PROTEIN AND MALIC ACID CONCENTRATIONS IN NATIVE AND INTERSPECIFIC HYBRID GRAPES USED FOR SPARKLING WINE PRODUCTION

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ABSTRACT

Wine grapes contain several classes of proteins which survive the winemaking process and have the potential to impact foaming qualities of sparkling wines. Thus far, no work has explored the ways grape-derived proteins found in white interspecific hybrid grapes impact sparkling wine foam quality. A first step to understanding this relationship is to determine the total protein concentration of white hybrid grapes, and compare this concentration to the total protein concentration of white *Vitis vinifera* and native grapes. Previous work has shown that red hybrid grapes contain a higher total protein content when compared to red *V. vinifera* cultivars, but white hybrids have not been comprehensively studied. Another component of foam quality is malic acid concentration, as previous work has shown that malic acid positively affects foaming height but negatively affects foam stability. Hybrid and native grapes have higher concentrations of organic acids than *V. vinifera*, suggesting that hybrid grapes will likely have higher malic acid concentrations. Thus, the aim of this work was to determine the total protein and malic acid levels of selected white interspecific hybrid grapes, with the hypothesis that white hybrid grapes would have a higher total protein content and malic acid concentration when compared to white *V. vinifera* cultivars. No significant difference, however, was found in the total protein concentration among cultivar, grape type (*V. vinifera*, French-American hybrid, Neo-American hybrid, cold-hardy hybrid, or native), and year of harvest. Similarly, no significant difference was found in malic concentration between grape type and year of harvest, but there was a significant difference when comparing certain cultivars. Since this study was limited by its small sample size, further work is required to form a more complete picture of total protein and malic acid concentrations in white hybrid grapes.

BIOGRAPHICAL SKETCH

Alexander received a Bachelor of Arts degree in Chemistry and Chemical Biology from Cornell University in May of 2017. During his undergraduate years he performed research on silica-coated nanoparticles in the Wiesner Group.

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CHAPTER 1

INTRODUCTION

Background

Thus far, few studies have reported the total protein and malic acid concentrations of interspecific white hybrid grapes. Since both of these parameters likely impact sparkling wine foam quality, a better understanding of their effects may aid in improving hybrid sparkling wine production.

Grape Proteins

Proteins are found mostly in grape pulp and skin, and make up approximately 0.05% of grape berry pulp by weight (Sarry et al. 2004). In *V. vinifera* cultivars, there are approximately 70 grape proteins that can be isolated from the pulp and characterized, of which 34% play a role in energy storage, 13% play a role in metabolism, and 19% play a role in defense, stress, and disease response regulation (Sarry et al. 2004). Lesser protein functions include protein destination regulation, transcription, signal transduction, cell division/DNA synthesis, protein synthesis, and cell organization. The differences in protein types across cultivars are minimal, with the exception of alcohol dehydrogenase, as different cultivars have varying isoforms of this protein (Sarry et al. 2004). The most abundant proteins are dehydrins, invertases, and pathogenesis-related proteins (chitinases and thaumatin-like proteins) (Sarry et al. 2004). These pathogenesis-related proteins (PRPs) play a role in grape antifungal defense (Sarry et al. 2004). Other proteins of note include glycoproteins and proteoglycans, which are found in grape cell walls (Waters et al. 1994).

Grape-Derived Proteins in Wine

Fermentation and associated winemaking activities have a significant impact on protein levels. Tannin interactions may cause protein precipitation (Somers and Ziemelis 1973). The addition of proteases can induce proteolysis, and pH changes can cause protein denaturation. Because proteolysis and denaturation lead to the degradation and eventual precipitation of grape proteins, only chitinases and thaumatin-like proteins (TLPs) survive in wine (Murphey et al. 1998). These proteins contain a large number of disulfide bonds, allowing them to effectively retain their shape; therefore, they are the main grape-derived proteins in wine (Marangon et al. 2014). Chitinases and TLPs have molecular weights ranging from 20-32 kDa, and with isoelectronic points between 4.1 and 5.8, are positively charged at wine pH (Brissonnet and Maujean 1993). The protein nitrogen content of wines typically falls in the range of 15 – 230 mg per liter (Ferreira et al. 2002). Wine proteins are not completely identical to their grape juice precursors, despite having identical molecular weights, as they may have different isoelectronic points (Pueyo et al. 1993). It is notable that wines prepared from different cultivars or with different vinification treatments have virtually identical types of chitinases and thaumatin-like proteins (Ferreira et al. 2000), approximately one-half of which are bound to grape phenolics (Somers and Ziemelis 1973).

Impact of Grape-Derived Proteins on Wine Stability

Grape-derived proteins have a significant impact on white wine stability. Protein instability refers to the potential of haze formation in bottled wine, which is visually unappealing and therefore unacceptable to consumers. Chitinases and TLPs have both been implicated in haze formation, a process induced by high temperatures (Vincenzi et al. 2011). All chitinase proteins

are unstable, and precipitate out more readily than TLPs, which can be stable or unstable depending on isoform. Stability is defined as the ability of the protein to reversibly unfold/refold after heating and cooling, and unstable proteins are those that irreversibly denature and unfold at high temperatures (Marangon et al. 2014).

Wine Instability Mechanisms

Although the mechanism of white wine haze formation is not fully understood, it is likely a multistep process. Proteins first unfold and become unstable in response to elevated storage temperatures immediately following clarification, then the unfolded, unstable proteins begin to self-aggregate through hydrophobic interactions. At this point, sulfate can modify the ionic strength of the solution, thereby favoring the bonding of unfolded proteins (Marangon et al. 2010). Finally, the sulfate acts to cross-link the protein aggregates, which grow large enough to be seen with the naked eye, forming a haze. These aggregates may then precipitate out of solution or remain suspended (Marangon et al. 2011).

Grape-Derived Proteins in Sparkling Wine

Past research has demonstrated that grape-derived proteins play a role in sparkling wine foam. Although grape-derived proteins do not cause foam on their own, they likely contribute to foam formation through their interaction with mannoproteins (Vincenzi et al. 2012). Mannoproteins are released by the lysing of yeast cells and stabilize white wines during lees aging (Pellerin et al. 1994). Experiments have demonstrated that the highest level of foam formation in sparkling wine occurs when all of the protein fractions (grape-derived proteins and mannoproteins) are combined together, which supports the notion that foam physics are likely partially governed by

synergistic effects between grape-derived proteins and mannoproteins (Vincenzi et al. 2012). The mechanism of this synergistic interaction may be charge based, wherein negatively charged mannoproteins interact with positively-charged grape-derived proteins, thereby forming macromolecular complexes that contribute to foam formation (Vincenzi et al. 2012). Proteins stabilize wine foam by forming stabilizing adsorption layers at the liquid-gas interface (Brissonet and Maujeen 1991). Furthermore, total protein content is correlated with foam formation – in other words, as total protein increases, wine foam formation increases (Brissonet and Maujeen 1991).

Malic Acid

In addition to protein, malic acid is thought to play a role in sparkling wine foam quality. Malic acid is one of the two most abundant organic acids found in wine grapes, and is influenced by growing region, cultivar, and climatic conditions such as light exposure and average temperature (Lamikanra et al. 1995). Native grapes and interspecific hybrid grapes with native parentage often have higher total acidity concentrations than *Vitis vinifera* grapes (Teissedre 2018). Indeed, previous work has demonstrated that red cultivars of the native grape species *Vitis riparia* and *Vitis labrusca* have higher titratable acidity values than red *Vitis vinifera* grapes (Waterhouse et al. 2016). Furthermore, some native grape species, such as *Vitis riparia,* also contain more malic acid than tartaric acid (García et al. 1967). Organic acids have also been implicated in contributing to sparkling wine foam quality. Previous work has shown that malic acid positively impacts foaming height but negatively impacts foam stability (Girbau-Sola et al. 2002).

CHAPTER 2

MATERIALS AND METHODS

Samples

Twenty-three white and red interspecific hybrid, native, and *V. vinifera* grapes were collected from vineyards across New York State as part of the annual Véraison to Harvest ripening surveys that were conducted during the 2017 and 2018 vintages (Table 1). Red cultivars Marquette (*Vitis spp)* and Niagara (*Vitis labrusca)* were sampled because both have been used to make sparkling rosé wines. When possible, samples of the same cultivar from the same vineyard were analyzed in both years.

Table 1 – Name, vineyard location, year of harvest, grape cultivar, soluble solids (in °Brix), grape color, and grape type of samples.

Sample Name	Location	Year of Harvest	Cultivar	°Brix	Grape Color	Grape Type
Riesling-2017	Finger Lakes	2017	Riesling	18.3	White	*V. vinifera*
Seyval blanc-2017	Hudson Valley	2017	Seyval blanc	18.7	White	French-American Hybrid
Seyval blanc-2018-1	Hudson Valley	2018	Seyval blanc	17.3	White	French-American Hybrid
Seyval blanc-2018-2	Finger Lakes	2018	Seyval blanc	15.2	White	French-American Hybrid
Vignoles-2017	Finger Lakes	2017	Vignoles	21.2	White	French-American Hybrid
Vignoles-2018-1	Finger Lakes	2018	Vignoles	18.5	White	French-American Hybrid
Vignoles-2018-2	Finger Lakes	2018	Vignoles	22.7	White	French-American Hybrid
Vidal blanc-2017	Finger Lakes	2017	Vidal blanc	20.0	White	French-American Hybrid
Vidal blanc-2018	Finger Lakes	2018	Vidal blanc	22.4	White	French-American Hybrid
Traminette-2017-1	Finger Lakes	2017	Traminette	21.0	White	Neo-American Hybrid
Traminette-2017-2	Finger Lakes	2017	Traminette	22.1	White	Neo-American Hybrid
Traminette-2018	Finger Lakes	2018	Traminette	19.5	White	Neo-American Hybrid
Aromella-2017	Finger Lakes	2017	Aromella	23.4	White	Neo-American Hybrid
Cayuga White-2017	Finger Lakes	2017	Cayuga White	17.8	White	Neo-American Hybrid
Cayuga White-2018	Finger Lakes	2018	Cayuga White	17.1	White	Neo-American Hybrid
Marquette-2017	Finger Lakes	2017	Marquette	24.7	Red	Cold-Hardy Hybrid

Marquette-2018-1	Finger Lakes	2018	Marquette	22.5	Red	Cold-Hardy Hybrid
Marquette-2018-2	Hudson Valley	2018	Marquette	23.4	Red	Cold-Hardy Hybrid
La Crescent-2017	Hudson Valley	2017	La Crescent	21.8	White	Cold-Hardy Hybrid
La Crescent-2018	Hudson Valley	2018	La Crescent	23.6	White	Cold-Hardy Hybrid
Niagara-2017	Lake Erie	2017	Niagara	14.2	White	Native
Niagara-2018	Lake Erie	2018	Niagara	14.2	White	Native
Catawba-2017	Finger Lakes	2017	Catawba	16.4	Red	Native

Berry samples were crushed and pressed following a standard protocol, and the juice was stored frozen at -10°C in 50 mL screw-cap tubes (Celltreat Scientific Products, Pepperell, MA) until needed.

Extraction of grape proteins

Samples were completely thawed at room temperature, then centrifuged at 14,000g and 4°C for 20 minutes. The supernatant (juice) was pipetted off and kept, while the precipitate was discarded. KDS Precipitation was performed based on previous methods (Vincenzi et al. 2005, Fusi et al. 2010). First, 4 µL of a 10% solution (w/v) of sodium dodecyl sulfate (MP Biomedicals, Solon, OH) was added to 2 mL of sample juice and the mixture was heated at 100°C for 5 min in a hot water bath. After heating, 505 µL of 2 M potassium chloride (VWR Life Science, Solon, OH) was added to the sample, which was then vortexed vigorously. The samples were then incubated at 4°C in a cold water bath for 45 min, and centrifuged at 14,000 g and 4°C for 20 minutes. After centrifugation, the supernatant was pulled off and discarded. The pellet was then resuspended in 2 mL of deionized water. Samples were prepared in triplicate.

Quantification of total grape protein

Total grape protein was quantified with a Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford, IL). Samples were prepared according to kit instructions, and the Standard Test Tube Protocol was used when creating standard dilutions. Spectrophotometric readings were taken on a Genesys 2 spectrophotometer (Thermo Electron Corporation, Waltham, MA). Malic acid concentrations were measured using an OenoFoss (Foss North America, Eden Prairie, MN). F-tests were performed with JMP Pro 14 (SAS Institute, Cary, NC).

CHAPTER 3

RESULTS

Concentrations

Protein concentration ranged from approximately 300 – 1600 mg/L, and there was as much variation within cultivar as between cultivars (Figure 1). Furthermore, almost all cultivars had protein concentrations that were higher than that of Riesling, which was used as the *V. vinifera* comparison (Figure 1). Malic acid concentration ranged from approximately 1 – 8 g/L, and several, but not all, cultivars had malic acid concentrations that were higher than that of Riesling (Figure 2).

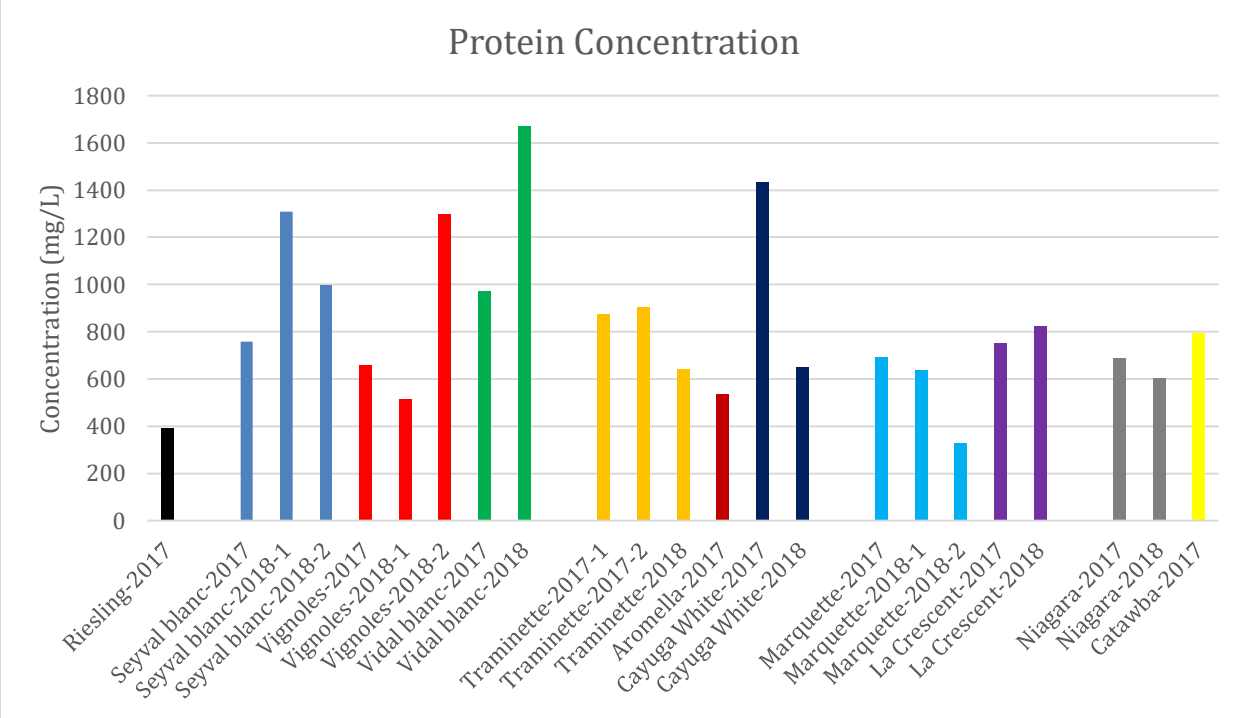

Figure 1 – Protein concentration (in mg/L) of each sample. Each color corresponds to one cultivar.

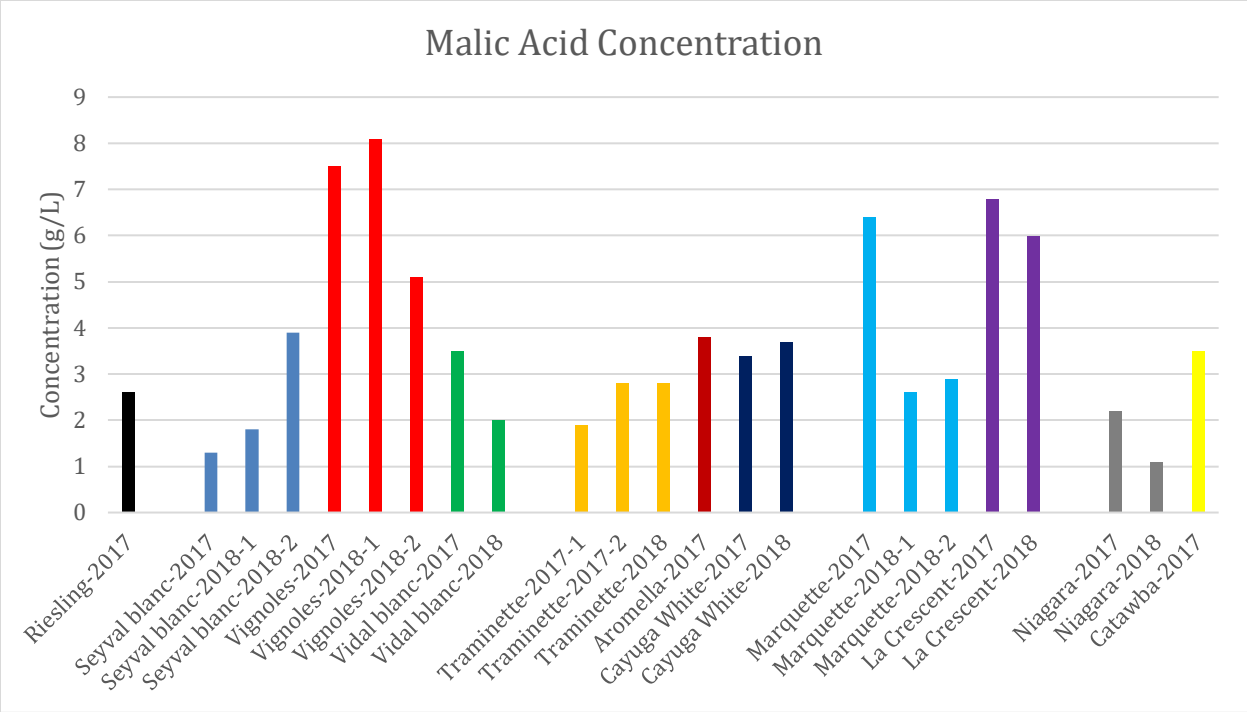

Figure 2 – Malic acid concentration (in g/L) of each sample. Each color corresponds to one cultivar.

Statistical Analysis

Table 2 – Significance of cultivar, grape type, and year versus protein and malic acid concentrations as analyzed by F-test.

	Analysis	**Probability > F**
Protein	Cultivar vs Protein	0.3339
	Grape Type vs Protein	0.1634
	Year vs Protein	0.6079
	Cultivar & Grape Type vs Protein	0.3339
	Cultivar & Year vs Protein	0.4526
	Grape Type & Year vs Protein	0.2743
Malic Acid	Cultivar vs Malic Acid	0.0141*
	Grape Type vs Malic Acid	0.3229
	Year vs Malic Acid	0.8417
	Cultivar & Grape Type vs Malic Acid	0.0141*
	Cultivar & Year vs Malic Acid	0.018*
	Grape Type & Year vs Malic Acid	0.3697

*indicates significance at $p < 0.05$

Most of the experimental variables (cultivar, grape type, year of harvest) did not correlate to significant differences in protein and malic acid concentration. The exception was the correlation observed when comparing cultivar to malic acid concentration; there were significant differences in malic acid concentration depending on which cultivars were being compared. In particular, Vignoles had concentrations of malic acid that were significantly higher than Niagara, Seyval blanc, Traminette, Riesling, Vidal blanc, Catawba, Cayuga White, and Marquette. Similarly, La Crescent had concentrations of malic acid that were significantly higher than Niagara, Seyval blanc, Traminette, Riesling, Vidal blanc, and Cayuga White (significance table not shown).

CHAPTER 4

DISCUSSION

Total Protein

Red interspecific hybrid grapes have been shown to have higher total protein concentrations than red *V. vinifera* grapes (Springer and Sacks 2014). Thus, it was hypothesized that grape type (*V. vinifera*, French-American hybrid, Neo-American hybrid, cold-hardy hybrid, and native) would be correlated with total protein concentration. Although statistical analysis demonstrated that grape type did not correlate to significant differences in protein concentration, almost all cultivars had higher total protein concentrations than the *V. vinifera* control Riesling. This trend may be explained by physiological differences between grape types, such that on average, white interspecific grapes have higher protein concentrations than white *V. vinifera* grapes. This physiological difference may be a consequence of native grape ancestry found in interspecific hybrid grapes. Since interspecific hybrids are often a cross between *V. vinifera* and other *Vitis* species, their higher protein content may be due to non-*vinifera* ancestry (Springer and Sacks 2014). If this trend between grape type and protein concentration is common, then it would have implications for sparkling wine production. Previous work has shown that an increase in total protein content is correlated with an increase in foam formation (Brissonet and Maujeen 1991). Thus, using grapes with higher total protein content would likely lead to a higher total protein content in the finished wine. As such, if white interspecific hybrid grapes have on average higher protein concentrations than white *V. vinifera* grapes, then sparkling wines made from hybrid grapes may foam more vigorously. Therefore, winemakers that produce sparkling wine out of hybrids may adjust their protein levels with fining during base wine production to reach a protein

level that would create the optimal amount of foam in the finished wine. However, recent work has demonstrated that even after bentonite fining, considerable amounts of protein may remain in finished wine (Lindstrom 2019). Thus, more research will be required to determine the best method of fining protein to achieve optimal foam. Protein concentration may also play a role in gushing. Gushing is the rapid, excessive production and overflow of foam upon opening a bottle of sparkling wine (Kemp et al. 2018). Empirical observation suggests that rosé sparkling wines made with the red Marquette grape tend to gush (Josie Boyle, Craig Hosbach, and Demi Perry, personal communications). Previous work has demonstrated that lipid transfer proteins associated with beer gushing have also been found in Riesling still wine (Kemp et al. 2015). Thus, if these lipid transfer proteins are present in sufficient quantities in sparkling wine, it is possible that they are partially responsible for gushing in Marquette sparkling wines.

Malic Acid

Native and interspecific hybrid grapes have also been found to have higher total acidity concentrations than *V. vinifera* grapes (Teissedre 2018), and some native grape species such as *Vitis riparia* contain more malic acid than tartaric acid (García et al. 1967). Based on these findings, it was hypothesized that grape type would be correlated with malic acid concentration. As with protein concentration, the data did not support this hypothesis. However, there were significant differences in malic acid concentration between some cultivars. In particular, interspecific hybrids Vignoles and La Crescent both had significantly higher malic acid levels than Niagara, Seyval blanc, Traminette, Riesling, Vidal blanc, and Cayuga White. These results support previous findings which show that Vignoles and La Crescent grapes contain relatively high levels of malic acid, with concentrations in the range of 4 – 8 g/L and 5 – 12.5 g/L,

respectively (Main et al. 2007, Thull and Luby 2016). In the case of La Crescent, this phenomenon may be explained by its parentage. La Crescent is a cross between St. Pepin and E.S. 6-8-25, which is a *V. riparia* x 'Muscat Hamburg' cross (Smiley and Cochran, 2016). Since *V. riparia* grapes often have higher concentrations of malic acid than tartaric acid, it is likely that the 1/4th *V. riparia* ancestry of La Crescent promotes comparatively high malic acid levels (García et al. 1967). Furthermore, hybrid grapes often have higher titratable acidity than *V. vinifera* grapes (Teissedre 2018). Combined, these factors likely contribute to the high malic acid content of La Crescent. On the other hand, although the parentage of Vignoles is commonly reported as a cross between Seibel and Pinot de Corton (a clone of Pinot noir), recent genetic testing has disproven this notion, so it is more difficult to form a hypothesis as to how the parentage of Vignoles may play a role in malic acid content (Bautista et al. 2008). It is possible, however, that the parentage of Vignoles includes some amount of *V. riparia*, which might explain its high malic acid levels.

Grape maturity

Sample grapes were harvested at varying maturity levels as defined by °Brix (Table 1). Previous work has shown that higher protein concentration is correlated with greater grape maturity (Pocock et al. 2000). Therefore, future studies should control for this experimental variable and harvest the grapes at identical maturity levels. Future studies should also employ more *V. vinifera* and native samples, so that the comparison between grape types can be performed with greater statistical confidence.

CHAPTER 5

CONCLUSION

While the sample size in this study was relatively small, the trends of higher protein and malic acids represent a starting point from which further research may be directed in order to provide useful data for producers of interspecific hybrid sparkling wine. Once the protein and malic acid concentrations of a large number of varying interspecific hybrid grapes are determined and correlated to sparkling foam quality, guidelines and recommendations on adjusting concentrations of these parameters for optimal foam quality can be developed. If total protein content is indeed correlated with foamability, winemakers will be able to make more informed decisions about fining base wine. If the impacts of malic acid on wine chemistry are of concern, then winemakers may choose which cultivars to grow, blend, or put through malolactic fermentation to optimize malic acid levels.

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