncture and tearing.

Due to the problems with the GoFermententor an alternative method of small-scale cap management is being investigated, which is feasible at the lab scale. Bench-top fermentations, which were adopted for objective 1 are also being tested for their ability to vary tannin extraction with different cap management practices and temperatures as well as the oxygen pickup of these ferments. This will allow high sample through-put and the ability to compare a large number of treatments. While there is reason to believe comparative differences will

be relevant to commercial production there will be a need to understand how these results scale to larger and commercial vinification in a future study.

Objective 3: Based on the optimized parameters and conditions from the bench-scale trials, we are currently setting up a pilot-scale winemaking trial to both corroborate those results found at bench-scale and to produce research wine in sufficient quantity for evaluation by winemakers at extension events, presentations, and workshops. The treatments that will be evaluated include:

1. Control treatment (= no added enzymes)
2. Combined treatment with pectinase and cellulase added pre-fermentation and papain post-maceration
3. Papain + Cellulase added pre-fermentation
4. Pectinase + Papain added pre-fermentation

Results from this project will be presented at the American Society of Enology & Viticulture – Eastern Section conference next year and will able be disseminated via the PWRMB symposium.
Financial reporting is provided by the Department of Research Accounting at PSU in accordance with the terms of the grant agreement.

SECTION 3 – SCOPE OF WORK

(Reasons why established objectives were not met, if applicable?)

Not applicable. Project deliverables are all reported as we were able to adapt our original plans despite the ongoing Covid-19 pandemic.

We are planning on publishing in an open-access journal which allows us to share our results freely without a paywall with stakeholders from the PA wine industry.

In collaboration with Co-PI Kelley, we are planning on disseminating our findings also through blog posts at the PSU Wine & Grapes website (https://psuwineandgrapes.wordpress.com), and at the next PWMRB Symposium.

SECTION 4 – DELAYS/RISKS

(Reasons for any problems, delays, or adverse conditions which will affect attainment of overall program objectives, prevent meeting time schedules or objectives, or preclude the attainment of particular objectives during established time periods. This disclosure shall be accomplished by a statement of the action taken or planned to resolve the situation?)

Not applicable.

SECTION 5 – SPECIAL NOTES

(What objectives and timetables are established for the next reporting period? Etc.)

References

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