PHENOLIC EXTRACTION FROM RED HYBRID WINEGRAPES

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by

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ABSTRACT

As the wine industry continues to expand, it has included rapid growth in the development of cool climate wine regions across the United States. This growth has been spurred by the use of native and hybrid winegrapes, along with the development of new hybrid cultivars for which there is little working experience.

Research efforts, along with many consumers, have focused on *Vitis vinifera* based wines. A great many studies have concentrated on the viticultural and oenological management of phenolics, attempting by manipulation to enhance or suppress certain phenolic characteristics across many *V. vinifera* cultivars. While *V. vinifera* cultivars have a greater economic impact globally, the local use of cold hardy winegrapes can profoundly affect tourism and customer knowledge in particular regions. As tourism is driven by consumers, it is of paramount importance that winemakers produce salable wines using the resources available to them, including regionally adapted cultivars, tools, and techniques to best create a pleasurable wine experience.

This research sought to assess the challenges winemakers face when using red hybrid winegrapes, and the ways that winemaking techniques influence the phenolic profile of hybrid cultivars Corot noir, Maréchal Foch, and Marquette.

To gauge current production practices, a survey was distributed to winemakers in Michigan, Minnesota, New York, Pennsylvania, and Wisconsin, asking them to characterize their techniques and challenges in using red hybrid grapes for wine production. Statistical analyses of the survey data showed that winemakers deal primarily with challenges in acidity, phenolics, and storage. A multinomial logit model was fit to the data to estimate the likelihood

of a winemaker encountering particular challenges based on winery size, location (state), and varietal use.

Grapes were vinified in triplicate lots for each treatment of Corot noir and Maréchal Foch, and in duplicate lots for each treatment of Marquette, following standardized winemaking protocols. The five treatments investigated were as follows: control, enzyme addition, exogenous tannin addition, cold soak, and hot press. These treatments were chosen based on survey data to provide winemakers with scientific research on the grapes they use, instead of relying on data used for *vinifera* wines. Grape musts and wines were analyzed via HPLC to quantify and compare differences in phenolic concentration for each winemaking technique.

Statistical analyses of the phenolic concentration data showed significant differences between treatments in Maréchal Foch musts and wines in the total tannin concentration, total monomeric concentration, and total anthocyanin concentration, though no significant differences were found between treatments for the mean degree of polymerization (mDP). Corot noir treatments showed no significant differences in the total concentration of monomeric phenolics, but statistically significant differences were found in anthocyanins, tannins, and the mDP, mostly between the hot press and tannin addition treatments. Few significant differences were found among treatments in the Marquette musts and wines.

BIOGRAPHICAL SKETCH

Céline Thérèse Marie Coquard Lenerz was born into a family passionate about wine. She and her two brothers were raised in southern Wisconsin and spent many hours running through their family vineyards and winery. Céline graduated from Sauk Prairie High School in 2006, and continued her education at the University of Wisconsin-Madison, receiving a B.S. in Natural Sciences-Horticulture in 2009. In 2010, Céline joined the lab of Dr. Anna Katharine Mansfield at Cornell University to pursue her interests in enology. Since that time, she has been investigating the extraction of phenolics from cold hardy winegrapes, and is planning to return to Wisconsin to continue her family's winery and support the local wine industry.

To Grandpa Bob,
whose courage in the face of adversity I hope to emulate

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Extended thanks go to Dr. Dave Manns who provided me with the indispensable assistance I needed to learn the HPLC analyses; I could not have asked for a better person from whom to learn chromatographic techniques.

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Last but not least, I am most thankful for my family's unconditional love and support that has motivated me through thick and thin. Finally, thank you to my husband. Without his love, encouragement, and willingness to leave behind real cheese curds, I would not have been able to do this.

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LITERATURE REVIEW

1.1 Introduction

Over the last forty years, the grape and wine industry has grown exponentially over the midwestern and eastern United States. Most of these areas are considered to be cool or cold climate wine regions, and so rely heavily on the use of cold-hardy winegrape cultivars where traditional *V. vinifera* cannot survive or produce consistently high quality fruit. Along with this economic reliance on hybrid cultivars, and the continued introduction of new winegrape cultivars, research addressing the challenges winemakers face in acidity, wine production, and phenolic management in red hybrid winegrapes has grown in importance.

1.2 Hybrid Grapes

A hybrid grapevine is a grape cultivar produced by crossing one species of grapevine with another, or by crossing two cultivars of the same species (Collin 2005). Many interspecific hybrids are a cross of not only two, but often three or more species.

1.2.1 Grape species and cultivars

Most commonly known species of commercial winegrapes are of American or European lineage. Native American vines include *Vitis riparia* (riverbank grape), *V. rupestris* (sand grape), *V. cinerea* (graybark grape), *V. muscadinia* (muscadine grapes), *V. rotundifolia* (round-leaved), *V. aestivalis* (summer grape), and *V. labrusca* (fox grape) (Monaghan 2008). Most popular wine grape cultivars (e.g. Pinot noir, Chardonnay, Syrah, etc.) are of European *V.*

vinifera descent, while interspecific crosses include germplasm from *V. vinifera* and other species such as *V. labrusca, V. cinerea, V. rupestris*, or *V. riparia*. Among these, four main species are used for wine production: *V. aestivalis, V. labrusca, V. riparia*, and *V. rotundifolia.*

Red hybrid cultivars of particular interest to the eastern and mid-western United States include Corot noir, Maréchal Foch, and Marquette.

Vitis rupestris

V. rupestris habitat ranges from southern Missouri to Kentucky, western Tennessee, Arkansas, Oklahoma, Texas and New Mexico. It thrives in dry, sandy, and rocky riverbeds and is locally known as the Mountain grape or Rocky grape (Oklahoma University 1999).

Its plant habit is a small, many-branched shrub form ranging six to eight feet in height. Clusters are small with 12 to 24 black or purple-black berries. The skin of the berries is very thin and the seeds quite small. Berries range from sweet to sour in taste with no foxy aroma. The vine blossoms in June and ripens early from June to August in the southwestern United States (Oklahoma University 1999).

V. rupestris is drought tolerant and can survive in areas with hot, dry, southern exposures. Due to its resistance to *Phylloxera* and lack of foxy aromas, it is widely used in grape breeding (USDA, NRCS 2011).

Vitis riparia

V. riparia is found along the banks of streams, rivers, and other bodies of water. It is the most widely distributed of all the native American grape species, and can be found across southern Canada to the southern United States (USDA, NRCS 2011).

V. riparia vines tend to be very vigorous with a climbing or trailing habit. The vine blossoms early, but ripens late in September; clusters are relatively small and compact with many small to medium-sized black berries. Berries tend to be very high in acidity (16-20g/L TA) though with high average sugar content (>20°Brix) (Hemstad and Luby 1997).

V. riparia is drought tolerant and very cold-hardy, withstanding temperatures as cold as -60°F. It is also more tolerant of excessively alkaline soils than other species. Like other native species, it is resistant to *Phylloxera* and mildews. Because of these traits, *V. riparia* has been used as rootstock and for grape breeding (Hemstad and Luby 2000; USDA, NRCS 2011).

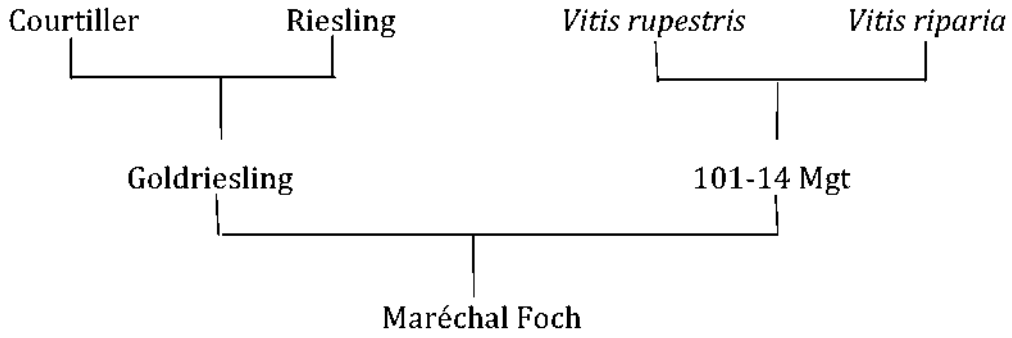
Maréchal Foch

Maréchal Foch (Kuhlmann 188.2) is a hybrid from the early 1900s, resulting from breeder Eugene Kuhlmann's cross of a *V. riparia* X *V. rupestris* selection with Goldriesling (*V. vinifera*), in Alsace, France (Lehman and Gerrath 2004). In 1946 it was introduced to Canadian vineyards with other French hybrids by Adhemar deChaunac, and imported to the eastern United States in 1951 by Philip Wagner (Pinney 2005). Its pedigree is shown in Figure 1.1.

Today, it is widely grown across the eastern United States, Canada, and the Midwest. In New York alone the acreage of Maréchal Foch increased from 79 acres in 1990 to 144 acres in 2006 (Martinson 2000, Stephen and Blair 2006). In Wisconsin, Maréchal Foch is one of the top ten grapes planted, with 46 acres, and the second most harvested, contributing 30 tons for the 2010 harvest (Rochester 2011). Confusingly enough, in North America Maréchal Foch is also colloquially known simply as 'Foch.' A study by Pollefeys (2003) has shown that this name has historically been assigned to two different vines that are probably siblings, though the extent to which each variant has been cultivated is unknown.

Maréchal Foch clusters are small and compact with dark-colored berries (Jackson 2000), which ripen early to mid-September. The vine is cold hardy to -35°F and is relatively resistant to disease, though is moderately susceptible to mildews, bunch rot, crown gall, and black rot. (Reisch and others 2000). One challenge is its tendency to over-crop (Fisher 1979; Pool and others 1978); in New York State, this tendency has affected fruit composition, wine quality, and vine vigor. Sun (2011) also found that Maréchal Foch has low skin and wine tannin concentration, with low tannin extractability.

Figure 1.1. Maréchal Foch pedigree



Corot noir

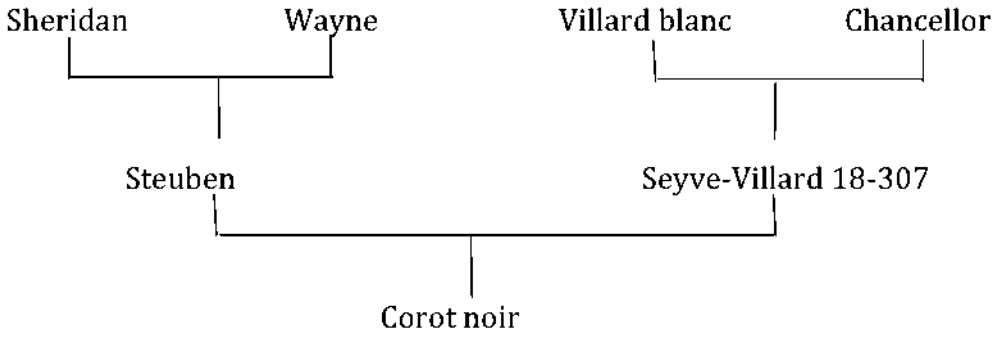
Corot noir was introduced by the Cornell University grape breeding program in Geneva, NY in 2006. It is a cross of Seyve Villard 18-307 and 'Steuben,' and is moderately winter-hardy with a deep red color. The pedigree is shown in Fig 1.2. This hybrid was bred by Bruce Reisch of Cornell University in 1970, first tested for wine characteristics in 1978, and released in 2006 (Reisch and others 2006).

Corot noir ripens mid- to late-season (end of September to early to mid-October) and is moderately winter-hardy, surviving lows of -10°F. According to data from the New York State Agricultural Experiment Station (NYSAES) it is more hardy than some hybrids but not as hardy as *riparia*-derived cultivars such as Maréchal Foch and Frontenac. It also shows moderate

resistance to fungal disease. Corot noir produces large, relatively tight clusters with large, dark red berries. Reisch and others (2006) reported that Corot noir gives favorable cherry or berry fruit aromas without hybrid aromas.

Most winemakers find Corot noir to be appropriate for use in monovarietal wines or for blending with other cultivars, though some report that it is difficult to work with in the vineyard due to vigorous vegetative growth, low cluster light exposure, and high fruit yield.

Figure 1.2. Corot noir pedigree

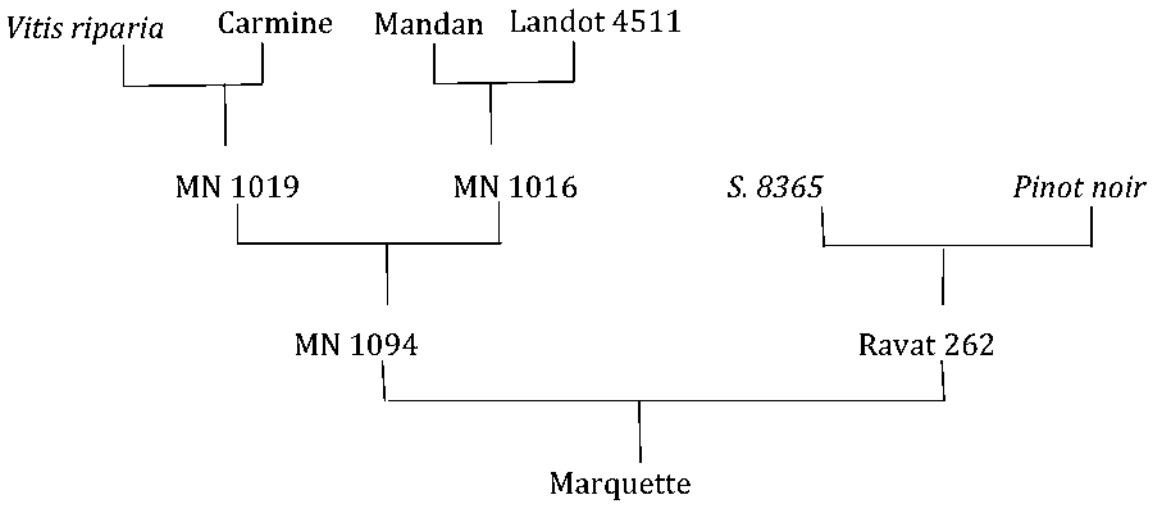


Marquette

Marquette (MN1211) is a complex interspecific hybrid of *V. riparia, V. vinifera,* and other *Vitis* species (Hemstad, private communication, 2012), released by the University of Minnesota grape breeding program in 2006. The pedigree is shown in Figure 1.3.

Clusters are small with small- to medium-sized berries. The berries have blue-black skin with light-pink pulp and ripen early to mid-September in the upper Midwest. Vines are moderately vigorous with an upright growth habit, providing good sun exposure, though early bud break leaves Marquette vulnerable to frost damage. The vines are very cold hardy, surviving temperatures as low as -30°F, and have low susceptibility to rots and mildews (Cook 2012; Hemstad and Luby 2005).

Figure 1.3. Marquette pedigree



1.2.2 Use of Hybrid Grapes

The main viticultural areas of the world are climatically limited between 30° and 50° N latitude and 30° and 40° S latitude (Mullins and others 1992), but within these areas there is a large variation in geography and topography allowing for many microclimates. In many of these areas, such as the eastern and midwestern portions of the United States, local climates can be challenging for viticulture. Even where *V. vinifera* grapes are widely grown, they may not consistently reach maturity each year. In contrast, many native American species of *Vitis* have been successfully grown in these regions, as have *V. labrusca* cultivars and interspecific hybrids of *V. vinifera* and native American species (Hedrick 1908).

Hybrids can occur in nature, but modern-day grape breeding programs came about in response to the susceptibility of *Vitis vinifera* to *Phylloxera vastatrix*, a root louse native to eastern North America, which destroyed thousands of acres of winegrapes across Europe in the mid to late 1800's. One means of combating *Phylloxera* susceptibility is to graft *V. vinifera* scions on the roots of a resistant native American grape species such as *V. riparia* or *V. aestivalis*. Alternatively, crosses are made with these resistant species, resulting in 'direct producers': hybrids that combine favorable fruit characteristics of *V. vinifera* while retaining

Phylloxera resistance without rootstock grafts (Janick and Paull, 2008). Hybrids that exhibit desirable traits are subsequently propagated asexually through cuttings (Reisch and Stewart 2001).

Hybrid grapes are grown for a variety of reasons, with climatic constraints (including sub-zero winter temperatures), short growing seasons, and severe disease pressures topping the list (Hancock 2008). This is important in northern growing regions, where minimum and maximum observed temperatures can be extreme (Table 1.1).

Table 1.1. Minimum and maximum temperature by state

State	Minimum temperature °F	Maximum temperature °F
Michigan	-51	112
Minnesota	-60	114
New York	-52	108
Pennsylvania	-42	111
Wisconsin	-55	114

Source: National Climatic Data Center.

Hybrid grape cultivars are often more cold hardy and disease resistant than *V. vinifera*, and subsequently are grown for their early ripeness and productivity (Pollefeys and others 2003). Most hybrid grapevines are unique in their ability to produce a commercial crop from secondary or tertiary buds in the event that frost or freezing has destroyed the primary bud, developing shoot, or flower cluster (Barret 1956; Smith and Mendall 1972). In humid climates with warm summers, such as can be found in Minnesota, Wisconsin, and New York, cultivars with high levels of resistance to downy mildew (*Plasmopara viticola*), black rot, and powdery mildew may be of great importance (Barret 1956). Recent hybrids of commercial importance have been developed by the late Elmer Swenson (Pollefeys and others 2003) and through breeding programs at Cornell University's NYSAES in Geneva, NY, and at the University of Minnesota in Excelsior, MN.

1.3 Regions of Economic Importance

Today, many *V. vinifera*, American *Vitis*, and other hybrids are grown across the eastern and mid-western United States, where locally produced wines generate revenue through agrotourism and excise taxes. For example, there are over 6,000 wineries in the United States, and just over half of them are located outside of California. Nationally, wine-related tourism expenditure is over $3 billion, and in New York State the full economic impact of the grape and wine industry tops $6,000,000,000 (MG&WII 2008).

More recently there has been an increase in 'buy local' movements (Skuras and others 2006). This, along with a growing interest in producing higher value horticulture crops such as grapes, has rapidly increased hybrid planting in cooler climate regions. Over the last forty years 4,000 acres have been planted in New York alone. Collectively, New York, Iowa, Wisconsin, Minnesota, and Missouri currently have about 5,600 acres of hybrid grapes (Martinson 2011).

1.3.1 American Viticultural Areas

Similar to the controlled appellation systems of Europe, an American Viticultural Area (AVA) encompasses a specific viticultural region with unique growing conditions and geographic features (Robinson 2006). The U.S. Alcohol and Tobacco Tax and Trade Bureau (TTB) defines and approves every AVA within the Code of Federal Regulations. As of March 2012 there were a total of 200 AVAs across the country (TTB), including 29 in the region of interest (Table 1.2).

Table 1.2. Approved American Viticultural Areas in selected cold- and cool-climate states

Michigan	Minnesota	New York	Pennsylvania	Wisconsin
Fennville Leelanau Peninsula Lake Michigan Shore Old Mission Peninsula	Alexandria Lakes Upper Mississippi River Valley	Niagara Escarpment Cayuga Lake Finger Lakes Hudson River Region Lake Erie Long Island North Fork of Long Island Seneca Lake The Hamptons, Long Island	Central Delaware Valley Cumberland Valley Lake Erie Lancaster Valley Lehigh Valley	Upper Mississippi River Valley Lake Wisconsin Wisconsin Ledge

1.4 Grape & Wine Chemistry

The main compounds found in grapes in order of magnitude are: water, sugars, phenolics, and volatiles (Sacchi 2005). Many compounds contribute little to the flavor of the berries and act purely as precursors for important volatile compounds in the wine (Moreno-Arribas and others 2009).

In contrast, the main compounds in wine, in order of magnitude, are: water, ethanol, sugars, organic acids, glycerol, phenolics, and minerals. (Moreno-Arribas and others 2009). Phenolics are of particular interest because of their impact on red wine quality and the continued difficulty in isolating and quantifying individual compounds. Further, little research has been performed on wines made from red hybrid winegrapes.

1.4.1 Phenolics

Phenolics are a class of chemical compounds sharing a basic structure comprised of at least one aromatic hydrocarbon with one or more hydroxyl substituents. Variation in the addition of chemical functional groups and in the degree of polymerization result in a wide variety of phenolic compounds. This class includes several hundred compounds that affect wine taste, aroma, color, and mouthfeel.

In dry red wines, phenolics are usually are the most plentiful constituents after alcohol, tartaric acid, and unfermentable sugars (Singleton and Noble 1976). Phenolics can be found in monomeric, oligomeric, or polymeric forms in the seeds, skins, and stems of the grape (Monagas and others 2005). Grape-derived phenolics may be modified by enzymes (Moreno-Arribas and others 2009) and exposure to crushing and preparation for fermentation may exacerbate this effect.

Phenolics can be broken down into two large groups: flavonoids and nonflavonoids (Figure 1.4).

Figure 1.4. Phenolic grouping

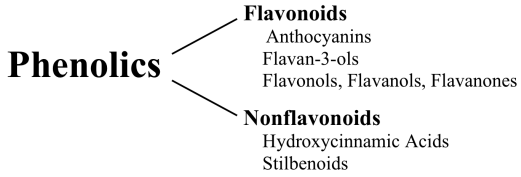
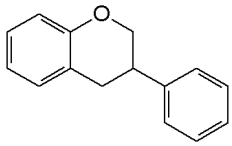


Figure1.5. Basic phenolic structure



Flavonoids are composed of two aromatic rings joined together by a heterocyclic ring structure (Figure 1.5). Flavonoids include anthocyanins, flavanols, flavonols, flavones, isoflavones, flavanones, and their derivatives. Particular flavonoid classes are defined by the oxidative state of the three-carbon chain. Most flavonoids are found in glycosylated form, with the exception of flavan-3-ols (*Current Protocols in Food Analytical Chemistry*). The three main classes of phenolic compounds found in grapes and wines include anthocyanins, flavonols, and proanthocyanidins (also known as condensed tannins). In grapes, proanthocyanidins are present in the greatest concentration, followed by anthocyanins and flavonols (Souqet and others 1996). Prodelphinidins are composed of gallocatechin monomers and found in high proportions in grape skins (Peyrot des Gachons and Kennedy, 2003). Non-flavonoids include stilbenes and phenolic acids.

Anthocyanins

Anthocyanins are water-soluble pigments occurring in the tissues of plants and are responsible for the red color of grapes and wine. They are composed of an anthocyanidin aglycone bound to a sugar—in grapes, most often a glucose molecule, though they can bind to arabinose, xylose, rhamnose, and galactose (Francis 1989). The main anthocyanidins found in grapes are cyanidin, pelargonidin, peonidin, petunidin, delphinidin (found high amounts in *V. labrusca)*, and malvidin (found in high amounts in *V. vinifera)*. Often, the sugar moiety is acylated or coumarylated (Haslam 1977); several of the most common acids involved in acylation include caffeic, *p*-coumaric, and *p*-hydroxybenzoic acids. Each grape cultivar has a unique compositional profile of anthocyanin accumulation (Mazza and Miniata 1993), which impacts color density and color stability of wine.

In order to release anthocyanins into the wine, maceration is required to rupture the berry cell walls and vacuoles in which anthocyanins are stored. During fermentation, anthocyanins are quickly extracted within the first few hours to days, while most other phenolics are slowly extracted throughout fermentation (Sacchi and others 2005). The ease of anthocyanin extraction into wine is based on the cell wall components of the berry, most notably on pectin and cellulose content (Ortega-Regules and others 2006). Berry size and skin-to-pulp ratio, in addition to fruit ripeness (Sims and Bates 1994; Canals and others 2005; Pérez-Magarino and González 2004) may influence extraction. Consequently, smaller berries with a higher proportion of skin surface to volume could result in a greater concentration of anthocyanins in wine (Matthews and Anderson 1988).

Once anthocyanins have been extracted into the wine matrix, they rapidly undergo numerous reactions and can form copigments (Brouillard and others 1994). The color expression

of anthocyanins is pH dependent (Boulton 2001). Several families of pigmented compounds have been identified and described by Salas and others (2005).

Proanthocyanidins

Proanthocyanidins, commonly known as condensed tannins, polyphenols, or polymeric phenols, encompass a complex range of structures that are classified into three groups of polyphenols: condensed tannins, hydrolysable tannins, and phlorotannins. These are naturally occurring polyphenolic compounds of high molecular weight, and are important to the mouthfeel and ageability of wine (Kennedy and others 2006; Noble 1990). In addition, they contribute to color stability by forming pigmented polymers with anthocyanins (Somers 1971). Their role in the sensory perception of wine is closely related to red wine quality (Amerine and Roessler 1976).

Condensed tannins are composed of flavan-3-ol monomer units. The most common monomers in wine are catechin, epicatechin, gallocatechin, and epigallocatechin. The galloylated forms of the monomers tend to be more astringent than their non-galloylated counterparts, and presence of a galloyl group increases the strength of copigmentation (Berké and Freitas 2005).

Condensed tannins have molecular masses ranging between 500 and 3000 daltons, and are located in the skin, seeds, and stem of the grape berry. Grape seeds contain (+)-catechin, (-)-epicatechin, and (-)-epicatechin-3-*O*-gallate flavan-3-ol monomers. Berry skin contains the monomers found in the seeds along with (-)-epigallocatechin, which is a tri-hydroxylated monomer (Prieur and others 1994; Romeyer and others 1986; Czochanska and others 1979). Skins differ from seeds in that they contain prodelphinidins, a lower concentration of flavan-3-ol

monomers, a lower proportion of galloylated subunits, and a higher degree of polymerization. In grape skins the primary flavan-3-ol is catechin (Kennedy and others 2001; Pastor del Rio and Kennedy 2006).

Proanthocyanidins can undergo acid catalyzed cleavage to release a flavan-3-ol unit containing an unstable carbocation, which can be reacted with a nucleophile such as a thiol or phloroglucinol as a method to identify and quantify tannin subunits (Haslam 1977; Kennedy and Jones 2001). In wine, the reactive carbocations can undergo other reactions forming pigmented polymers.

There are hundreds of possible condensed tannin structures formed through various linkage locations, branching, and elongator and initiator units. Each condensed tannin chain must start with an initiator flavan-3-ol monomer followed by elongating monomers. In wines, condensed tannins have been found with up to 80 polymerized monomers (Kennedy 2006).

One of the measures of condensed tannins is the degree of polymerization, quantifying the number of subunits that are part of one tannin molecule. The mean degree of polymerization is the average number of subunits over all condensed tannins present. This is more convenient to measure than the actual distribution of subunits from each individual tannin molecule. However, this measure does not give an indication of branching or exact astringency.

A method based on the presence of epigallocatechin tannin extension subunits, which exist only in grape skins, has been developed to analyze the percent extraction of seed and skin tannins in wine (Peyrot de Gachons and Kennedy 2003). Although grape seeds contain a higher total amount of flavan-3-ols and condensed tannins than skins, extraction of condensed tannins from seeds is generally low. Only a small portion of phenolics in grapes are extractable, and

studies suggest that only half of those extractable phenolics in grape seeds are transferred into wine (Singleton and Draper 1964; Sun and others 1999).

Hydrolysable tannins are comprised of a central glucose molecule substituted with gallic acid moieties. These tannins are derived from non-grape woody plant matter, and are added to wine through the use of barrels, oak chips, or commercial tannin additives (Harbertson and others 2012).

Flavonols

Flavonols are found in grape skins and are highly responsive to light exposure. They are thought to function as UV protectants (Price and others 1995; Spayd and others 2002; Downey and others 2004).

Glycosides and gluconurides of quercitin, kaempferol, myrcetin, and isorhamnetin (Ribereau-Gayon 1964; Makris and others 2006) are also found in grape skins. In wine, flavonols function as a factor that can copigment with anthocyanins (Asen and others 1972; Sceffedldt and Hrazdina 1978) and as free radical scavengers (Markham and others 1998). Typical flavonol levels in wine have been reported to range from 4.6 to 41.6 mg/L (Morag and others 1998).

Phenolic acids

Of the phenolic acids found in wine, the hydroxycinnamic acids (HCAs), which include *p*-coumaric, caffeic, ferulic, and sinapic acids, are most abundant. These acids most frequently occur as tartaric acid esters, glucose esters and quinic acid esters, rather than in a free state (Morreno-Arribas and others 2009), and are rapidly extracted during processing (Arnold and Noble 1979).

Phenolic acids can also act as copigmentation cofactors, polymerize with other phenols, and are precursors for volatile phenols (Scheffeldt and Hrazdina 1978; Chatonnet and others 1993). In wines, the presence of phenolic acids decreases due to oxidation reactions and adsorption to insoluble solids, soluble solids, and lees and barrels (Moreno-Arribas and others 2009).

Role of Phenolics

The phenolic makeup of grapes and wine has been studied due to its impact on the major organoleptic wine qualities of taste, color, flavor, mouthfeel and stability (Kennedy and others 2006; Moreno-Arribas and others 2009) and its possibilities of health benefits (Blanco 1998; Monagas and others 2005). Phenolic concentrations in wines are altered by enological and viticultural practices throughout the grape growing and winemaking process (Kennedy and others 2006; Thimothe 2007).

Aroma and Mouthfeel

Astringency and bitterness in wine is provided primarily by flavan-3-ols and proanthocyanidins originating from the fruit (Noble 1994). These compounds are also important for their role in the formation of long-term color stability and pigmented polymers in wine (Somers 1971). Astringency is the drying, roughening, puckering sensation in the mouth, caused when salivary proteins interact with wine tannins to form a precipitate (Kennedy 2006; Lawless and others 1994). Salivary proteins are rich in proline and exist in rigid planar formations (Luck and others 1994). Variations in wine tannin composition, the level of galloylation, and formation of derivatives can affect both bitterness and astringency by differentially interacting with these

salivary proteins (Lesschaeve and Noble 2005). Astringency in wine increases with increasing tannin polymerization (Arnold and others1980), and skin tannins have greater polymerization than seed tannins (Labarbe and others 1999). As wine ages, condensed tannins undergo reactions of acid catalysis, de-polymerization, and re-polymerization, resulting in more branched structures and lower astringency. Tannins that are younger and have undergone less recombination and branching have more surface area; subsequently, they react more readily with proline chains and are more easily precipitated, leading to a more pronounced sensation of astringency (Kennedy 2011). Seed mean-degree-of-polymerization (mDP) was found to be in the range of 5-9 (Pastor del Rio and Kennedy 2006; Kennedy and others 2000b). Skin tannins have a higher mDP than seed tannins, and are the major source of polymeric tannins in wine (Sun and others 1999).

Monomeric flavan-3-ols such as phenolic acids are primarily bitter, though as molecular weight increases with polymerization, astringency predominates over bitterness (Noble 1994; Peleg and others 1999). The differences in sensory properties between phenolics are related to the type of monomeric unit, linkage sites, degree of galloylation, and the formation of derivatives (Peleg and others 1999; Vidal and others 2003; Lesschaeve and Noble 2005.)

Many phenolic compounds have been found to impact the sensory character of wines, contributing odor, astringency, pungency, sweetness, and bitterness (Singleton and Noble 1976). They also have been reported to contribute to the level of liking of a particular wine (Lesschaeve and Noble 2005). Other compounds in wine modify the perception of astringency through enhancement or suppression. The addition of acid increases the astringency of wines (Kallithraka and others 1997) and increases perceived sourness, but had no effect on bitterness (Fischer and Noble 1994). Increasing the ethanol content increased the intensity of bitterness but

had no effect on astringency (Fischer and others 1994). Increasing sucrose content in wines decreased the perception of astringency (Ishikawa and Noble 1995). As of yet, the impact of pigmented polymers on wine astringency has not been determined.

Pigments

Polymeric pigments are formed through the direct combination of flavonoids and nonflavonoids. Polymeric pigments are responsible for the color of older wines and are formed during winemaking and aging. The predominant pigments in aged red wines are formed through the direct condensation of a condensed tannin and an anthocyanin (Remy and others 2000), which only takes place in the presence of oxygen. Polymeric pigments produce a red-brick color, and a mix of their many forms typically account for 50 to 70% of the pigments in a year-old wine (Ribereau-Gayon and others 1970; Somers 1971).

Copigmentation is important for the color of wines up to two years old. The copigmentation interaction is not particularly strong, and can bleach in the presence of sulfites, as sulfites are a better nucleophile to anthocyanins than most cofactors. The level of copigmentation is dependent on the amount of anthocyanins extracted from the grapes; as such, color cannot be increased through copigmentation if enough anthocyanins are not present.

Pyranoanthocyanins are formed through the combination of an anthocyanin and ketone or aldehyde, and are found in older wines (Fulcrand and others 1998). At wine pH they are orange–red pigments, and are resistant to oxidation and bisulfite bleaching (Lee and others 2004). Other families of pigmented compounds have been identified and described by Salas and others (2005).

1.4.2 Volatile Compounds

The main aroma components of grapes and wines can be grouped into three chemical classes: ethyl esters, acetate esters, and higher alcohols (Morreno-Arribas and others 2009). Other compounds, such as various norisoprenoids, can also contribute significant aromas (Vinholes 2009).

Some phenolic compounds also lend aromas to wines, or are precursors to volatile phenols. Of particular note are the free hydroxycinnamic acids, which some *Saccharomyces cerevisiae* yeast strains enzymatically decarboxylate into 4-vinylphenol and 4-vinylguaicol, contributing spice and clove aromas, respectively (Chatonnet and others 1993).

1.4.3 Differences between Hybrids and V. viniferas

The forms of anthocyanins found in *V. vinifera* and hybrid grapes and their wines is strikingly different—the diglucoside anthocyanin form is found in *V. riparia* and in *V. rupestris,* but not in *V. vinifera* (Webb and others 1974). Though anthocyanin forms and solubility may be different, it has not been demonstrated that different processing methods are required to extract color from hybrid grapes. Empirically, wines produced from hybrid grapes have been described as having characteristic "radish," "cabbage," or "hybrid funk" aromas not found in wines produced from *V. vinifera*.

1.5 Influencing Phenolic Concentration in Wine

1.5.1 Processing Methods

The processing methods and yeast selection used during wine production can affect the types and concentration of phenolic and volatile compounds in the resulting wines. In addition

to the amount of phenolic compounds in the berry, the extractability of compounds and their location within the berry influence final wine profile.

Fermentation conditions or pre-fermentation treatments can affect the concentration of compounds found within a red wine to a greater or lesser degree. The bulk of research on processing methods has been performed on *V. vinifera*, and little has been published concerning their effects on the hybrid cultivars of interest in this research.

Effects of Time on Fermentation

If two identical musts are fermented on the grape solids and one is stopped early while the other is allowed to ferment to dryness, a greater amount of alcohol will be present (Sacchi and others 2005). The presence of ethanol increases the solubility of phenolics into the wine matrix (Moreno-Arribas and others 2009), so extended contact of grape solids with ethanol may increase final phenolic concentrations.

Maceration Time

Research suggests that a period of extended maceration would increase tannin concentration, but not that of anthocyanins (Sacchi and others 2005). Extending maceration time prolongs skin contact with the wine after it has been fermented to dryness, and is believed to increase extraction of phenolics from the skins and seeds (Sacchi and others 2005). A study that compared maceration of Cabernet Sauvignon for 7, 13, and 21 days found that total phenols, gallic acid, and flavonols all increase with skin contact time (Auw and others 1996). Also, increased tannin extraction may lead to greater polymeric pigment formation (Sacchi and others 2005), which stabilizes color during aging.

Fermentation temperature

Overall higher fermentation temperatures have been reported to increase phenolic extraction due to increased permeability of the hypodermal cells releasing anthocyanin, and increased solubility of other phenolics in the wine solution (Sacchi and others 2005). The increase in polymeric pigment appears to be due more to the preferential extraction of tannins over anthocyanins, since anthocyanin levels drop early in fermentation. If adequate tannin levels are not present at this stage to bind with anthocyanins, final polymeric pigment concentrations in the wine will be lower (Sacchi and others 2005).

Thermovinification

During thermovinification, grape must is heated to 60-70°C for a short time, allowing greater extraction of skin compounds prior to pressing and cooling pre-fermentation (Auw and others 1996). The heat damages the hypodermal cell membranes, releasing anthocyanins, and denatures polyphenol oxidase, preventing browning (Lee and others 1983). Since there is no alcohol present at the time of heating, this treatment would not be expected to increase tannin extraction, and it has been reported that, compared to fermentation on the skins, thermovinification leads to improved anthocyanin extraction but much lower phenolic extraction overall (Auw and others 1996). In a study comparing three Italian winegrape cultivars, thermovinification was found to increase anthocyanin content for all three and decrease catechin and total phenols for two of the wines (Sacchi and others 2005). When hot skins are pressed immediately after thermovinification, lower levels of anthocyanins, catechins, and total phenols were reported (Leone and others 1983). For heat treatments to be successful in increasing

extraction, it appears to be necessary to have the skins in contact with the juice during or after heating (Leone and others 1983).

Cold Soak

During a cold-soak treatment, the must is held at low temperatures, usually 10-15°C, for several days before fermentation (Sacchi et al 2005). The rationale offered by winemakers is that prolonged aqueous extraction improves wine color. Reported results are inconsistent, suggesting that cold soak treatment alone seems to be ineffective in extracting more phenolics and pigments, but that a cold soak treatment in the presence of 50mg/L or greater of sulfur dioxide increases the anthocyanin content of young wines and total phenols in finished wines (Sacchi and others 2005).

Macerating enzymes

Enzymes are used in grape musts and wine fermentations for settling and clarification, and can be used in an attempt to increase wine color by breaking down skin cell walls to release pigments. In the majority of studies, pectinases, enzymes used to break down pectin, do not seem to increase anthocyanins extraction but do seem to increase extraction of other phenolics, including tannins (Leone and others 1983). They have also been found to increase polymeric pigment formation (Sacchi and others 2005).

With the many commercial preparations of enzymes available, winemakers and enologists can exploit specific enzyme activities, such as the use of pectinases to provide ease of must filtration, or beta-glucosidases which, according to commercial claims, can release terpenes

and other bound aromas by catalyzing the hydrolysis of β-glucose bonds found in grape cell wall components.

SO_2 Effects

Sulfur dioxide (SO_2) is a common wine additive used pre- and post fermentation to prevent browning and microbial spoilage. Usually it does not affect the extraction of phenolics under levels and temperatures normally used for red wine fermentations, though some studies have observed increased extraction of phenolics with higher levels of SO_2 and at lower temperatures (Sacchi and others 2005). However, SO_2 can also have negative effects on color through reversible bisulfite bleaching of anthocyanins (Jurd 1964).

Punch –down and Pump-over Effects

During fermentation on the skins, carbon dioxide release causes grape solids to rise to the top of the fermentation tank and create a cap. This has two potentially negative consequences: heat is trapped in the cap, and there is reduced contact between the bulk juice and the skins and seeds (Sacchi and others 2005). Traditionally, cap temperature is thought to be important to wine quality, but literature on the subject is sparse. To overcome issues of reduced contact, skins and juice are usually mixed several times a day, either by pushing the grape solids below the juice surface (punch down), or by pumping juice out of the bottom of the vessel and spraying it over the top of the skins (pumpover). The effect of a pumpover is dependent upon the timing during fermentation, as it will be influenced by both fermentation temperature and whether skins are circulated through the pumps (Sacchi and others 2005).

Addition of Exogenous Tannins

Commercial tannin products may be added for a variety of reasons, and are one of the many additional input costs in producing wine. Winemakers use them in an attempt to modify mouthfeel, for clarification, increased color stability, the removal of off-aromas, or antioxidant properties.

A variety of exogenous tannins are commercially available for use at various steps in the winemaking process. Commercial products can be roughly classified into two groups: oenological tannins and ellagic tannins. Oenological tannins are produced from grape material, and formulations may contain a mixture of polyphenols, HCAs, and low molecular weight phenols that may cause bitterness and contribute condensed tannins that enhance astringency. According to Harbertson and others (2012) most commercial preparations of oenological tannins contain 12-48% tannin. Ellagic tannins originate from woody material commonly sourced from oak (*Quercus*), chestnut (*Castanea*), or exotic woods (*Schinopsis, Acacia)*, which contribute hydrolysable tannins and decrease astringency as wine ages.

According to manufacturers' recommendations, tannin additions should be specifically timed for each formulation, though Harbertson and others (2012) found that most suggested addition rates were far too low to add any perceptible change in sensory characteristics, and that added tannins may adsorb to sugar, pectins, and solids in the wine, resulting in instant loss post-addition.

1.6 Instrumental Analysis of Phenolics

Phenolic analysis is commonly performed with high-performance liquid chromatography (HPLC) using reversed-phase (RP) columns packed with C18 or C8 material and a binary or

ternary eluent system consisting of water, methanol, or acetonitrile and an acid. (Bonerz and others 2008). Though currently the best means of identifying and quantifying phenolic compounds, analysis of large or polymerized molecules is difficult, even the latest technology in HPLC, low pressure liquid chromatography (LPLC), and high performance thin layer chromatography (HPTLC), and results are imprecise (Ribéreau-Gayon and others 2006). Many methods rely on direct injection of the wine sample following simple dilution and filtration (Bonerz and others 2008; Lamuela-Raventos and others 1994), though more recently, sample preparation and processing techniques have evolved (Jeffery and others 2008; Stalikas 2007). The use of solid phase extraction (SPE) is one technique commonly used to isolate single compounds or select classes of compounds from the wine matrix. However, some techniques may require multiple steps, including dealcoholization and pH adjustment of the sample prior to fractionation (Jeffery and others 2008).

With proper wine sample fractionation, HPLC identification and quantification of anthocyanins, monomeric phenolics, and polyphenolics becomes easier due to simpler chromatographic behavior and increased apparent resolution. Each fractionated group of phenolics requires analysis at a specific wavelength to determine the particular compounds present: 280nm shows most classes of phenolics, while hydroxycinnamic acids, flavonols, and anthocyanins are observed at 320nm, 360nm, and 520nm, respectively.

Other techniques involve solvent-assisted flavor evaporation, a gentle distillation technique that separates aroma extracts into volatile and non-volatile components, cleaning the sample and preventing artifact formation (Engel and others 1999). Solid phase micro extraction (SPME) is typically a solvent-free method of volatile isolation. A fiber coated with adsorbent material is exposed either to the headspace of a product or immersed within it. Its advantages

include high experimental reproducibility and a lack of harmful solvents (Pozo-Bayón and others 2001).

1.7 Rationale

Little work has been done to assess the quality characteristics of red hybrids grown across the eastern and mid-western United States. More specifically, because of the interspecific background of these cultivars, the wines produced from them may vary from familiar sensory characteristics associated with *V. vinifera* wines.

The concept of 'quality' in red hybrid wines, even more than in 'traditional' wines, is difficult to grasp. Research on wine quality has shown that wine drinkers feel wine has distinct quality components (Charters and others 2007). A study on Spanish wine drinkers has shown that people break the concept of wine quality into seven dimensions: origin, balance, flavor and bouquet, vintage, aging ability, image, presentation, and "acuteness"—the aromatic complexity and intensity (Verdú-Jover and others 2004). Other scientists and researchers define quality as the absence of faults, or base it on the amount of pleasure derived from drinking a wine (Peynaud 1987). Another view of quality comes from elements that make up the wine. The most common quality indicators are balance, length, intensity of flavor, complexity, and varietal purity (Amerine & Roessler 1976; Basset 2000; Broadbent 1988; Jackson 2000; Peynaud 1987).

In red hybrid wines, the impacts of taste and aroma profile on perceived quality are areas of continued research. Though each hybrid has a unique genetic mixture, both parent cultivars generally contribute compounds commonly found in their singular form.

Grape and wine phenolic management has been poorly studied to date, and existing work has focused largely on *V. vinifera* cultivars. For economies where red hybrid wines are very

important, more work needs to be done to help winemakers and grape growers understand how to manage both viticultural and enological aspects of phenolic development and extraction. It is important to many of these wineries to establish an identity based on quality wines from hybrid grapes, and to understand how their individual viticultural and winemaking practices affect this quality.

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CHAPTER 2
SURVEY OF WINEMAKING TECHNIQUES AND CHALLENGES

2.1 Introduction

Over the last forty years, the grape and wine industry has grown exponentially across the midwestern and eastern United States. Most of these areas are considered to be cool or cold climate wine regions, where *V. vinifera* either cannot survive or consistently produce high quality fruit. Spring frost and severe winters can damage the harvest, as can sudden changes in temperature, severe storms, winds, precipitation, or hail which can obliterate a crop. Subsequently, hybrid grape cultivars are the mainstay of these regions, where they are grown for their cold hardiness, disease resistance, early ripeness and productivity (Pollefeys, 2003).

The concept of 'quality' in red hybrid wines, and even in more 'traditional' wines, has been difficult to grasp on either a consumer and professional level. Research on wine quality has shown that wine drinkers feel that wine has distinct quality components (Charters *et al.,* 2007). Some scientists and researchers define quality as the absence of faults, or base it on the amount of pleasure derived from drinking a wine (Peynaud, 1987). Another view of quality comes from more esoteric elements, including hard-to-define concepts like balance, length, intensity of flavor, complexity, and varietal purity (Amerine and Roessler, 1976; Basset, 2000; Broadbent, 1979; Jackson, 2000; Peynaud, 1987) in which phenolic compounds play the greatest role (Gawel, 2000).

The phenolic makeup of grapes and wine impact the major organoleptic wine qualities of taste, color, flavor, mouthfeel, and stability (Kennedy *et al.,* 2006; Moreno-Arribas *et al.*, 2009) and their possibilities of health benefits (Blanco, 1998; Monagas *et al.,* 2005). The concentration of phenolic compounds in wine is altered by viticultural and enological practices throughout the grape growing and winemaking process (Kennedy *et al.,* 2006; Thimothe, 2007). These

practices include, but are not limited to crop management, canopy management, maceration time, temperature manipulation, and the use of additives such as enzymes and tannins. As weather varies from vintage to vintage, the accumulation of phenolics can be greatly affected, especially in cold-climate growing regions where weather extremes and disease pressures may be unpredictable.

As perceived wine quality is key in consumers' purchasing decisions, winemakers and grape growers must understand how to manage both viticultural and enological aspects of phenolic development and extraction. In economies where red hybrid grapes are of high value, wineries must establish an identity based on quality wines. More recently there has been an increase in 'buy local' movements (Skuras *et al*., 2006). This, along with a growing interest in producing higher value horticulture crops such as grapes, has increased the importance of products that are locally produced. In these cooler climate regions, hybrid plantings have rapidly increased--over the last forty years 4,000 acres been planted in New York alone. Collectively, New York, Iowa, Wisconsin, Minnesota, and Missouri currently have about 5,600 acres of hybrid grapes (Martinson, 2011). With this rapid increase, research has been slow to follow. To date, only a few studies have addressed viticultural or enological practices and consumer preferences for red hybrid winegrapes, though there has been no comprehensive survey across states to assess challenges winemakers face with certain cultivars, winery size, consumer education, and winemaking techniques.

The objectives of this study were three-fold: to investigate the challenges and techniques in red hybrid wine production, to estimate the probability of certain challenges against particular factors, and to relate this information to a SCRI-funded research project involving the phenolic extraction of red hybrid winegrapes. This work was designed to test the hypothesis that certain

red hybrid winegrapes pose challenges over the course of winemaking and that through years of trial and error, winemakers have adopted certain techniques to alleviate some of these challenges. Ultimately, this information will contribute to a greater understanding of the challenges and techniques winemakers deal with in the production of quality wine, so that future research projects will provide practical applications to this group of hybrid users with the goal of improving profitability and to achieve sustained growth of the industry.

2.2 Method

Given that techniques and growing region greatly impact the quality of wine, an initial survey was made done in collaboration with winemakers across Michigan, Minnesota, Wisconsin, New York, and Pennsylvania. The objectives were as follows: to investigate the challenges and techniques in red hybrid wine production, to determine the likelihood of certain challenges with respect to varietal, winery size and state, and to relate this information to a SCRI-funded research project involving the phenolic extraction of red hybrid winegrapes.

To collect data for this study, a survey was supplied to winemakers through their local extension agents in each state; responses were collected from June 2011 to July 2011. The survey was created using Cornell University's online Qualtrics System, and was distributed electronically via email.

The survey comprised two parts: general information on location, winery production size, consumer education, and a screening question on the use of hybrid winegrapes. If respondents answered "no" to using hybrid winegrapes, they were asked whether they might use hybrids in the future. If respondents answered "yes", they continued onto the last set of questions treating which hybrids they grew or bought, prices paid for hybrid grapes at harvest, production methods,

grape cultivars, and the challenges associated with hybrid wine production. Responses that were free-form text entries were categorized and enumerated manually. A multinomial logit model was created based on the data to estimate the likelihood of particular challenges occurring. All statistical analyses were performed using R (Version 2.14.2).

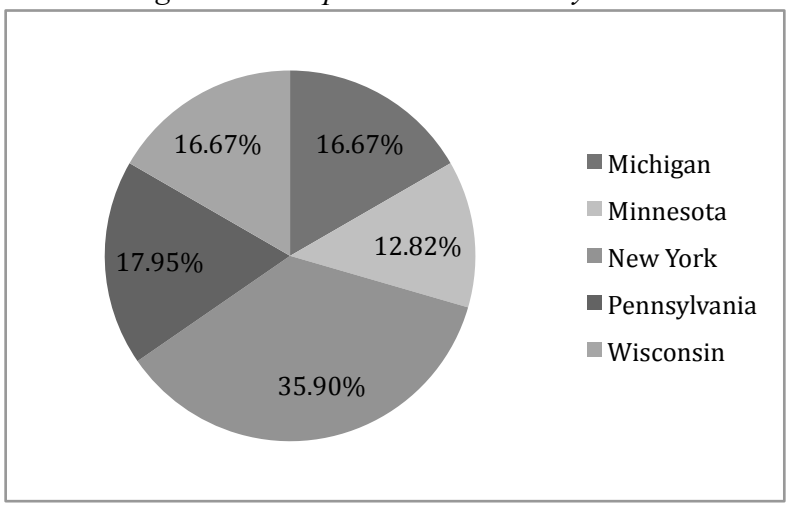
2.3 Results and Discussion

This study identified the major challenges winemakers deal with, and how the use of particular cultivars, the size of the winery, or the state in which wine is being produced affects those challenges.

2.3.1 Responses

Across the five states, a total of 78 responses were collected from winemakers; the breakdown of response by state can be seen in Figure 2.1. Respondents were selected by their local enology extension agents as winemakers of commercial wineries in Michigan, Minnesota, New York, Pennsylvania, and Wisconsin.

Figure 2.1. Response breakdown by state



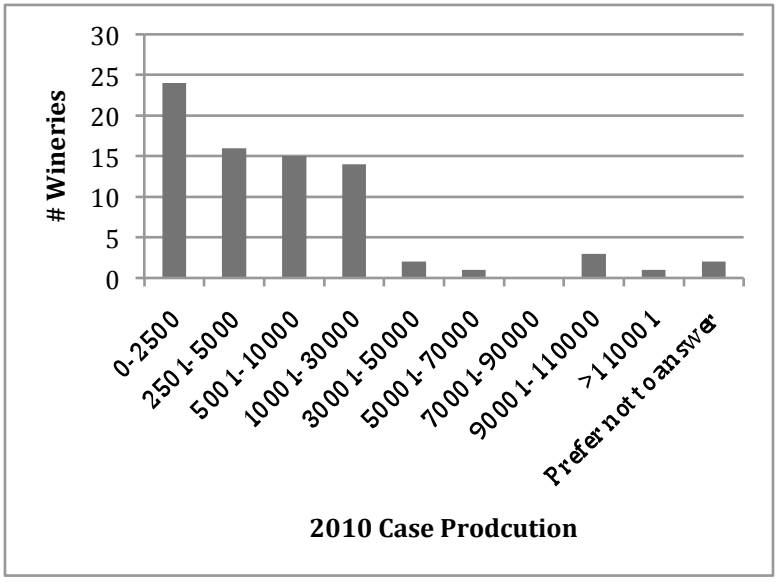
2.3.2 Size Distribution of Wineries

While the major share of wine is produced by very large wineries, the majority of wineries in each state are very small. In some cases, this is simply a result of regional history, as in New York, where the Farm Winery Act of 1976 enabled grape producers to find a higher value market for their grapes if they turned to making wine commercially, rather than selling their grapes to large wineries.

A large number of small wineries are selling primarily through direct-to-consumer retail (out of the tasting room) or through small restaurants and retailers within their region or state. These wineries tend to be visitor-driven, relying on their location near a wine trail or as a "destination winery" to sell wine (Motto, Kryla and Fisher, 2008).

A majority of respondents were from small wineries, defined as those producing less than 10,000 cases of wine from the 2010 harvest. Overall, about one quarter of the respondents produced more than 10,000 cases. The size distribution of wineries across respondents can be seen in Figure 2.2.

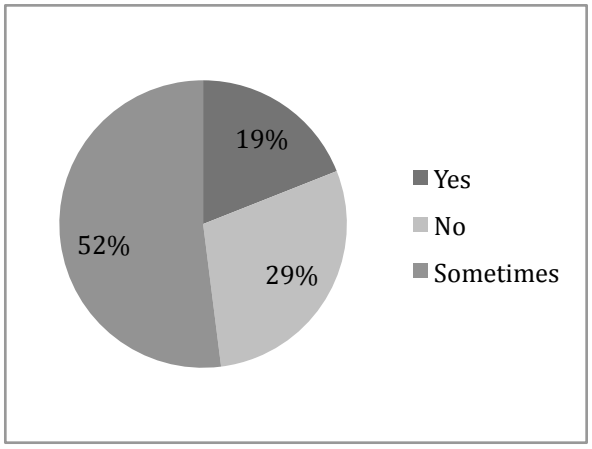
Figure 2.2. Size distribution of winery respondents



2.3.3 Consumer Familiarity

It is commonly believed in the industry that red hybrid grapes are less well known than vinifera grapes, simply due to lower market share, and thus, less exposure. In the regions of interest, small wineries sell a majority of their product directly out of the tasting room, where interaction with consumers is high. As consumers drive sales, winemakers were asked whether they felt their core consumers were generally familiar with red hybrid grapes and wines. The 80% of their consumers either had some or no knowledge of red hybrid grapes, while only 20% were reported to be familiar with red hybrid grapes for wine production.

Figure 2.3 Core consumer familiarity with hybrid grapes and wines



2.3.4 Respondents Working with Red Hybrid Wines

Of the respondents, 86% produced wines from red hybrid grapes. The remaining 14% were asked if they were planning to produce wine from red hybrid grapes in the future. Of those, 27% said yes, 18% said no, and 55% said maybe. Those who were not currently working with red hybrid wines completed the survey, while the other respondents were given another set of questions dealing with cultivars, prices, winemaking techniques, and challenges.

2.3.5 Red Hybrid Grape Cultivars Used for Wine Production

There is a wide variety of red hybrid grapes suitable for cultivation in the climates of Michigan, Minnesota, New York, Pennsylvania, and Wisconsin; however, there are large variations in climate and topography across the states. While some hybrids may survive in Pennsylvania or certain regions of New York, they may not be able to withstand the colder climates of Minnesota or Wisconsin; Corot noir is one such example, as it is only cold hardy to -10°F (Reisch *et al*., 2006), and would not survive a Wisconsin winter. Table 2.1 summarizes the range of cultivars and the percentage of winemakers using those cultivars for red hybrid wine production in each state. It is important to note that this information is not an indication of total acreage or of total production.

Table 2.1. Percentage of winemakers using listed red hybrid cultivars in each state

Michigan		Minnesota		New York		Pennsylvania		Wisconsin	
Baco noir	12.50%	Baco noir	3.45%	Baco noir	11.29%	Baco noir	7.55%	Frontenac	25.93%
Chambourcin	18.75%	Chambourcin	3.45%	Chambourcin	14.52%	Chambourcin	20.75%	Leon Millot	7.41%
Chancellor	12.50%	DeChaunac	3.45%	Chancellor	3.23%	Chancellor	5.66%	Maréchal Foch	18.52%
Chelois	6.25%	Frontenac	37.93%	Chelois	1.61%	Chelois	1.89%	Marquette	14.81%
DeChaunac	6.25%	Leon Millot	6.90%	Corot noir	9.68%	Corot noir	3.77%	Sabrevois	3.70%
Frontenac	6.25%	Maréchal Foch	17.24%	DeChaunac	14.52%	DeChaunac	5.66%	St. Croix	22.22%
Leon Millot	6.25%	Marquette	27.59%	Frontenac	6.45%	GR7	1.89%	Steuben	3.70%
Maréchal Foch	6.25%			GR7	1.61%	Landot noir	7.55%	Worden	3.70%
Noiret	6.25%			Maréchal Foch	14.52%	Leon Millot	13.21%		
Regent	6.25%			Marquette	4.84%	Maréchal Foch	11.32%		
St. Croix	6.25%			Noiret	12.90%	Noiret	13.21%		
St. Vincent	6.25%			Rougeon	1.61%	Rosette	1.89%		
				Vincent	3.23%	St. Croix	3.77%		
						Vincent	1.89%		

2.3.6 Source of Red Hybrid Grapes for Wine Production

In visiting commercial wineries it is not uncommon to see rolling vineyards associated with the property; grapes grown and processed onsite are considered 'estate grown,' and can be labeled as such (TTB, 2012). While many winemakers are able to source some or all of their

grapes from their own estate, land, financial, labor, or climate limitations may force winemakers to source their grapes from other local, or even national, growers.

In our group of respondents, 89% grow some or all, and 63% buy some or all, of the red hybrid grapes used in their wine production. Those who buy or sell grapes were also queried on the average price they pay or demand for red hybrid winegrapes. The average price paid for red hybrid wine grapes in each state can be seen in Table 2.2.

Table 2.2 Average red hybrid grape prices by state

State	Average Price ($/ton)
Michigan	$750
Minnesota	$1350
New York	$700
Pennsylvania	$740
Wisconsin	$1150

A wide variation in average price can be observed, most likely due to overall supply, local farm winery laws, and different viticultural practices required for different cultivars. When improving grape quality to yield high quality wines, viticulturists reduce yields, shoot thin, selectively remove leaves, and/or invest heavily in trellising systems. The prices of grapes also vary by state and region, most likely due to their level of reliance on hybrid grapes to produce wines—in Wisconsin and Minnesota, no *V. vinifera* consistently survive the winters, so winemakers rely only on locally and estate grown hybrid grapes, along with out-of-state *V. vinifera* imported for wine production.

2.3.7 Challenges Associated with Red Hybrid Wine Production

Winemakers were asked to list specific challenges they faced when working with red hybrid wines. Most commonly, winemakers report issues in storage, production, and acidity, though cases had not been officially documented. Challenges were fit to six categories based on

the responses received: acidity, aroma, color stability, low tannin, production and storage. Wines that were a challenge with acidity included those whose pH or titratable acidity was too high. Aroma challenges included the production of off-odors or overpowering varietal character. Color stability and low tannin were mentioned where wines dropped color in the bottle, had a lack of mouthfeel, or a lack of density. Issues in production and storage included ease of filtering and handling, oxidation susceptibility, maturation, film yeast presence, and shelf life.

Challenges were referenced by state, by varietal, and by winery size, assuming that particular cultivars grown in different states may present different challenges. According to the survey, winemakers' biggest challenges were with color stability, acidity, and low tannin content. Figure 2.4 shows a breakdown of all the challenges mentioned in red hybrid wine production, and Figures 2.5, 2.6 and 2.7 show the challenges associated by grape cultivar and by state. Of all the grape cultivars, the greatest number of incidences was reported in Chambourcin and Frontenac. Overall, winemakers in New York and Pennsylvania reported the most issues, followed by Wisconsin, Michigan, and Minnesota, respectively. Other questions were designed to determine whether the size of a winery would impact the challenges winemakers faced, perhaps due to financial freedom or success in the marketplace. Results suggested that smaller wineries were concerned mostly with acidity, while larger wineries deal with issues in color stability and storage; these data may suggest that regions growing high-acid *V. riparia* based hybrids, e.g. Minnesota and Wisconsin, had a larger proportion of small wineries. The largest wineries did not report dealing with any particular challenges.

Figure 2.4. Challenges in red hybrid wine production, based on the percentage of incidences mentioned. Greatest challenges in color stability, acidity, and low tannin content.

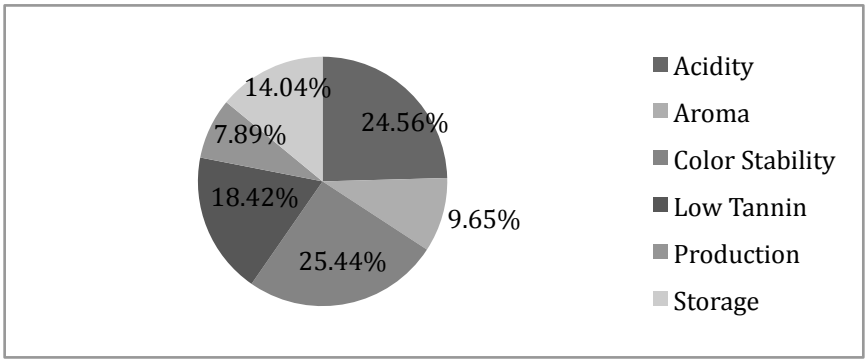


Figure 2.5. Incidence of challenge by cultivar. Majority of challenges deal with acidity and low tannin content.

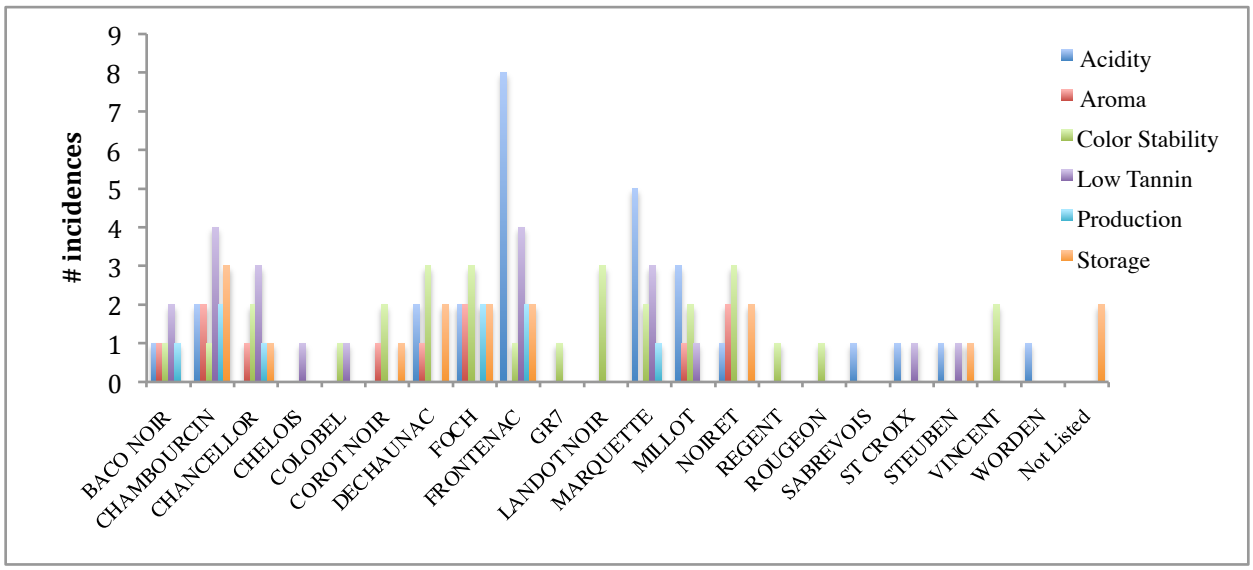


Figure 2.6. Challenges by state. Wisconsin and Minnesota dealt mostly with challenges of acidity, while New York faced its greatest challenges with storage issues. Michigan and Pennsylvania faced their greatest challenges in low tannin and color stability, respectively.

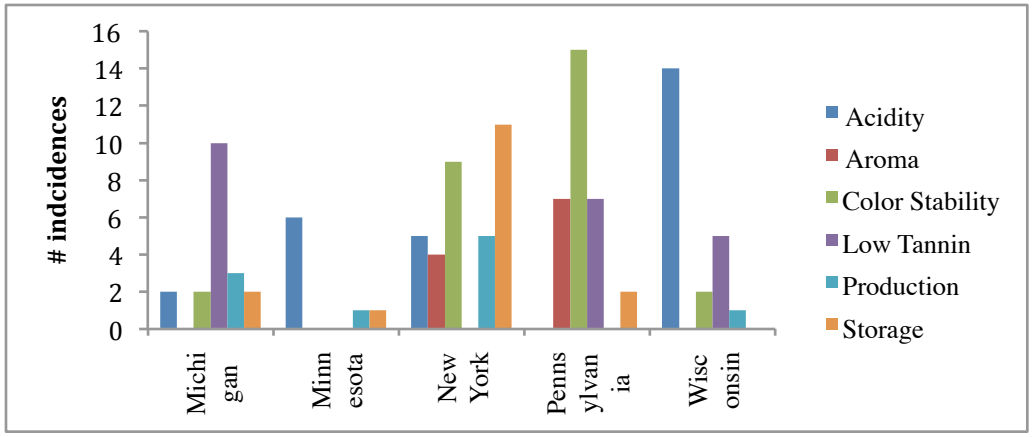
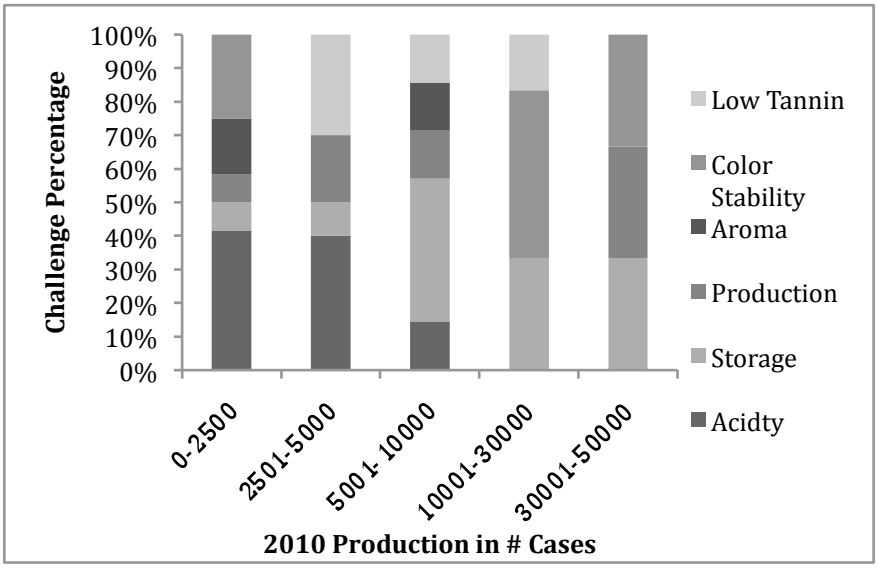


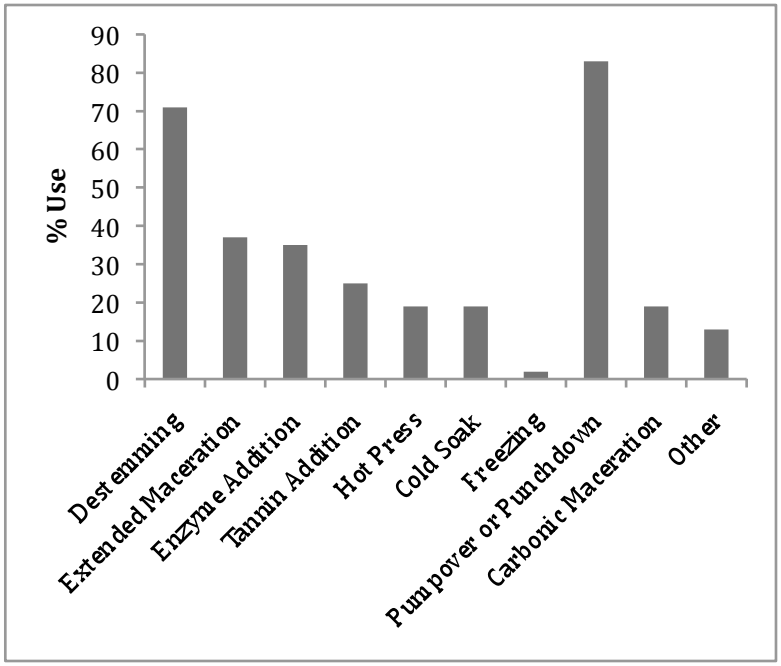
Figure 2.7. Comparison of challenges by state according to winery size. Data shows that smaller wineries deal mostly with acidity while larger wineries deal with issues in color stability and storage. The largest wineries did not indicate dealing with any issues.



2.3.8 Processing Techniques

Through thousands of years of trial and error, winemakers have put particular techniques to use in order to influence particular characteristics of wine. Some of these techniques are regional, and may be selected for stylistic expression, like the use of carbonic maceration in Beaujolais, France which yields light, fruity wines. Other techniques address more physical issues in winemaking, such as the removal of stems from grapes to prevent extraction of harsh green flavors and tannins, or cap management techniques (pumpover, punchdown) to provide a more even temperature and extraction profile during fermentation. Winemakers selected their responses from a list of techniques: destemming, extended maceration (extra juice contact with grape solids), enzyme addition (to aid extraction or processing), tannin addition (to increase mouthfeel or color stability), hot press (high temperature treatment followed by immediate pressing), cold soak (cold treatment before fermentation), freezing (to aid extraction), carbonic maceration (whole berry fermentation), and other (unlisted choice). They were asked to list all that applied.

Figure 2.8 Use of processing techniques. Percent use of each winemaking technique is shown. Destemming and cap management treatment are most widely used, followed by extended macerations and enzyme additions.



2.3.9 Assessing Challenges in Red Hybrid Winemaking: A Multinomial Logit Model

While this information in raw form may be helpful in assessing the current state of the industry, it does not indicate what challenges a winemaker may face in a particular state, winery size, or when using a specific red hybrid grape cultivar. Providing a model that would allow for estimation of the likelihood of challenges may be particularly useful in an extension application and to people interested in starting a wine business. The objective of the multinomial logit model was to determine the likelihood of challenge incidence based on winery size, varietal use, and region in the United States.

For determining regions, Michigan, Minnesota, and Wisconsin were grouped as Midwest while New York and Pennsylvania were grouped as Northeast. Challenges were characterized as: none, acidity, aroma, phenolic (color, mouthfeel), production, and storage. Size of the winery

and cultivars were also considered, though neither factor was significant to the model due to the small number of observations. Models were run to determine which factors had significance by challenge, state and region.

Model 1--State x Presence of any challenge: The reported existence of any challenge was of near significance ($p<0.7$) to the model only for Wisconsin. Wisconsin is nearly 0.6 to 1.7 times more likely to face any challenge than were other states. The other states were not significantly different from each other.

Model 2--State x Specific challenge: This model was found to be insignificant due to large standard errors and too few observations.

Model 3--Region x Presence of any challenge: Outcomes of having a challenge ($p=0.5$) were approximately equal in comparing regions. This indicates that each region has approximately the same probability in reporting a challenge when working with red hybrid grapes.

Model 4--Region x Specific challenge: The Midwest and Northeast were compared across each category of challenge. Based on the survey responses, the Midwest is nearly ten times more likely to report challenges in acidity, 3.7 times more likely to face challenges in aroma, and four times more likely to face challenges in color stability, a lack of tannin, and storage than the Northeast. The probability of a production challenge was insignificant due to few observations in that category.

2.4 Conclusion

It is clear that the main challenges winemakers face deal with acidity, phenolic character (color stability, density, tannins), and storage, especially in the Midwest. Currently, the cultivar

and winery size are not statistically significant in determining the likelihood of facing a particular challenge. Due to the limited number of responses, replication of this study to with larger pool of respondents would make multinomial logit models more manageable by decreasing standard error.

Continued replication of this study over multiple years would also be beneficial in determining trends in the use of winemaking techniques and challenges, with the addition of data on the use of viticultural techniques and challenges and winemakers' years of experience to see how they relate.

Most beneficial would be to tie this data to customer satisfaction in the tasting room and to the quality of the wines based on challenges or techniques the winemakers face. The industry's consumer satisfaction will help achieve sustained growth of the industry.

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CHAPTER 3
PHENOLIC EXTRACTION FROM COLD HARDY RED WINEGRAPES: MARECHAL FOCH, COROT NOIR, & MARQUETTE

3.1 Introduction

The concept of 'quality' in red hybrid wines, and even in more 'traditional' wines, has been difficult for many to grasp. Research on wine quality has shown that wine drinkers feel that wine has distinct quality components (Charters and others 2007). A study of Spanish wine drinkers found that consumers break the concept of wine quality into seven dimensions: origin, balance, flavor and bouquet, vintage, aging ability, image, presentation, and "acuteness"—the aromatic complexity and intensity (Verdú-Jover and others 2004). Other scientists and researchers define quality as the absence of faults, or base it on the amount of pleasure derived from drinking a wine (Peynaud 1987). Another view of quality includes more esoteric elements, including such hard-to-define concepts as balance, length, intensity of flavor, complexity, and varietal purity (Amerine and Roessler 1976; Basset 2000; Broadbent 1988; Jackson 2000; Peynaud 1987). Gawel and others (2000) suggest that phenolic compounds play the greatest role in affecting organoleptic qualities.

The phenolic makeup of grapes and wine impact the major organoleptic wine qualities of taste, color, flavor, mouthfeel, and stability (Kennedy and others 2006; Moreno-Arribas and others 2009) and their possibilities of health benefits (Blanco 1998; Monagas and others 2005). The concentration of phenolic compounds in wine is altered by viticultural and enological practices throughout the grape growing and winemaking process (Kennedy and others 2006; Thimothe 2007). These practices include, but are not limited to, crop management, canopy management, maceration time, temperature manipulation, and the use of additives such as enzymes and tannins.

In dry red wines, phenolics are usually the most plentiful constituents after alcohol, tartaric acid, and unfermentable sugars (Singleton and Noble 1976). Phenolics can be found in monomeric, oligomeric, or polymeric forms in the seeds, skins, and stems of the grape (Monagas and others 2005). Grape-derived phenolics may be modified by enzymes (Moreno-Arribas and others 2009) and the process of crushing and preparation for fermentation may exacerbate these changes.

Phenolics can be broken down into two large groups: flavonoids and nonflavonoids. Flavonoids include anthocyanins, flavanols, flavonols, flavones, isoflavones, flavanones, and their derivatives. Non-flavonoids include stilbenes and phenolic acids.

Anthocyanins are water-soluble pigments occurring in the tissues of plants and are responsible for the red color of grapes and wine. The main anthocyanidins found in grapes are cyanidin, pelargonidin, peonidin, petunidin, delphinidin (found in high amounts in *V. labrusca)*, and malvidin (found in high amounts in *V. vinifera)*. These are most often found complexed to glucose or other sugar moieties. Often, the sugar moiety may be acylated or coumarylated (Haslam 1977); the most common acids involved in acylation include caffeic, *p*-coumaric, and *p*-hydroxybenzoic acids. Each grape cultivar has a unique compositional profile of anthocyanin accumulation (Mazza and Miniata 1993), which impacts color density and color stability of wine. *V. vinifera* and hybrid grapes exhibit different forms of anthocyanins, with the diglucoside form present in *V. riparia, V. rupestris*, and interspecific hybrid grapes, but not in *V. vinifera* (Webb and others 1974).

Proanthocyanidins are naturally occurring, high molecular weight polyphenolic compounds, are important to the mouthfeel and ageability of wine (Kennedy and others 2006; Noble 1990) and contribute to color stability by forming pigmented polymers with anthocyanins

(Somers 1971). Their role in the sensory properties of wine is closely related to red wine quality (Amerine and Roessler 1976).

Flavonols are found in grape skins and are highly responsive to light exposure. They are thought to function as UV protectants (Price and others 1995; Spayd and others 2002; Downey and others 2004). Glycosides and gluconurides of quercitin, kaempferol, myrcetin, and isorhamnetin (Ribereau-Gayon 1964; Makris and others 2006) are also found in grape skins. In wine, flavonols function as factors that can copigment with anthocyanins (Asen and others 1972; Sceffedldt and Hrazdina 1978) and as free radical scavengers (Markham and others 1998).

Phenolic acids are rapidly extracted into wine (Arnold and Noble 1979), and can act as copigmentation cofactors, can polymerize with other phenols, and are precursors for volatile phenols (Chatonnet and others 1993). In wines, phenolic acid concentrations decrease through oxidation reactions and adsorption to insoluble solids, soluble solids, and lees and barrels (Moreno-Arribas and others 2009).

Grape and wine phenolic management has been poorly studied to date, and existing work has focused largely on *V. vinifera* cultivars. For wine regions where Maréchal Foch, Marquette, and Corot noir are important economically, an improved understanding of the effects of viticultural and enological management of phenolic development and extraction in these cultivars is key to production of quality red hybrid wines.

3.2 Materials and Methods

3.2.1 Grape Selection and Harvest

Corot noir, Maréchal Foch, and Marquette were sourced from the Finger Lakes region of New York in 2011 from vineyards using standard cultural practices of the region. Fruit samples

were collected from all cultivars weekly following véraison. Harvest date was determined based on soluble solids, titratable acidity, and pH analyses, along with considerations of weather and wildlife pressures. Titratable acidity was analyzed with a Titrino Plus 848 doser and 869 autosampler (Metrohm USA, Riverview, FL) and pH was analyzed with an Accumet Excel XL25 pH meter (Thermo-Fisher Scientific, Waltham, MA). Brix measurements were performed in duplicate with a handheld Atago Alpha-PAL refractometer (Bellevue, WA).

Maréchal Foch was hand-harvested from the Cornell University campus orchard (Ithaca, NY) on September 4, 2011 and Marquette from Black Diamond Farm (Trumansburg, NY) on October 3, 2011. The commercial winery (Cayuga Lake, NY) donating Corot noir harvested the grapes mechanically on October 10, 2011. In each instance, grapes were stored overnight in a cooler (2°C) before processing.

3.2.2 Wine Production

Wines were made in triplicate for each treatment produced with Corot noir and with Maréchal Foch, and in duplicate for wines produced with Marquette. For each replicate, 21 kg fruit was mechanically crushed and destemmed (Rossi e Cama, Prospero, Pleasantville, NY) and treated with 50mg/L sulfur dioxide. A Chemwell 2910 multianalyzer with Software Version 6.3 (Awareness Technology, Palm City, FL) was used for yeast assimilable nitrogen (YAN) determination by enzymatic analyses (Unitech Scientific, Hawaiian Garden, CA), and FermaidK, GoFerm, and diammonium phosphate (DAP) (Lallemand) were added accordingly to attain 200mg/L YAN minimum, as recommended by the Scott Laboratories Fermentation Handbook (2011).

Maréchal Foch and Corot noir were divided equally into a control and four treatments: pectolytic enzyme addition, exogenous tannin addition, cold soak, and hot press. Due to small lot size, Marquette was only used for control and hot press treatments. For all treatments but the hot press, fermentation on the solids was performed in 13-gallon stainless steel pots to accommodate the small size of replicates. All wines followed protocols identical to the control unless otherwise noted in the treatment protocol (Table 3.1). The must was inoculated with R2 yeast (Lalvin) after warming to room temperature, and was held in a 20°C temperature controlled room where caps were punched down manually twice a day. After seven days, each wine lot was pressed by hand and transferred to a 3-gallon glass carboy to complete fermentation.

Table 3.1 Wine treatments and protocol for red wine fermentations

Treatment	**Protocol**
Enzyme Addition	70mL/ton ColorPro(Scottzyme) to crushed must
Tannin Addition	40g/hl BioTan (Laffort) to crushed must
Cold Soak	Held at 5°C 24 hours prior to inoculation
Hot Press	Crushed must heated to and held at 65°C in steam kettle, pressed immediately (Mori PZ.82, TCW Equipment, Napa Valley, CA), treated with 25ppm SO_2

Fermentation was considered complete when residual sugar dropped below 5% as measured by Clinitest tablets (Bayer, Etobicoke, ON, Canada). At dryness, wines were inoculated with malolactic bacteria (Alpha, Lalvin) according to the manufacturer's guidelines. Upon completion of malolactic fermentation (MLF), wines were racked and sulfur dioxide was added to maintain 40 mg/L free SO_2 before being cold stabilized at 2°C for eight weeks prior to bottling. The same time and temperature profile was maintained during all fermentations and storage. Free and total SO_2 were measured prior to bottling using a FIAstar 5000 system (Foss, Eden Prairie, MN). Titratable acidity was adjusted to relative equivalence over each cultivar's replicates through the addition of tartaric acid or potassium carbonate after cold stabilization.

The wines were screened for faults by an expert panel prior to bottling in 750mL olive green glass bottles with screw-cap closures and were stored at 20°C until needed for further analysis.

Ethanol analysis was performed using gas chromatography with flame ionization detection (GC-FID) (Hewlett Packard GS 5890 Series II, GMI Inc., Ramsey, MN) equipped with a FactorFour ™ VF-WAXms column, 30 m x 0.25 mm x 1.0 µm (Varian, Inc., Palo Alto, CA). The method for ethanol determination was adapted from the AOAC gas chromatographic method for ethanol in wines (AOAC Official Method 983.13). 1-Butanol was used as the internal standard (ACS grade, Sigma-Aldrich, St. Louis, MO), and ethanol was quantified using a 10% (v/v) ethanol standard (Sigma-Aldrich).

3.2.3 Sampling Protocol

Samples were taken of pre-crush grape clusters, post-crush must, post 24-hour cold soaked must, post hot pressed must, post alcoholic fermentation, post pressing solids after alcoholic fermentation, and post malo-lactic fermentation. Following crushing, must samples were collected and strained through cheesecloth to remove skins and seeds, after which soluble solids, titratable acidity, and pH were determined as described above. To inhibit enzymatic degradation in the must, ascorbic acid (Presque Isle Wine Cellars, North East, PA) was added to 1% w/v. All samples were frozen at -20°C until analysis.

3.2.4 Analysis of Phenolics

Instrumentation

All solvents, including methanol, acetonitrile, ethyl acetate, formic acid (Thermo-Fisher Scientific, Waltham, MA) were HPLC grade. An Agilent Model 1260 Infinity series HPLC (Palo

Alto, CA) consisting of an in-line vacuum degasser, autosampler, binary pump, diode array detector, and thermostatted column compartment were used. The HPLC was equipped with two separate columns: a Kinetex C18 column (100mm, 2.6 µm particle size, 4.6 mm inside diameter) and Kinetex PFP column (2.6 µm particle size, 2.1 mm inside diameter), each fitted with a KrudKatcher guard filter (Phenomenex, Torrance, CA). A computer workstation with Chemstation software (Version 3.04.02SP1 with spectral pack) was used for chromatographic analysis.

Sample Preparation

Must and wines samples were thawed and centrifuged for five minutes at 10K RCF before undergoing solid-phase extraction (SPE). A 3 cc, 60 mg Oasis HLB cartridge (Waters, Milford, MA) was conditioned with 2mL 100% HPLC-grade methanol followed by 2mL of acidified water (0.01M HCl). After conditioning, 1mL of wine sample diluted with 1mL of acidified water was loaded onto the column and rinsed with 2 mls of 0.01N HCl to remove sugars and alcohols. The cartridge was then dried while pulling under a vacuum for five minutes. The initial rinse fraction (F1) was removed and discarded. A 40mL portion of acetonitrile:0.01M HCl (95:5) was run through the cartridge to remove and collect the monomeric phenolic fraction (F2). This fraction was evaporated under high purity nitrogen gas in a 30°C water bath. After evaporation, the monomeric fraction was extracted three times using 3mL 100% ethyl acetate in order to remove the non-anthocyanin components. The ethyl acetate rinse was filtered through a Corning™ regenerated cellulose syringe filter (15mm diameter, 0.2µm, Sigma-Aldrich) and subsequently dried under high purity nitrogen gas in a 30°C water bath. The non-anthocyanin residue was resuspended in 1mL of 20% methanol and filtered

through a 0.2 μm polyethersulfone (PES) membrane (Krackeler Scientific, Inc., Albany, NY) prior to immediate HPLC analysis. The anthocyanins remaining in the tube containing the original F2 monomeric fraction was then resuspended in 1mL of 0.01M HCl prior to immediate HPLC analysis. This fraction also contained 2-S glutationyl caftaric acid, commonly known as grape reaction product (GRP).

To isolate the polymeric fraction (F3), the cartridge was removed from the vacuum chamber and put into a test tube. A 300μL aliquot of neat formic acid was quickly pushed through followed immediately with 3mL of a methanol:water (95:5) solution. The tube was homogenized and evaporated under nitrogen gas in a 30°C water bath. Proanthocyanidin isolates were characterized by acid-catalysis in the presence of excess phloroglucinol using a previously described method (Kennedy and Jones 2001). Phloroglucinolysis provides information on subunit composition, conversion yield and mean degree of polymerization.

Reversed-Phase HPLC of Phenolics

Prior to HPLC analysis, all samples were filtered using CellTreat™ syringe filters (PES, 0.22μm, 13mm diameter, Krackeler Scientific, Inc.). Total flavan-3-ol monomer content and post-phloroglucinolysis polyphenolic content in grape must and wine was measured with the Kinetex C18 column while the anthocyanin identification and quantification was performed using the PFP column described above. Eluting flavan-3-ol monomers and polymeric substituents were identified and quantified using catechin, epicatechin, gallic acid, protocatechuic acid, dihydroxybenzoic acid, caffeic acid, vanillin, coumaric acid, ferulic acid, cinnamic acid, rutin, naringenin, and quercitin standards, either directly from the known standards, or semi-quantitatively based on standards of a similar chemical nature. The

proportion of hydrolyzed condensed tannin components extracted into wine was calculated using via catechin equivalents using a previously described method (Peyrot des Gachons and Kennedy 2003). Anthocyanins were identified and quantified using malvidin-3-glucoside and malvidin-3,5-diglucoside standards as measures of equivalents for mono- and di-glucosides, respectively.

3.2.5 Statistical analyses

Statistical data analysis was performed using an analysis of variance (ANOVA) and TukeyHSD to find statistically different values between treatments at a significance level of <0.05. All statistical analyses were performed using R (Version 2.14.2).

3.3 Results and Discussion

3.3.1 Harvest and Wine Parameters

Harvest parameters are listed in Table 3.2. Differences between the control and hot press musts were observed in the grape must where heat treatments increased °Brix, pH, and yeast assimilable nitrogen (YAN) values, though there was no significant carryover in the wine (Table 3.2, 3.3).

Table 3.2 Harvest metrics of grape musts before fermentation

Grape Must	Harvest Date	Harvest Location	°Brix	pH	Titratable Acidity (g/L)	YAN[1] (mg/L)
Corot noir	10.10.2011	Winery, Romulus, NY	17.8	3.34	11.61	213
			18.4*	3.54*	9.40*	254*
Maréchal Foch	9.4.2011	Cornell University Orchards, Ithaca, NY	20.1	3.10	11.40	119
			20.5*	3.42*	10.72*	150*
Marquette	10.3.2011	Black Diamond Farm, Trumansburg, NY	21.9	3.09	9.37	329
			22.2*	3.27*	10.52*	412*

*Denotes post hot pressed must values if applicable.
[1]YAN additions were made to reach 200mg/L for sufficiency.

Table 3.3. Wine chemistry across treatments and cultivars

Corot noir	pH	Titratable Acidity (g/L)	Alcohol (%v/v)
Control	3.69	5.91	10.8
Enzyme Addition	3.78	5.73	10.9
Tannin Addition	3.82	5.55	10.8
Cold Soak	3.90	5.89	10.9
Hot Press	3.75	5.16	11.1

Maréchal Foch	pH	Titratable Acidity (g/L)	Alcohol (%v/v)
Control	3.63	8.10	10.8
Enzyme Addition	3.62	8.20	10.7
Tannin Addition	3.66	8.05	10.7
Cold Soak	3.64	7.86	10.6
Hot Press	3.55	8.23	10.9

Marquette	pH	Titratable Acidity (g/L)	Alcohol (%v/v)
Control	3.37	6.25	11.6
Hot Press	3.53	6.90	11.9

3.3.2 Observed Phenolic Compounds

Phenolic compounds were identified by HPLC and quantified individually. Table 3.4 lists identified phenolic compounds of interest observed at their respective optimal wavelengths. Due to the unavailability of commercial standards, some compounds were semi-quantitatively measured using standards that were structurally and chemically similar. Caftaric acid and grape reaction product (GRP) were quantified in caffeic acid equivalents, coutaric acid as coumaric acid equivalents, and fertaric acid as ferulic acid equivalents. Anthocyanins were quantified using malvidin-3-glucoside and malvidin-3,5-diglucoside standards as measures of equivalents for mono- and di-glucosides, respectively.

Table 3.4 Observed phenolic compounds in red hybrid winegrapes, viewed at optimal HPLC wavelengths

280nm (C18)	320nm (C18)	360nm (C18)	520nm (PFP)
Gallic acid	Dihydroxybenzoic acid	Rutin	Cyanidin-3-glucoside
Protocatechuic acid	Caffeic acid	Quercitin	Cyanidin-3,5-diglucoside
Catechin	Caftaric acid		Delphilnidin-3-glucoside
Vanillin	Coumaric acid		Delphinidin-3,5-diglucoside
Epicatechin	Coutaric acid		Malvidin-3-glucoside
Cinnamic acid	Ferulic acid		Malvidin-3,5-diglucoside
Naringen	Fertaric acid		Peonidin-3-glucoside
	Caffeic acid ethyl ester		Peonidin-3,5-diglucoside
	Coumaric acid ethyl ester		Petunidin-3-glucoside
	Grape Reaction Product		Petunidin-3,5-diglucoside

Non-vinifera hybrids have long been distinguished by the existence of diglucoside anthocyanins, which are rare in *V. vinifera* (Webb and others 1974). All diglucoside anthocyanin forms were observed in these cultivars studied, along with other phenolic compounds common to *V. vinifera* grapes.

A minute number of HPLC chromatogram peaks were unidentifiable and non-quantifiable. These may be indicative of phenolics unique to hybrid grapes, like the delphinidin modified by a hexose sugar and *p*-coumaric acid identified via HPLC-MS at the University of Minnesota by Meyer and others (2011).

3.3.3. Monomeric Phenolics

Maréchal Foch

Phenolic compounds in Maréchal Foch must and wine appear to be selectively extracted, likely based on solubility due to temperature variations and the presence of ethanol (Auw and others 1996; Netzel and others 2003), as their presence differs between musts and wines. Both the cold soak and hot press treatments demonstrate greater concentrations than the control, with the hot press treatment showing the most variation in extraction profile (Table 3.5, 3.6).

Flavonols (quercitin and rutin) were non-detectable in the control must. It appears from the data that contact on the skins in the presence of ethanol is required for quercitin. Flavonols are highly insoluble in water alone, and require organic solvents such as alcohols for solubilization (Jeffery and others 2008). Treatments that increased skin contact through time or through cellular breakdown showed the highest amounts of quercitin, with the hot press treatment showing the highest overall level of flavonols. Naringenin, commonly found in citrus fruits and grapes, and ferulic acid were not detected in the musts or wines.

Caftaric and coutaric acids were detected with a concentration ranging from 3-47mg/L and 0.1-7mg/L, respectively (Table 3.5); these concentrations are lower than the 62mg/L t-caftaric acid and 4mg/L t-coutaric acid reported previously in Maréchal Foch. Singleton and others reported these analytes ranging from 47-130mg/L for t-caftaric acid and 2-44mg/L for t-coutaric acid in various samples (1986). Differences may be attributed to growing conditions, winemaking protocols, or advances in HPLC technology and sample preparation.

In musts and wines, respectively, 2-60mg/L and 10-65mg/L of total hydroxycinnamic acids were measured (Table 3.5, 3.6). According to Moreno-Arribas (2009), the concentration of hydroxycinnamic acids (HCAs) in wine is 10-25% of that found in the juice, with total concentrations of less than 50mg/L, due to a loss from oxidation reactions, interactions with grape insoluble solids, and adsorption to lees or barrels. The levels of non-esterified HCAs are usually ten percent of the HCA tartrate esters, which also does not appear to hold true in Maréchal Foch, as about 95% of the HCAs are in tartrate ester form.

Table 3.5 Monomeric phenolics in Maréchal Foch grape must as measured by HPLC

Compound, mg/L	Control Must	Cold Soak Must	Hot Press Must
Gallic acid	0.04±0.0	0.24±0.0	4.57±0.0
Protocatechuic acid	1.02±0.0	1.28±0.0	1.88±0.0
Catechin	1.92±0.0	2.44±0.0	420.78±0.0
Vanillin	ND	ND	0.46±0.0
Epicatechin	ND	1.59±0.0	89.31±0.0
Cinnamic acid	0.13±0.0	0.13±0.0	ND
GRP	8.35±0.0	5.57±0.0	3.45±0.0
Caftaric acid	3.49±0.0	14.36±0.0	47.54±0.0
Caffeic acid	ND	ND	3.56±0.0
Coutaric acid	ND	0.14±0.0	7.26±0.0
Fertaric acid	1.67±0.0	1.28±0.0	2.63±0.0
Rutin	ND	0.32±0.0	13.14±0.0
Total Monomeric	**16.62±0.0[a]**	**27.36±0.0[b]**	**594.59±0.0[ab]**

Values followed by a letter indicate significant difference between treatments
Mean ± standard deviation of replicates
ND=not detected.

Table 3.6 Monomeric phenolics in Maréchal Foch wine as measured by HPLC

Compound, mg/L	Control	Enzyme Addition	Tannin Addition	Cold Soak	Hot Press
Caffeic acid	3.05±0.8	3.73±0.5	2.79±0.5	4.02±0.8	2.92±1.6
Caffeic acid ethyl ester	0.35±0.1	0.54±0.0	0.36±0.1	0.61±0.2	ND
Caftaric acid	8.52±1.3	12.51±0.8	12.00±0.9	14.97±2.9	44.12±0.4
Catechin	35.96±10.6	61.08±6.6	52.37±14.3	68.28±0.6	337.24±24.0
Cinnamic acid	0.24±0.0	0.37±0.1	0.25±0.0	0.27±0.0	ND
Coumaric acid	3.54±0.9	4.82±0.2	3.68±0.6	4.38±0.4	0.40±0.1
Coumaric acid ethyl ester	0.52±0.1	0.70±0.0	0.49±0.0	0.71±0.1	ND
Coutaric acid	1.18±0.5	2.43±0.5	1.67±0.5	3.07±0.9	10.57±0.3
Dihydroxybenzoic acid	1.14±0.4	2.07±0.1	1.36±0.8	2.09±0.2	ND
Epicatechin	11.53±4.2	19.90±2.5	18.73±6.8	23.13±2.8	62.07±1.0
Fertaric acid	1.48±0.2	2.12±0.1	1.68±0.5	2.15±0.2	2.65±0.2
Gallic acid	9.04±2.0	12.58±1.3	11.91±2.3	12.61±1.0	6.19±0.4
GRP	2.71±0.3	2.57±0.0	2.37±0.1	2.43±0.2	3.84±0.2
Protocatechuic acid	1.68±1.0	1.54±0.3	1.41±0.4	1.64±1.0	1.61±0.4
Quercitin	0.54±0.3	1.03±0.3	0.76±0.4	1.29±0.3	0.99±0.0
Rutin	0.82±0.5	2.81±0.2	1.85±1.4	2.97±0.5	11.34±0.5
Vanillin	0.39±0.1	0.55±0.2	0.55±0.2	0.98±0.2	0.79±0.1
Total Monomeric	**81.87±20.5^{aef}**	**130.02±12.6^{be}**	**106.92±17.9^{c}**	**141.77±14.89^{df}**	**480.31±30.9^{abcd}**

Values followed by a letter indicate significant difference between treatments
Mean ± standard deviation of replicates
ND=not detected

Corot noir

Like Maréchal Foch, Corot noir shows low levels of monomeric, non-anthocyanin compounds (~80-140mg/L). No significant differences were found among treatments in the wines for non-anthocyanin monomeric phenolics, but a higher concentration was found in the hot pressed musts (Table 3.7, 3.8).

Both the cold soak and hot press treatments show greater overall concentrations than the control, though the hot press treatment shows the most variation in phenolic profile. Caftaric acid was found with high concentrations in the must, and decreased after fermentation. Quercitin was non-detectable in the control must.

Table 3.7 Monomeric phenolics in Corot noir grape must as measured by HPLC

Compound, mg/L	Control Must	Cold Soak Must	Hot Pressed Must
Caffeic acid	ND	0.91±0.0	4.57±0.0
Caftaric acid	8.65±0.0	23.92±0.0	41.85±0.0
Catechin	2.87±0.0	4.54±0.0	28.31±0.0
Cinnamic acid	0.14±0.0	0.18±0.0	ND
Coumaric acid	ND	0.30±0.0	0.49±0.0
Coutaric acid	0.25±0.0	1.33±0.0	11.55±0.0
Dihydroxybenzoic acid	1.63±0.0	0.94±0.0	2.09±0.0
Epicatechin	ND	4.24±0.0	33.26±0.0
Fertaric acid	1.23±0.0	0.83±0.0	2.07±0.0
Gallic acid	0.23±0.0	0.54±0.0	2.98±0.0
GRP	8.61±0.0	7.47±0.0	9.21±0.0
Naringenin	0.42±0.0	0.54±0.0	2.72±0.0
Protocatechuic acid	0.63±0.0	ND	1.54±0.0
Rutin	1.61±0.0	1.83±0.0	13.01±0.0
Total Monomeric	**26.28±0.0[a]**	**47.58±0.0[b]**	**153.66±0.0[ab]**

Values followed by a letter indicate significant difference between treatments
Mean ± standard deviation of replicates
ND=not detected

Table 3.8 Monomeric phenolics in Corot noir wine as measured by HPLC

Compound, mg/L	Control	Enzyme Addition	Tannin Addition	Cold Soak	Hot Press
Caffeic acid	7.76±2.6	11.50±0.3	8.67±3.2	8.82±1.5	4.40±1.2
Caffeic acid ethyl ester	0.77±0.3	1.20±0.0	0.79±0.3	1.17±0.1	0.05±0.0
Caftaric acid	10.14±1.2	8.65±1.0	7.67±1.0	16.34±1.0	30.40±1.6
Catechin	19.26±6.4	36.83±3.0	32.75±14.7	34.46±0.7	23.33±4.4
Cinnamic acid	0.17±0.0	0.25±0.0	ND	ND	ND
Coumaric acid	10.01±3.0	14.23±0.4	10.43±3.5	13.40±0.7	1.36±0.2
Coumaric acid ethyl ester	1.83±0.5	2.02±0.9	1.68±0.5	2.32±0.0	0.07±0.0
Coutaric acid	2.09±0.7	3.14±0.2	2.328±1.3	6.20±0.7	13.50±4.1
Dihydroxybenzoic acid	0.60±0.0	1.27±0.2	1.41±0.1	1.32±0.2	1.61±0.8
Epicatechin	16.42±6.0	32.84±2.1	29.60±15.4	32.40±3.0	21.62±6.5
Fertaric acid	0.54±0.2	1.09±0.2	0.79±0.4	1.45±0.2	1.76±0.2
Ferulic Acid	ND	ND	1.38±0.0	1.15±0.0	1.28±0.0
Gallic acid	6.48±2.3	10.68±0.7	10.29±4.5	10.38±0.4	4.64±0.4
GRP	0.82±0.2	0.61±0.1	0.69±0.1	1.14±0.1	3.68±1.1
Naringenin	0.42±0.2	1.07±0.2	0.75±0.4	0.86±0.3	1.37±0.4
Protocatechuic acid	0.80±0.3	1.30±0.2	1.12±0.5	1.29±0.0	0.93±0.1
Quercitin	0.30±0.0	0.50±0.1	0.46±0.1	0.70±0.2	ND
Rutin	0.65±0.3	2.04±0.3	1.54±1.0	2.34±0.1	7.72±2.2
Vanillin	0.24±0.0	0.28±0.3	0.24±0.1	0.23±0.0	0.33±0.0
Total Monomeric	**78.48±21.6**	**116.51±27.9**	**111.06±47.2**	**135.14±3.9**	**117.23±14.8**

Mean ± standard deviation of replicates
ND=not detected

Marquette

No statistically significant differences were found between the control and hot press treatments in the wines for the concentration of non-anthocyanin monomeric phenolics, though the hot press treatment did affect the concentration found in the musts.

The hot press treatment shows that about 12 times greater concentration of catechin was extracted and about 20 times greater concentration of epicatechin was extracted into the hot pressed must, when compared to the control must. Low levels of phenolic acids and flavonols were present in the must and wine. Concentrations of all other phenolic acids and flavonols were higher post-fermentation, except for the hot press treatment, which had a lower concentration. Hot press treatments showed a greater concentration in overall phenolic compounds than the control, though there was less variety in compounds when compared to treatments that fermented on the grape solids Table 3.9, 3.10). This loss may be due to binding with anthocyanins or other phenolics present in the wine (Scheffeldt and Hrazdina 1978).

Table 3.9 Monomeric phenolics in Marquette grape must as measured by HPLC

Compound, mg/L	Control Must	Hot Pressed Must
Caffeic acid	ND	3.93±0.0
Caftaric acid	34.23±0.0	53.19±0.0
Catechin	2.02±0.0	36.62±0.0
Cinnamic acid	ND	0.19±0.0
Coumaric acid	ND	0.27±0.0
Coutaric acid	1.02±0.0	8.88±0.0
Epicatechin	ND	21.47±0.0
Fertaric acid	0.87±0.0	1.73±0.0
Ferulic Acid	ND	0.45±0.0
Gallic acid	ND	3.73±0.0
GRP	8.25±0.0	8.07±0.0
Protocatechuic acid	ND	0.79±0.0
Rutin	7.97±0.0	2.09±0.0
Total Monomeric	**54.15±0.0[a]**	**141.41±0.0[a]**

Values followed by a letter indicate significant difference between treatments
Mean ± standard deviation of replicates, ND=not detected

Table 3.10 Monomeric phenolics in Marquette wine as measured by HPLC

Compound, mg/L	Control	Hot Press
Caffeic acid	6.64±0.4	2.98±1.7
Caffeic acid ethyl ester	1.17±0.1	0.01±0.0
Caftaric acid	26.71±0.0	58.80±2.1
Catechin	13.93±0.9	29.96±0.5
Coumaric acid	2.25±0.2	0.43±0.2
Coumaric acid ethyl ester	0.37±0.0	ND
Coutaric acid	4.79±0.2	7.24±3.8
Dihydroxybenzoic acid	1.47±0.1	ND
Epicatechin	5.55±0.6	14.42±8.1
Fertaric acid	1.23±0.0	1.33±0.7
Ferulic Acid	0.70±0.1	0.92±0.9
Gallic acid	8.00±0.5	4.45±2.5
GRP	3.09±0.2	6.64±0.2
Naringenin	0.81±0.0	1.23±0.8
Protocatechuic acid	0.82±0.0	0.57±0.4
Quercitin	ND	0.16±0.0
Rutin	4.68±6.1	2.07±0.3
Vanillin	0.29±0.1	0.04±0.0
Total Monomeric	**82.50±3.2**	**101.17±17.3**

Mean ± standard deviation of replicates
ND=not detected

Cultivar Comparison

Overall, Maréchal Foch, Corot noir, and Marquette show lower levels of phenolic acids and flavonols in musts when compared to *V. vinifera* and hybrids studied by Singleton and others (1986).

Though found in the wine, vanillin, quercitin, coumaric acid ethyl esters, and caffeic acid ethyl esters were not detected in any of the musts. This suggests that contact time with the grape solids is necessary for extraction (Sacchi and others2005). Ferulic acid was not detected in Maréchal Foch must or wine or Corot noir must, though small amounts of fertaric acid are observed. Naringenin was not observed in either the musts or wines of Maréchal Foch or Marquette.

The higher levels of monomeric phenolics in the hot pressed treatments were likely due to increased solubility from heat exposure, cellular breakdown, and to the blocking of oxidative reactions between phenolic acids and browning agents, as browning agents in must and wine are deactivated by heat treatment (Lee and others 1983). Treatments that increased skin contact time or increased cellular breakdown showed the highest amounts of quercitin, with the hot press treatment showing the highest level of flavonols overall. Catechin and epicatechin were found to be present in the greatest amounts in comparison to all other monomeric phenolic compounds observed. The hot press treatment led to the greatest concentration of overall monomeric phenolics into the must, though it was not a lasting effect through fermentation.

While sensory evaluation will be necessary to determine what effect the varying levels of monomeric phenolics will have on aroma, mouthfeel, and color perception in finished wines, the likely impact of these low weight monomeric phenolic compounds is perceived bitterness (Harbertson and others 2012).

3.3.4 Anthocyanins

Maréchal Foch

Though hot press juices showed statistically significant higher total anthocyanin concentrations pre-fermentation, no significant differences in total wine anthocyanins were found across treatments (Table 3.11, 3.12). Overall, a total of 150-280 mg/L anthocyanin material was quantified in these wines, similar to that reported by Sun (2011).

Table 3.11 Anthocyanins in Maréchal Foch grape must as measured by HPLC

Anthocyanin, mg/L	Control Must	Cold Soak Must	Hot Pressed Must
Delphinidin-3-glucoside	4.55±0.0	20.24±0.0	187.18±0.0
Cyanidin-3-glucoside	4.68±0.0	7.59±0.0	30.44±0.
Petunidin-3-glucoside	6.78±0.0	15.92±0.0	119.76±0.0
Peonidin-3-glucoside	2.97±0.0	4.61±0.0	13.50±0.0
Malvidin-3-glucoside	25.38±0.0	45.04±0.0	186.83±0.0
Delphinidin-3,5-diglucoside	1.04±0.0	2.43±0.0	5.26±0.0
Cyanidin-3,5-diglucoside	0.94±0.0	1.66±0.0	2.41±0.0
Petunidin-3,5-diglucoside	1.12±0.0	2.77±0.0	8.67±0.0
Peonidin-3,5-diglucoside	1.98±0.0	6.61±0.0	9.47±0.0
Malvidin-3,5-diglucoside	10.66±0.0	38.84±0.0	73.08±0.0
Total Anthocyanins	**60.13±0.0[a]**	**145.71±0.0[b]**	**636.60±0.0[ab]**

Values followed by a letter indicate significant difference between treatments
Mean ± standard deviation of replicates

Table 3.12 Anthocyanins in Maréchal Foch wine as measured by HPLC

Anthocyanin, mg/L	Control	Enzyme Addition	Tannin Addition	Cold Soak	Hot Press
Delphinidin-3-glucoside	12.58±0.9	16.18±1.0	15.74±0.6	17.04±1.3	56.27±34.1
Cyanidin-3-glucoside	1.14±0.0	1.26±0.1	1.34±0.1	1.22±0.1	2.21±0.8
Petunidin-3-glucoside	16.11±0.9	19.70±0.6	19.34±1.0	19.95±0.8	48.96±25.2
Peonidin-3-glucoside	1.62±0.0	1.77±0.0	1.79±0.1	1.75±0.1	3.39±1.4
Malvidin-3-glucoside	53.42±2.1	45.13±25.1	58.54±1.9	60.48±1.0	89.64±25.4
Delphinidin-3,5-diglucoside	2.88±0.3	3.58±0.2	3.26±0.2	3.65±0.3	7.39±0.7
Cyanidin-3,5-diglucoside	1.98±02	2.13±0.1	2.08±0.1	2.13±0.0	2.39±0.7
Petunidin-3,5-diglucoside	4.71±0.5	5.67±0.1	5.21±0.3	5.67±0.7	6.66±1.5
Peonidin-3,5-diglucoside	6.12±0.3	6.44±0.4	6.52±0.6	6.49±0.1	7.01±0.5
Malvidin-3,5-diglucoside	50.46±2.4	56.10±3.0	54.41±5.2	54.92±1.2	56.93±2.7
Total Anthocyanins	**151.03±7.6[a]**	**157.97±28.3[b]**	**168.23±9.8[c]**	**173.30±3.4[d]**	**280.86±92.3[abcd]**

Values followed by a letter indicate significant difference between treatments
Mean ± standard deviation of replicates

Corot noir

The hot press treatment shows the greatest concentration of anthocyanins in must, with levels about 4 times higher than the control, probably due to heat-mediated breakdown of cells in the grape berry (Sacchi and others 2005). The cold soak treatment shows a 2.5 times greater concentration, likely due to extra time in contact with skins (Sacchi and others 2005). Overall differences between anthocyanin levels in wine are very slight, though significant differences were found between the tannin addition and cold soak, between the hot press and the control, between and hot press and enzyme addition, and between the hot press and tannin addition (Table 3.13, 3.14).

Table 3.13 Anthocyanins in Corot noir grape must as measured by HPLC

Anthocyanin, mg/L	Control Must	Cold Soak Must	Hot Pressed Must
Delphinidin-3-glucoside	6.84±0.0	23.20±0.0	90.73±0.0
Cyanidin-3-glucoside	4.16±0.0	6.74±0.0	15.63±0.0
Petunidin-3-glucoside	5.83±0.0	13.15±0.0	43.07±0.0
Peonidin-3-glucoside	1.50±0.0	2.20±0.0	4.09±0.0
Malvidin-3-glucoside	6.25±0.0	11.52±0.0	25.42±0.0
Delphinidin-3,5-diglucoside	10.32±0.0	32.76±0.0	62.46±0.0
Cyanidin-3,5-diglucoside	7.36±0.0	15.24±0.0	22.68±0.0
Petunidin-3,5-diglucoside	13.80±0.0	38.47±0.0	63.24±0.0
Peonidin-3,5-diglucoside	15.82±0.0	27.11±0.0	35.41±0.0
Malvidin-3,5-diglucoside	61.01±0.0	125.46±0.0	189.12±0.0
Total Anthocyanins	**132.89±0.0^{ac}**	**295.86±0.0^{ab}**	**551.88±0.0^{bc}**

Values followed by a letter indicate significant difference between treatments
Mean ± standard deviation of replicates

Table 3.14 Anthocyanins in Corot noir wine as measured by HPLC

Anthocyanin, mg/L	Control	Enzyme Addition	Tannin Addition	Cold Soak	Hot Press
Delphinidin-3-glucoside	16.07±0.5	17.48±0.5	16.44±1.0	17.95±1.2	14.48±0.4
Cyanidin-3-glucoside	1.40±0.1	1.47±0.1	1.54±0.2	1.38±0.3	1.00±0.0
Petunidin-3-glucoside	12.05±0.3	12.47±0.3	11.99±0.8	13.60±0.9	16.69±0.4
Peonidin-3-glucoside	0.99±0.0	1.031±0.0	1.05±0.1	0.99±0.1	0.77±0.0
Malvidin-3-glucoside	12.76±0.2	12.90±0.2	12.16±0.4	13.81±0.5	17.01±0.2
Delphinidin-3,5-diglucoside	37.34±0.8	37.78±0.2	34.04±1.7	42.66±1.8	48.99±1.4
Cyanidin-3,5-diglucoside	16.49±0.3	16.88±0.2	15.74±0.2	17.51±1.9	19.83±0.7
Petunidin-3,5-diglucoside	48.18±0.9	48.26±0.2	44.93±1.3	52.98±1.9	62.29±0.7
Peonidin-3,5-diglucoside	25.65±0.3	26.07±0.7	25.63±0.3	27.04±2.2	29.49±0.0
Malvidin-3,5-diglucoside	150.36±1.7	151.31±2.4	148.14±3.1	157.53±8.9	163.14±0.0
Total Anthocyanins	**321.29±0.7^{ae}**	**325.65±4.3^{b}**	**311.69±8.9^{cf}**	**345.45±17.4^{deg}**	**373.68±3.0^{abcd}**

Values followed by a letter indicate significant difference between treatments
Mean ± standard deviation of replicates

Marquette

The hot press must showed a seven-time greater concentration of anthocyanins than the control, likely due to increased solubility and cell breakdown under heat treatment. However, this effect was not lasting, as anthocyanin concentration was lower after fermentation in the hot press treatments. There appears to be no difference in overall concentration between the control and hot press treatments in wine, as they both have about 250mg/L anthocyanins (Table 3.15, 3.16)

Table 3.15 Anthocyanins in Marquette grape must as measured by HPLC

Anthocyanin, mg/L	Control Must	Hot Pressed Must
Delphinidin-3-glucoside	4.43±0.0	51.27±0.0
Cyanidin-3-glucoside	1.88±0.0	7.61±0.0
Petunidin-3-glucoside	4.18±0.0	37.90±0.0
Peonidin-3-glucoside	1.50±0.0	5.07±0.0
Malvidin-3-glucoside	9.51±0.0	57.04±0.0
Delphinidin-3,5-diglucoside	2.37±0.0	20.17±0.0
Cyanidin-3,5-diglucoside	1.35±0.0	4.39±0.0
Petunidin-3,5-diglucoside	2.73±0.0	28.02±0.0
Peonidin-3,5-diglucoside	7.09±0.0	20.81±0.0
Malvidin-3,5-diglucoside	30.92±0.0	164.99±0.0
Total Anthocyanins	**65.95±0.0[a]**	**397.26±0.0[a]**

Values followed by a letter indicate significant difference between treatments
Mean ± standard deviation of replicates

Table 3.16 Anthocyanins in Marquette wine as measured by HPLC

Anthocyanin, mg/L	Control	Hot Press
Delphinidin-3-glucoside	6.72±0.4	14.92±0.5
Cyanidin-3-glucoside	0.78±0.0	0.84±0.0
Petunidin-3-glucoside	9.38±0.6	16.56±0.5
Peonidin-3-glucoside	0.87±0.6	1.39±0.2
Malvidin-3-glucoside	28.92±2.3	31.13±0.6
Delphinidin-3,5-diglucoside	12.18±0.6	14.97±0.4
Cyanidin-3,5-diglucoside	3.60±0.3	3.47±0.1
Petunidin-3,5-diglucoside	22.38±1.7	23.82±0.8
Peonidin-3,5-diglucoside	17.79±1.1	15.26±0.5
Malvidin-3,5-diglucoside	152.79±10.2	127.97±4.2
Total Anthocyanins	**255.41±16.9**	**250.34±7.5**

Mean ± standard deviation of replicates

Cultivar Comparison

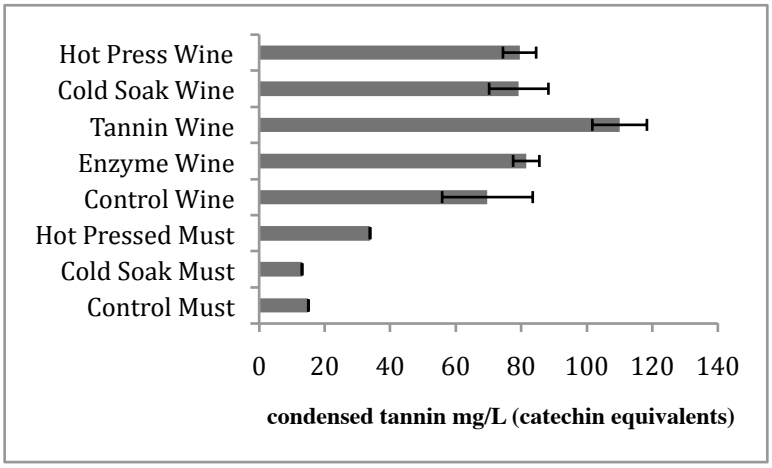
Young wines produced from these cultivars are empirically reported to be a blue-purple color, likely due to the high ratio of delphinidin, petunidin and malvidin to cyanidin and peonidin present in these wines, as they contribute purple, mauve, and blue hues (Romero and others 2008). The diglucoside form of anthocyanins dominated, though monoglucoside forms were present. In Marquette and Corot noir, malvidin-3,5-diglucoside was the primary anthocyanin, while in Maréchal Foch malvidin-3-glucoside dominated in the must and was of near equal proportions to its diglucoside form in the wines. The surprisingly high concentration of malvidin-3-glucoside, known for being the primary anthocyanin in wines of *vinifera* origin (Webb and others 1974), may be due to Maréchal Foch's complex genetic heritage with a strong *vinifera* background (Lehman and Gerrath 2004).

3.3.5 Condensed Tannins

Maréchal Foch

The hot pressed must shows the highest concentration of condensed tannins (Figure 3.1), though this does not last through fermentation. Among wines, the tannin addition treatment showed the highest concentration of condensed tannins, reaching total levels of about 110mg/L catechin equivalents compared to other treatments that ranged from 70-85mg/L catechin equivalents. Instances of wide variation between all wine replicates were observed, though the cause is unknown.

Figure 3.1 Total tannin concentration in Maréchal Foch



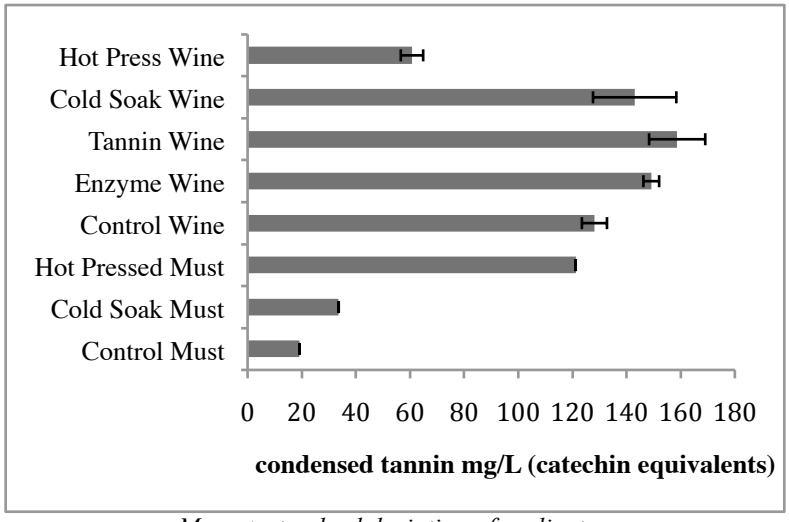
Mean ± standard deviation of replicates

Corot noir

In Corot noir, the hot pressed must shows the highest concentration of condensed tannins, with about six times greater concentration than the control, though it has the lowest concentration after fermentation (Figure 3.2) In the wines, the tannin treatment shows the highest concentration of condensed tannins, reaching total levels of 160mg/L catechin equivalents, in comparison to the other treatments, which ranged from 60-150mg/L catechin equivalents. The cause of wide variation between all wine replicates is unknown, though the control, enzyme addition, tannin addition and cold soak treatments all had a significantly greater concentration of

condensed tannins than the hot press treatment. A statistically significant difference was found between the control and tannin addition treatments, where the tannin addition had a greater concentration of condensed tannins than the control wine.

Figure 3.2 Total tannin concentration in Corot noir

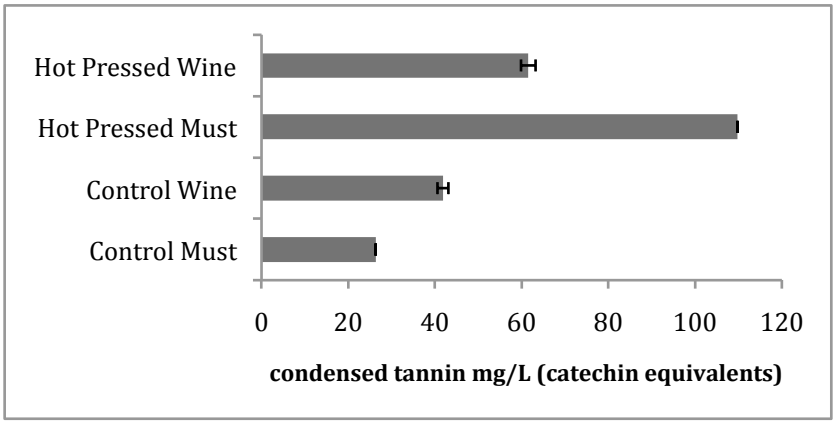


Mean ± standard deviation of replicates

Marquette

Significant differences in condensed tannin concentration are observed between treatments (Figure 3.3). Hot press treatment shows greater concentrations of condensed tannins in both the must and wine.

Figure 3.3 Total tannin concentration in Marquette



Mean ± standard deviation of replicates

Cultivar Comparison

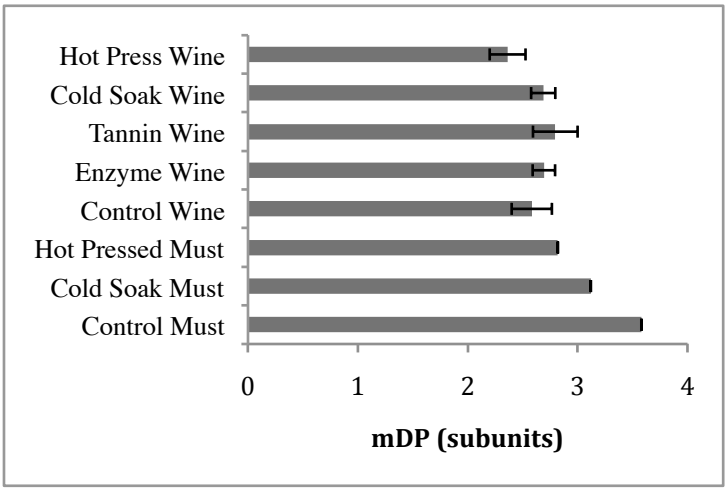
It is unknown whether the differences across treatments in polymeric phenolic content will have a lasting, stable effect, as polymeric compounds often depolymerize under acidic conditions (Kennedy and Jones 2001). The tannin treatment evinced the highest concentration of polymeric phenolic material across all cultivars, and as the addition was made with grape-based condensed tannins and not-hydrolysable tannins, generalizations cannot be applied to the use of wood-based hydrolysable tannins.

3.3.6 Mean Degree of Polymerization (mDP)

Maréchal Foch

A low mDP was observed across all must treatments (mDP 2-4 units), with the lowest in the hot pressed treatment, though it was not significant (Figure 3.4). Wines also evinced a low mDP in all treatments, with no significance difference among treatments, suggesting that no treatment utilized in this study favors the extraction of longer or shorter tannins.

Figure 3.4 Mean degree of polymerization in Maréchal Foch musts and wines

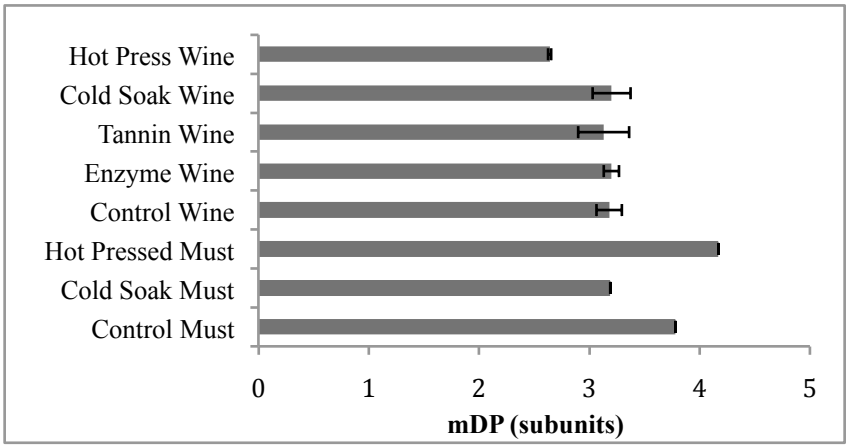


Mean ± standard deviation of replicates

Corot noir

A low mDP was observed across all must treatments, with the highest in the hot pressed must treatment, which was statistically significantly different from the other musts. Corot noir wine has a low mDP in all treatments, with the hot press wine significantly lower than the control, enzyme, and cold soak wines (Figure 3.5).

Figure 3.5 Mean degree of polymerization in Corot noir musts and wines

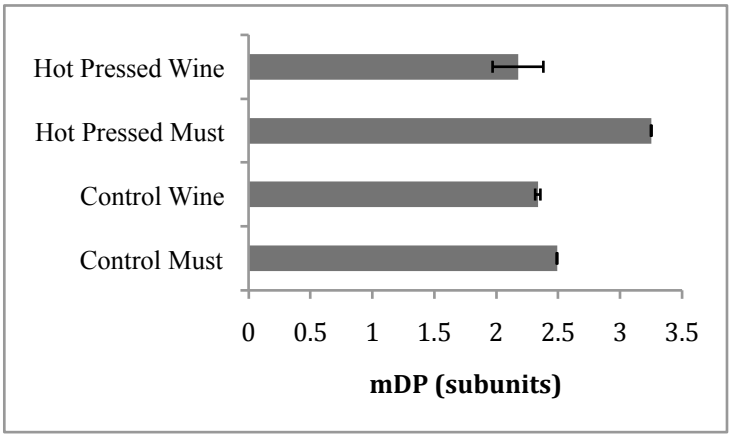


Mean ± standard deviation of replicates

Marquette

A low mDP is observed across both must treatments, with the highest in the hot pressed treatment. Marquette has a low mDP in both wine treatments with no one treatment leading to a significantly higher or lower mDP (Figure 3.6).

Figure 3.6. Mean degree of polymerization in Marquette musts and wines



Mean ± standard deviation of replicates

Cultivar Comparison

A low mDP was observed across all cultivars, with an average of 2-4 units. In comparing our hybrid musts to *V. vinifera* musts observed by Kennedy and others (2001), 2-4 units is much lower than the mDP of 11-27 that they found. It is possible that these low weight polymeric phenolic compounds will contribute more bitterness than astringency (Peleg and others 1999).

3.4 Conclusion

While statistically significant differences related to phenolic extraction were identified among treatments, the practical significance of this over time is yet unknown. Phenolic composition analysis by HPLC showed relatively low overall levels of phenolic compounds in all three cultivars in comparison to *V vinifera* (Singleton and others 1986). Hybrid grapes showed a higher concentration of anthocyanin in diglucoside, rather than monoglucoside, form. Malvidin, delphinidin, and petunidin forms dominated, resulting in deep blue or purple-colored wines. Tannin concentration was low, and the mean degree of polymerization indicated the presence of very short tannins (<3 subunits) that could lead to increased bitterness, rather than astringency.

Many winemakers are currently using these winemaking techniques with Corot noir, Maréchal Foch, and Marquette, but not all are practical depending on the vintage and condition of the grapes, so investigating the effects viticultural practices and other winemaking techniques could be beneficial. A consumer and professional sensory analysis of these wines that would tie back to the treatments could help winemakers choose which styles of wines could be most suited to their customers.

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CHAPTER 4

FUTURE WORK

Most winemakers go through years of trial and error to decide what works best for each cultivar in their microclimate using the tools they have available. In a growing industry where new or little-known hybrid grape species are used for wine production, there is limited research of practical use accessible to winemakers. Replication of this study over several years, along with companion studies of applicable significance, could shed further light on the complexities of phenolic extraction in red hybrid winegrapes. A greater understanding of phenolic management by winemakers is of immediate importance, as consumers are looking for local wine in emerging markets that rely on cold hardy red hybrid winegrapes. As weather varies from vintage to vintage, the accumulation of phenolics can be greatly affected, especially in cold-climate growing regions where weather extremes and disease pressures may be unpredictable. A relevant example came directly from our 2011 harvest where, due to frequent and heavy rains, both Marquette and Corot noir were damaged through berry splitting and cracking which allowed for *Botrytis* and other rot-inducing fungi to create additional damage, and caused a dilution in grape berry components.

Winemakers have many techniques to choose from, and only a small portion have been investigated here. Additional research on the influence of winemaking techniques like the use of sulfur dioxide, temperature, and time on phenolic extraction from red hybrid winegrapes could provide additional insight. While some work has been done by Harbertson and others (2012) on exogenous tannin additions and enzyme additions, their use in red hybrid winemaking has not been investigated fully. Due to differing chemical and insoluble solid content, the effect

contributed to wines by exogenous tannins or enzymes may vary greatly. Including a range of commercial tannins and enzymes would enhance our understanding of the chemical interaction these may have with hybrid grapes along with their sensory effects.

Chemically there are still areas in wine that we seek to understand. With the extraordinary variety of phenolics, many have yet to be identified, including many structures of phenolic acids and polymeric phenols. Phenolics also take part in complicated interactions in the wine matrix, and as hybrid wines present particular challenges, the stability of hybrid wine phenolics over time has not been thoroughly researched. The wide variety of anthocyanins is also somewhat of a mystery, as different ratios and forms may affect the color of the wine.

One of the most significant issues in academia is the practical application of research treatments to "real life" winemaking. While in research it is easiest to work with small lots of wine, treatments and testing may not be financially viable on a larger scale. It is also unknown how the complex interactions of chemical wine components and microbes will play out when scaled up for production.

Many winemaking techniques influence the amount of phenolics found in a wine, but their concentration can also be manipulated in the vineyard. Investigating the effects that viticultural practices and growing conditions have on the chemical and sensory characteristics of a range of red hybrid winegrape cultivars may shed light on our understanding of phenolic accumulation in the vineyard. Due to the complexities of enology and viticulture, multivariate analysis and careful experimental design are critical for the practical application of treatments and techniques for red hybrid winegrapes in the production of quality wines.

As winemakers produce wines with the ultimate goal of selling it to consumers who will enjoy it, consumer preferences are important to consider. A consumer sensory analysis of these

wines that would tie back to winemaking treatments could help winemakers choose which styles of wines would be most suited to their customers. Due to the relatively small regional distribution of red hybrid wines, many consumers are not familiar with their characteristics, so the level of liking might be regionally defined. A formal assessment with industry professionals and trained panelists may provide useful data for the descriptive attributes associated with particular treatments such as exogenous tannins.

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APPENDIX

Survey of Winemakers in MI, MN, NY, PA, WI: Questions

In which state do you produce wine?
- Michigan
- Minnesota
- New York
- Pennsylvania
- Wisconsin

How many cases did you produce in the 2010 harvest?
- 0-2500
- 2501-5000
- 5001-10000
- 10001-30000
- 30001-50000
- 50001-70000
- 70001-90000
- 90001-110000
- >110001

Do you produce any wines from red hybrid grapes?
- Yes
- No

If you are not currently making win with hybrid grapes, are you planning to do so in the future?
- Yes
- No
- Maybe

What hybrid varieties are most popular in your area of the state?

Do you feel that your core consumers are familiar with red hybrid grapes and wines?
- Yes
- No
- Sometimes

Please list the red hybrid grapes you use for winemaking

Of the red hybrids used in your wines, which do you grow?
- None
- We grow:
- Prefer not to answer

Of the red hybrids used in your wines, which do you buy?
- None
- We grow:
- Prefer not to answer

If you buy red hybrid winegrapes, how much do you pay, on average, per ton?
- <200
- 201-400
- 401-600
- 601-800

- 801-1000
- 1001-1200
- 1201-1400
- 1401-1600
- 1601-1800
- 1801-2000
- >2000
- Prefer not to answer

Please list they style of wines you produce with each red hybrid grape you use.
Pleas list any problems you have with processing, vinifcation, or storage of red hybrid wines.
In order to determine the most common processing methods used in red wine production, we have included a list of processing methods below. Pleas check the box beside any processing method you use for any of your red hybrid wines (check all that apply).

- Pre-maceration grape destemming
- Extended maceration
- Pectolytic enzyme addition: please list commercial preparation
- Tannin addition: please list commercial preparation
- Hot press or thermovinification
- Cold soak
- Freezing (fruit or juice)
- Pumpover or punchdown
- Carbonic maceration
- Other: list

How often do you perform malolactic fermentation on red hybrid wines?

- Always, sometimes, never

If you perform MLF on red hybrids, why?

- For stylistic expression
- To reduce acidity
- All of the above
- Other

What red hybrid processing steps would you like more information about?
Please list any research or extension ideas below.

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