What are the challenges to producing high quality red wines from interspecific grapes?

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Abstract. In the U.S. Midwest region, interspecific cold hardy grape cultivars have been developed to be resistant to the harsh cold winter, late spring frosts, and hot and humid summer. However, interspecific grape red wines tend to have higher acidity and lower tannins content than *Vitis vinifera* wines. This leads to unbalanced wines and an increase of the risk of oxidation and therefore impact the overall quality over time. The content and type of phenolic compounds differ in interspecific grapes and wines, depending on the cultivars, the viticultural practices, the environmental conditions and the wine making process. Because the chemical properties of red wines produced from interspecific grapes compared to *Vitis vinifera* is not well known, it is a challenge to determine the best wine making practices to produce a high quality wine that remains stable over time. This study focuses on evaluating phenolic compounds, oxidation-related compounds, and sulfur dioxide content in Marquette and Frontenac wines aged in bottles for up to 9 years. The goal is to help improve quality of red wines made from interspecific cold-hardy grape cultivars to increase consumer acceptance and develop optimal wine making practices.

1 Introduction

The goal of the wine industry is to produce high quality wines that could been joyed by the consumers at the time of purchase or after aging for a few years. Wines in Iowa and the U.S. Midwest are produced from cold-hardy grapes that are different than *Vitis vinifera*, the most commonly grown grapes in the world. Cold-hardy red wines tend to have high acidity levels, high color intensity, and low tannin content. It has been previously shown that a high quality red wine is positively correlated with high phenolic sand tannin content, in Cabernet sauvignon and Shiraz [1]. Phenolic compounds are a large family of secondary metabolites in grapes. Condensed tannins are phenolic compounds responsible for the astringent mouth feel, color stability and antioxidant properties of red wines. This helps protect wines against oxidation, thus maintaining quality over time. Wine quality is very subjective but is related to the consumer acceptance and associated with what a glass of wine looks like, smells like, and tastes like without any defect and in which all components work in harmony. Because the chemistry of red wines produced from cold-hardy grapes compared to *Vitis vinifera* is not well known and is very different, such as about 15-fold less tannin in interspecific wines[2], it is a challenge to determine the best wine making practices to produce a high-quality wine from those cold-hardy grape scapable of aging for a few years.

In the past ten years, many studies focused on better understanding the extraction and retention of phenolic compounds, especially anthocyanins and tannins, throughout red wine making from interspecific grape cultivars [3–7]. Among winemaking techniques commonly used on *Vitis vinifera* grapes, including cold soak [6], enzymes [6], and enological tannins addition [5] did not show promising results for interspecific grape cultivars. It has been suggested that macromolecules such as polysaccharides and pathogenesis-related proteins [7] might be involved in the lower tannin extract ability of those grape cultivars [8]. Therefore, other techniques, such as the use of bentonite to bind to proteins and the application of high-power sonication to change the conformation of proteins have been evaluated to help improve tannin extraction and retention [4, 9]. However those two techniques did not impact the tannin content much either. Other techniques to break down cell wall material and help the release of tannins are currently ongoing but is a continuous challenge that some states and countries are facing. As mentioned above, the main challenge is about cold-hardyred wine quality as it relates to tannin content, which is very low in those wines. The wine industry commonly uses sulfur dioxide as an antioxidant and antimicrobial to protect wines against oxidation and microbial spoilage, however the rational use of sulfur dioxide in wine making is based on the chemistry of *Vitis vinifera*. The main focus of this study was to evaluate the evolution of cold-hardy red wine quality over time, focusing on oxidation-related faults.

2 Material and methods

Chemicals

Sodium hydroxide 1.0N and 0.1N solutions and triethanolamine were purchased from Aqua Solutions Inc. (Texas, USA). SO_2 titrant, SO_2 reactant solution, and SO_2 acidsolution (7.3% Hydrochloric Acid) was purchased from Vinmetrica (California, USA). Potassium metabisulfite was purchased from Acros Organics (Germany). Hydrochloric acid was purchased from Sigma Aldrich (Missouri, USA).

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Glacial acetic acid, sodium dodecyl sulfate, and ferric chloride was purchased from Fisher Scientific (New Jersey, USA).

Wine samples

Commercial wines of Marquette (9 wines) and Frontenac (14 wines) cultivars of different vintages between 2013 and 2020, were provided by three Iowa wineries (A, B, and C). The details of the vintage for each variety and the pH and free sulfur dioxide content at bottling are shown in Table 1 as provided by the wineries. One bottle of each condition was provided by the wineries.

Table 1. Basic details about the wines at bottling, as provided by the involved wineries.

Cultivar	Winery/ Vintage	Closure type	pH	Free SO_2 (mg/L)
Frontenac	A 2013	screw cap	3.54	110
	A 2014		3.75	75
	A 2016		3.64	62
	A 2017		3.63	63
	A 2018		3.78	84
	A 2020		3.39	33
	B 2016	cork	3.48	40
	B 2017		3.50	45
	B 2018		3.59	40
	B 2019		3.41	40
	C 2015		3.58	32
	C 2016		3.60	40
	C 2017		3.48	28
	C 2019		3.45	30
Marquette	A 2013	screw cap	3.92	117
	A 2015		3.58	60
	A 2016		3.61	56
	A 2017		3.44	42
	A 2020		3.14	18
	B 2014	cork	3.65	45
	B 2015		3.64	45
	B 2016		3.50	40
	B 2017		3.69	32

Basic chemical analysis

A pH meter (Thermo Scientific Orion Star A211 pH meter) was used to determine the pH as well as the titratable acidity (TA) using a titration method to get a pH endpoint of 8.2. Using a UV-VIS spectrophotometer (Thermo Scientific Genesys), color intensity was defined as the sum of absorbance at 420 nm, 520 nm, and 620 nm, and the hue, which was defined as the ratio of the absorbance values at 420 nm to520 nm, were determined. The content of ethanol in all the wines was analyzed by high-performance liquid chromatography with a refractive index detector (HPLC-RID) as previously published (Cheng et al., 2022).

Total phenolic compounds and tannin content

Total iron-reactive phenolic content was determined in all the wines following a previously described procedure [10]. Briefly, 75µL of centrifuged wine samples were added to 800 µL resuspension buffer (pH 9.4) in Visible 1 cm cuvettes, vortexed and incubated for 10 min prior to analysis at 510 nm. Then 125 µL ferric chloride was added, vortexed, incubated for 10 min and analyzed at 510 nm. The content was expressed as (+)-catechin equivalent using a calibratio curve. Total tannin content was determined in all centrifuged wines using the previously described method by HPLC-DAD [11].

Acetaldehyde content determination

The acetaldehyde content in all the wines was determined after derivatization with 2,4-dinitrophenylhydrazine (DNPH) by HPLC-DAD. Briefly, 100 µL of wine was added to 25 µL of fresh sulfur dioxide (1120 mg/L, from potassium metabisulfite) and 20 µL of sulfuric acid 25% and 140 µL of DNPH reagent (8 g/L) [12]. The solution was then placed at 65°C for 15 min and cooled down in an ice bath prior to centrifugation for 5 min at 13,000 g. Fifteen µL of supernatant was then injected by HPLC-DAD and quantified at 365 nm using a calibration curve of acetaldehyde after derivatization. A 1260 infinity II Agilent Technologies HPLC-DAD with a Zorbax SB-C18 rapid resolution HT (4.6 ×100 mm, 1.8 µm) column wasused. The gradient used was with solvent A (0.5% v/v formic acid in water) and solvent B (100% acetonitrile) as follows: 0min (35%B), 8min (60%B), 13 min (95%B) 15min (95%B), 16 min (35%B), 20 min (35%B). The oven temperature was set at 35°C and the flow rate was 0.75 µL/min.

Sulfur dioxide determination

Free and total sulfur dioxide (SO_2) contents were determined by two methods, the aeration/oxidation method recommended by the OIV (Oiv-Ma-As323-04a.Pdf) as well as by titration using a Vinmetrica SC-300 analyzer (Manual_SC300.Pdf). Following the titration procedure, SO_2 acid and SO_2 reactant reagents were added to 25 mL of

wine and titrated using SO_2 titrant. Titration was complete once the titrant caused the display to exceed 50, and the red LED and beeper to stay on for longer than 15s.

Statistical analysis

Allthe analyses were carried out in triplicate for each wine. The data were analyzed using JMP Pro 15.1.0 software (SAS, Cary, NC, USA).

3 Results and Discussion

Overall, the pH of Frontenac wines ranged from 3.29 to 3.75 and the pH of Marquette wines ranged from 3.43 to 3.77 (Table 2). The titratable acidity (TA) ranged from 6.7g/L to 9.6 g/L with 2015 Frontenac wine being the highest TA value. The alcohol content varied from 10.5% to 13.9% with 2013 Frontenac and 2013 Marquette wine A being the highest. Those basic parameters were in agreement with previously published data of Frontenac and Marquette wines [13].

Table 2. Basic chemical properties of aged wines.

Sample name		pH (±0.01)	Titratable acidity (g/L tartaric)	Ethanol (%v/v)
Frontenac	A 2013	3.51	7.7±0.3	13.8
	A 2014	3.75	7.2±0.4	11.1
	A 2016	3.58	7.5±0.1	11.4
	A 2017	3.69	7.8±0.1	11.5
	A 2018	3.99	7.9±0.2	11.6
	A 2020	3.44	7.3±0.1	12.8
	B2016	3.52	7.8±0.3	11.8
	B2017	3.70	8.0±0.1	12.7
	B2018	3.67	9.3±0.3	12.2
	B2019	3.53	8.7±0.2	12.8
	C2015	3.55	9.3±0.2	10.5
	C2016	3.62	7.3±0.1	13.2
	C2017	3.60	8.2±0.2	12.2
	C2019	3.29	9.1±0.1	12.0
Marquette	A 2013	3.77	7.8±0.8	13.9
	A 2015	3.59	7.3±0.2	11.5
	A 2016	3.52	7.0±0.1	12.5
	A 2017	3.54	7.1±0.2	12.9
	A 2020	3.43	7.9±0.3	12.5
	B2014	3.69	6.8±0.1	13.3
	B2015	3.46	9.4±0.1	12.6
	B2016	3.50	7.6±0.1	12.0
	B2017	3.76	7.8±0.1	13.3

The hue of Frontenac wines significantly decreased from 2013 to 2020, suggesting that more browning was observed in 2013 Frontenac wines than in 2020 Frontenac wines (Fig. 1). The hue of Marquette wines was not different across ging and the color intensity of all wines did not follow any specific trends (data not shown).

The amount of phenolic compounds, especially tannins, did not follow any trend throughout aging in bottle. But was still very low compared to the average content of tannins (~500 mg/L) in Pinot noir wines, a *Vitis vinifera* variety known to below in tannin content (Fig. 2). The content of phenolics in Frontenac and Marquette wines varied from 500 to 1400 mg/L which was in agreement with [11]. The content of tannin was also in agreement with the previously published data and was about 240 mg/L epicatechin eq. in Frontenac wines (Fig. 2A) and about 400 mg/L epicatechin eq. in Marquette wines (Fig. 2B).

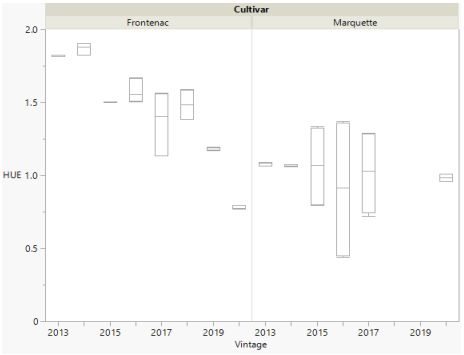

Figure 1. Hue values in Frontenac and Marquette wines from 2013 to 2020.

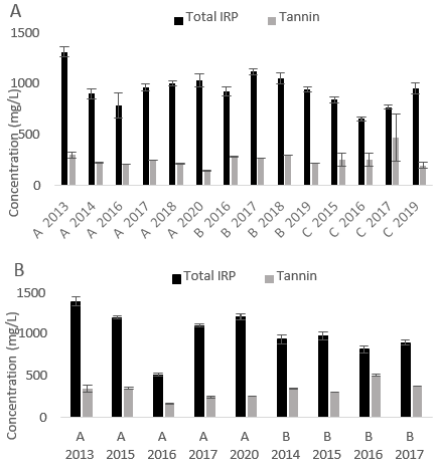

Figure 2. Concentration of total iron-reactive phenolic compounds (IRP) and tannins in Frontenac (A) and Marquette (B) aged wines.

Because the content of tannins was very lowin those wines and the addition of sulfur dioxide (SO_2) was based on *Vitis vinifera* winemaking practices, it was hypothesized that the risk of oxidation would occur more in those aged wines. The expected amount of free SO_2 at bottling was calculated based on the wine pH at bottling for a target of 0.5 mg/L and

0.8 mg/L molecular SO_2 considering an extra 40% of free SO_2 at bottling, which would bind with other compounds such as acetaldehyde, in all of those wines. This was compared to the free SO_2 content at bottling as provided by the involved wineries. Only 3 out of 23 wines showed lower free SO_2 content compared to a target of 0.5 mg/L molecular SO_2, as is recommended for red winemaking (Fig. 3). However, when considering a target of 0.8 mg/L molecular SO_2, 20 wines out of 23 wines showed a much lower free SO_2 content.The higher target of 0.8 mg/L molecular SO_2 is agreement with a previous study [15]. However, the other wines did not follow any trend of loss of free SO_2. The amount of acetaldehyde was evaluated in all the wines and Recommended for white wine making [14] because of low tannin content, similar to the analyzed cold-hardyred wines.

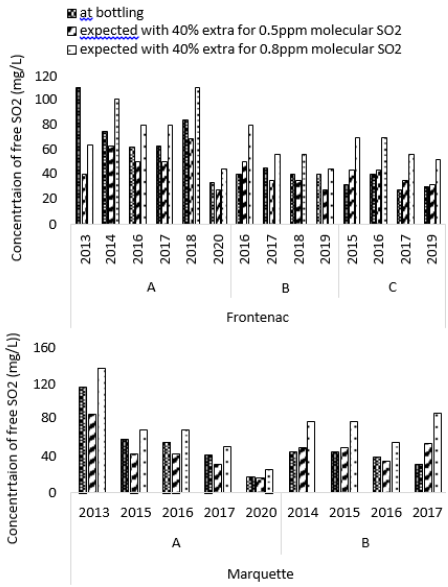

Figure 3. Concentration of free SO_2 in mg/L in wines at bottling and after calculation of expected amount at bottling.

The loss of free SO_2 in Marquette wines of winery A was linear over time from 80% loss in 2013 wines and 40% in 2020 Marquette wines (data not shown), which was in compared to the amount of free SO_2 remaining in the wines and with phenolics and tannin content (Fig. 4).

In Frontenac wines, the component 1 and 2 explained less than 50% of the total variance. And in the Marquette wines, the component 1 and 2 explained about 55% of the total variance. In both cultivars, the acetaldehyde content was negatively correlated to the free and total SO_2 content and the total ironreactive phenolic compoundsin wines (Fig. 4). Overall, the presence of high amount of acetaldehyde, acetic acid and higher hue were negativelycorrelated to the content of free SO_2 and iron reactive phenolic compounds remaining in the wines after aging, as expected and previously published in *Vitis vinifera* wines [16].

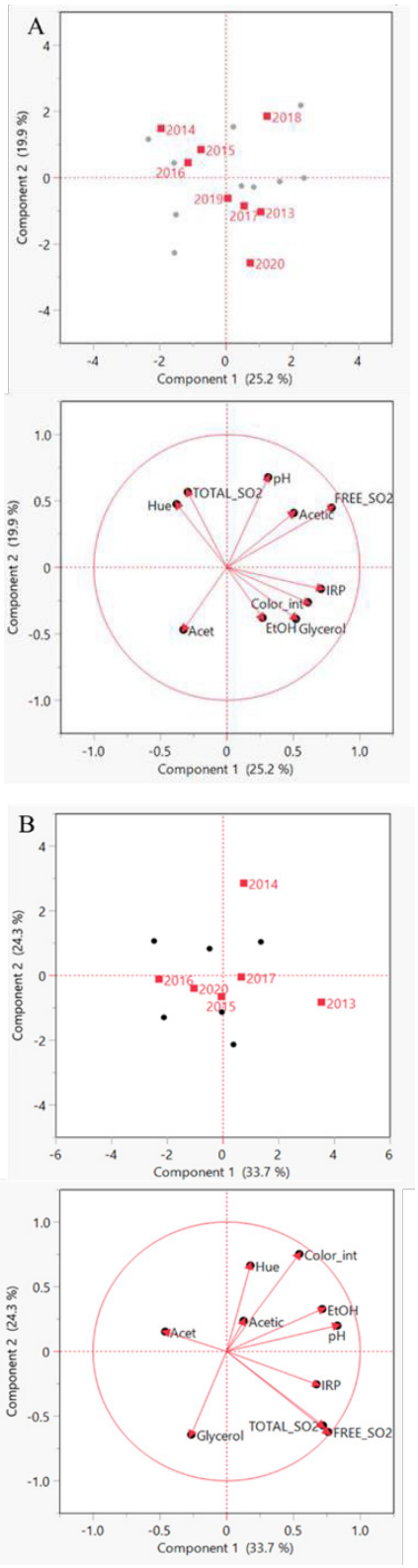

Figure 4. Principal component analysis of Frontenac wines (A) and Marquette wines (B) including the amount of free SO_2, phenolics (IRP), basic chemistry and oxidation related fault including acetaldehyde and acetic acid.

4 Conclusions

The content of tanninsin cold hardy red wines cv. Marquette and Frontenac were very low and much lower than in *Vitis vinifera* wines, as previously observed and discussed. The use of sulfur dioxide in those cold-hardy red wine varieties is commonly based on the wine making practices for red wines made from *Vitis vinifera* cultivars that are rich in tannins and other phenolic compounds. However, it was observed that the amount of free SO_2 added at bottling in the cold-hardy cultivars was too low to protectred wines against oxidation. This resulted in wines rich in acetaldehyde and acetic acid as well as a brown color, especially in 2014 Frontenac wines. Further work will focus on winemaking practices to improve the content of phenolics, especially tannins, and on the rational amount of free SO_2 to add in cold-hardy dry red wines at bottling to maintain their quality over time.

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