Characterization of Key Volatile Compounds in Red Table Wines Produced from Frontenac Grapes (*Vitis* spp.)

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DEDICATION

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ABSTRACT

Frontenac (*Vitis spp.* MN 1047) is a new, cold-hardy red wine grape that is currently the most-planted grape cultivar in much of the Upper Midwest. Though typically described as having notes of cherry, black currant and spice, the volatile characteristics of Frontenac wine have not been investigated, and no structured evaluation of common sensory characteristics has been performed. To develop a standard set of aroma descriptors that characterize red Frontenac table wines, descriptive analysis was performed on six products. Thirteen sensory descriptors were developed and defined with reference standards; correlation plots indicated that attributes were discrete and not redundant. All 13 attribute descriptors were useful for describing and/or distinguishing between red Frontenac table wines.

In order to determine odor active compounds, eight Frontenac table wines were evaluated using stirbar sorptive extraction (SBSE) combined with concurrent gas chromatography-olfactometry/ mass spectrometry (GCO/MS). Twenty-four volatiles perceived by panelists were identified via mass spectra comparison, and included five alcohols, fourteen esters, one lactone, two acids and two volatile phenols. Twenty-four of these were confirmed with LRI comparisons in separate GC/MS analyses using a C_6 to C_{16} carbon ladder, and 23 were quantified in runs using a known concentration of internal standard. Analyses of wines produced from *V. riparia* clone #89, a parent of Frontenac, identified 16 volatiles common to Frontenac wines. Tentative identification, via GC/MS, of Frontenac juice with two days of skin contact suggested that four volatiles found in the wine may originate in the fruit.

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Hypotheses

1. The pool of perceptible volatile compounds identified in red Frontenac table wines by gas chromatography-olfactometry/mass spectrometry will be qualitatively and quantitatively similar to those identified in other monovarietal wine types.

2. Aromas described as black cherry, black currant, plum and herbaceous are present in a majority of red table wines produced from Frontenac.

Objectives

1. To To identify volatile compounds in red Frontenac wines which can be perceived by GCO panelists.

2. To develop a sensory vocabulary that can be used to describe red Frontenac table wines.

CHAPTER ONE

Literature Review

1.1. Climatic conditions for winegrape production

The production of *Vitis vinifera*, the "European" wine grape, is largely limited to the world's two 'grape belts,' generally represented as the areas bounded by 30˚ to 50˚ N latitude in the northern hemisphere and 30˚ to 40˚ S latitude in the southern hemisphere (Mullins *et al.* 1992). These boundaries serve only as general guidelines, however, as variations in weather and geography may make *V. vinifera* production difficult even in localities within this range. This is true in the Upper Midwest region of the United States, which lies largely between 39˚ and 50˚N latitude and exhibits a humid continental climate, typified by hot or warm summer daytime temperatures and winter temperatures below -3°C (Peel *et al.* 2007). Much of the region is classified as USDA Zones 3a-4b (USDA) or Köppen Dfa & Dfb, the former indicating that one or more summer months has a mean temperature >22°C, and the latter, that at least four months exhibit mean temperatures >10°C (Mitchell & Kienholz 1997). While the fruit of several *V. vinifera* cultivars will reach maturity in these summer temperatures, winter temperatures often drop far below the classifying temperature of -3°C, making winter vine survival difficult. In Minneapolis (44.83˚N), for example, the mean daily minimum during January is -15˚C, with a record low of -36˚C and an average of 30.8 days with a daily minimum lower than 0˚C (NCDC). The mean minimum yearly temperature for Excelsior, the site of the UM Grape Breeding project, is -33°C (Hemstad & Luby 2000). White Riesling (*V. vinifera* L.), commonly considered the most cold-hardy of the traditional wine grapes, shows primary bud T_{50} at -17˚C (Schnabel & Wample 1987), suggesting that *V. vinifera*

in general are unsuited in the region, except under extraordinary circumstances. Subsequently, grape producers in the northern United States and much of Canada have been limited to a small selection of grape cultivars, growing either *V. vinifera* or direct producer hybrid cultivars that are marginally hardy and require costly winter protection, or cultivars from other species that produce wines of inferior quality. For this reason, attempts to develop commercial wine industries in these areas have been erratic and often unsuccessful, despite the American public's growing interest in wine.

1.2. Frontenac history and importance

Historically, breeders at the University of Minnesota's Fruit Breeding Farm have worked sporadically on cold-hardy grape stock, but early introductions like Edelweiss and Bluebell were intended for home growers and consumption out-of-hand. Focus shifted to wine grape development in the late 1970's, at which time the native 'river grape' species *Vitis riparia* was investigated as breeding stock for cold-hardy wine grape development. Of the 26-30 grape species native to North America, *V. riparia* ranges furthest north, with an upper limit stretching north of the Great Lakes and into central Canada (Hedrick 1908, Hemstad & Luby 2000). *V. riparia* has been used as a parent in the breeding of moderately cold-hardy wine grape cultivars, including Baco Noir (*V. vinifera* cv. Folle blanche X *V. riparia*) and Maréchal Foch [(*V. riparia* X *V. rupestris*) X *V. vinifera* cv. Goldriesling], and in the very cold-hardy table and juice grape Beta (*V. riparia* X *V. lubrusca* cv. Concord) (Brooks & Olmo 1972, Pfaender 1912, Pierquet 1977). The relatively high acid of *V. riparia* requires that it be bred with lower acid cultivars of *V. vinifera, V. amurensis, V. acerfolia,* or direct producer (so-called 'French-American')

hybrids to produce interspecific hybrids combining cold hardiness, disease resistance and appropriate chemistry for wine production.

In 1996, the University of Minnesota breeding program released Frontenac, a red wine grape arising from a cross of the direct producer variety Landot 4511 (Landal L.244 X Villard blanc) and *Vitis riparia* clone #89, found growing wild near Jordan, MN (Luby & Hemstad 2006.) Originally tested as MN 1047, Frontenac has been reported to survive winter minima as low as -30°C (ASHS 1997). Frontenac produces small, bluish-black berries in medium-sized, fairly loose clusters, and is a vigorous and productive vine (ASHS 1997). Due to its extreme cold-hardiness and suitability for wine production, Frontenac is currently the most-planted grape cultivar in Minnesota, with a reported 34,260 vines making up 20% of the total vineyard plantings in 2007 (Tordsen *et al.* 2007).

As Frontenac is a hybrid incorporating more than two species, sensory profiles of the wines it produces are often very different than those produced from traditional *V. vinifera* wine grapes. As an F_1 offspring of *V. riparia*, it can be assumed that Frontenac shares some sensory characteristics with its parent, and the juice does exhibit soluble solids and titratable acidity (TA) higher than those found in classic *V. vinifera* cultivars. Juice from grapes produced on mature vines at the University of Minnesota's Horticultural Research Center yielded a five-year mean of 25.5°Brix, 15.7 g/L TA in tartaric acid equivalents, and 3.06 pH (Mansfield, 2007). Frontenac juice also exhibits more color intensity and lower perceptible astringency than red wines produced from *V. vinifera* cultivars (Luby *et al.* 2006).

While early writers described *V. riparia* fruit to be "juicy, pure and vinous," (Pierquet 1977) and key volatile compounds in fruit from one *V. riparia* cultivar have been identified (Schreier & Paroschy 1980), the vast number and variation among *V. riparia* genotypes (Pierquet 1977) make any comparisons to *V. riparia* #89 tenuous. While wines produced from Frontenac are commonly described as having notes of cherry, black currant, and herbaceous character (Reisch and Henick-Kling 1997, Leahy 2007), no formalized sensory evaluation has been published, and little work has been done to characterize the volatile compounds responsible for these characteristics.

1.3. Wine flavor

As with any food product, the sensory character of wine is dictated by a matrix of compounds that can be perceived by taste, chemesthesis and smell. In wines, non-volatile organic acids, sugars, phenolic compounds and minerals are the most common tastants (Schreier 1979), eliciting sweet, sour, and bitter responses. In some wines, phenolic compounds and high levels of ethanol may also result in trigeminal or chemesthesis sensations of astringency and false heat, respectively. More importantly, the wine matrix includes an estimated 600-1000 volatile compounds (Rapp 1998, Noble 1990). While the relative amount of such compounds is small, representing a total of 0.8-1.2 g/L in the average wine (Rapp 1998), they often are the primary means of differentiating wines produced from different cultivars (Rapp 1990). For this reason, the study of wine volatiles and their generation has been pursued since the work of Henning and Villforth in the 1940's (Rapp & Gûntert 1986). The complexity of wine aroma profiles can be attributed to the diverse mechanisms from which odor-active compounds

are derived. Key components arise from four major mechanisms: i) grape metabolism, influenced by environment and cultural management; ii) pre-fermentation events occurring during extraction and maceration, including oxidation and hydrolysis; iii) fermentation metabolism; and iv) chemical and enzymatic changes occurring during aging and additional processing (Ebeler 2001, Schreier 1979). To characterize the aroma of a finished wine type, it is necessary to consider the contributions of each of these sources and their relationship to the parent grape.

1.3.1. 'Varietal character' or 'Typicity' of wine

In specialty products like sparkling or fortified wines, the final beverage is meant to express a specific processing style, and defining sensory characteristics are derived largely from processing methods. In contrast, still table wines are often judged by their expression of varietal character or typicity, that is, the extent to which the wine reflects the sensory characteristics of its parent grape. It is well known that the largest portion of wine flavor is derived from yeast metabolism, rather than grapes, and that the majority of these yeast products are present in all wines, thereby playing a negligible role in the distinguishing characteristics of a particular varietal (Montedoro & Bertuccioli 1986, Nykänen 1986, Noble 1990, Scharpf *et al.* 1986). Thus, despite their considerably smaller volume, volatile compounds of grape origin likely dictate varietal character in wines, though the transfer from fruit or juice to wine may not be as simple as originally thought. Some compounds seem to survive fermentation and appear unchanged in the finished wine. Kotseridis and Baumes (2000) reported eighteen impact odorants identified in Cabernet Sauvignon juice, sixteen of which were also important to the aroma

of Cabernet Sauvignon wine; whether all compounds found in the wine originated in the grape was undetermined. Other volatile compounds key to varietal character are known to develop during fermentation; in Sauvignon blanc wines, for example, thiols important to varietal expression have been found to increase during fermentation, due to the degradation of cysteinylated precursors by *Saccharomyces cerevisiae* yeast (Dubourdieu 06). While these compounds are produced by yeast, their quantity and subsequent impact is dictated by the precursors found in the grape, and concentrations in the final wine vary widely among cultivars.

In a few cases, varietal character can be attributed to a single volatile compound, or to a single chemical family; this is the case with the 2-methoxy-3-isobutylpyrazine (MIBP) that characterizes Cabernet Sauvignon, or the monoterpenes that characterize Muscat and Gewürztraminer (Ebeler 2001, Noble 1990, Rapp 1990). More commonly, a wine's distinctive aroma profile is dictated not by a single impact compound, but by specific ratios of several volatile constituents (Noble 1990, Caven-Quantrill & Buglass 2006). Each cultivar may possess differing proportions of compounds common to many wines, giving rise to differences in the perceived aroma (Fang & Qian 2006). Definitive assessment of the constituents of varietal character in such cultivars is significantly more difficult, though comparison of the monoterpene 'fingerprint patterns' in gas chromatograms has been proposed as a means to differentiate between some young aromatic white wines produced from various cultivars (Rapp 1990).

1.3.2. Grape-derived volatiles

Like most fruits, grape berries begin the accumulation of volatile compounds during the ripening phase, a period marked by a climacteric rise in respiration (Reineccius 2006). In grapes, this period is termed *engustment,* which begins at veraision and is concurrent with the accumulation of color and softening of the berries. The formation of metabolic products in grapes is primarily determined by grape species and cultivar, and may be further influenced by degree of ripeness and environmental conditions during ripening, especially water stress (Matthews and Anderson 1988, Schreier 1979, Rapp 1990). More than 1300 different volatile compounds have been identified in various cultivars of *V. vinifera* (Ebeler 2001); these have been classified into five main groups: the monoterpenes, norisoprenoids, benzenoids, aliphatic compounds and methoxypyrazines, and include glycosylated forms of all but the last group (Williams *et al.* 1996).

Monoterpenes were the first group of grape-derived volatiles linked to wine aroma. Terpenes are generated by both carbohydrate and lipid metabolism in several higher plants (Reineccius 2006), and are likely synthesized in grape cell plastids (Jackson, 2000). Approximately 50 terpene compounds have been identified in *V. vinifera* grapes; monoterpene alcohols are among the most important. Of these, linalool, geranial, α-terpineol, nerol, citronellol, hotrienol, and *cis*-rose oxide [4-methyl-2-(2-methyl-1-propenyl)tetrahydropyran] have the greatest sensory impact (Ebeler 2001; Strauss *et al.* 1986, Noble 1990, Rapp 1990). As a group, they contribute floral notes like rose, iris and the self-defined 'muscat-like' aroma to wines. As much as 90% of grape terpenes may present as non-aromatic, glycosidically-bound precursors, which can be hydrolyzed by enzymes or acid during fermentation and aging (Wilson *et al.* 1986).

Monoterpenes have been identified as the key impact odorants in the varietal character of *V. vinifera* cultivars in Gewürztraminer, Riesling, and the Muscat family, and play a minor role in the floral or flowery notes found in Pinot gris, Viognier, Muscadelle, Albariño and Muller-Thurgau. Several non-aromatic cultivars, including Chardonnay, Sauvignon blanc, Syrah, Cabernet Sauvignon, Cabernet Franc, and Merlot, produce monoterpenols, but levels are generally below perception threshold (Ribereau-Gayon *et al.* 2001).

Methoxypyrazines are aroma compounds of grape origin responsible for varietal character in some red and white wines. Of primary importance to wine aroma is a trio of 3-alkyl-2-methoxypyrazines, namely the isopropyl, *sec*-butyl, and isobutyl forms (Ribereau-Gayon *et al.* 2001, Sala *et al.* 2005). The perception threshold of these compounds in wines is very low (Kotseridis *et al.* 1998, Maga 1990), and they are associated with the characteristic herbaceous notes found in Cabernet Sauvignon, Sauvignon blanc, Cabernet Franc and Merlot (Ebeler 2001, Ribereau-Gayon *et al.* 2001, Sala *et al.* 2005). At high levels, the sensory impact of 2-methoxy-3-isobutylpyrazine is considered to be an off-flavor.

The biological mechanism for methoxypyrazine production in plants is largely unknown; in grapes, a balance of biological formation and photo-degradation may dictate final concentrations in the fruit. Light exposure, such as that found under typical vineyard conditions, is reported to promote formation of methoxypyrazines in immature grapes, but effect photodecomposition in ripening berries (Hashizume & Samuta 1999), and the decline of methoxypyrazine concentrations in ripening fruit is well documented (Galet 2000).

In addition to the monoterpenes and methoxypyrazines, some individual compounds are thought to be fully or partially responsible for varietal typicity in specific wines. Benzaldehyde, an aromatic aldehyde, is thought to originate in the Gamay grape, and produce the bitter-almond odor characteristic of the wine (Jackson 2000). While grape derived lactones generally do not participate in wine aroma, 2-vinyl-2-methyltetrahydrofuran-5-one has been associated with characteristic aromas of Riesling and Muscat wines (Schreier & Drawert 1974).

In contrast to these specific, typifying notes, some families of grape-derived volatile compounds may make more general, non-characterizing contributions to wine aroma. Norisoprenoids are oxidative degradation products of carotenoids; in grapes, they are thought to arise during ripening, and are biosynthesized independently in both leaves and berries (Ribereau-Gayon *et al.* 2001, Ebeler 2001, Wirth *et al.* 2001). C_{13}-norisopreniods, those containing 13 carbons, exist as megastigmane (oxygenated) or non-megastigmane forms, with several volatile compounds in each group. In the former group, the ketones β-damascenone (oxygenated on carbon 7) and β-ionone (oxygenated on carbon 9) have been found in both aromatic and non-aromatic grapes (Jackson 2000). β-Damascenone is thought to be present to some degree in all grapes, though concentrations are higher in red wines than whites, with the exception of Muscat *vins doux naturel*. β-Ionone is also thought to be present in all grape cultivars, but has negligible effects on the aroma of white wines (Ribereau-Gayon *et al.* 2001); berry concentrations are variable, and not dictated by cultivar. In wine, the megastigmanes contribute a complex array of aromas, including flowers, tropical fruit, stewed apple, violet, lime, tea, grass, honey, and pineapple (Ribereau-Gayon *et al.* 2001, Ebeler 2001).

Glycosylated C_{13}-norisoprenoids exist in large quantity, largely as monoterpenes; they have been found to be somewhat unresponsive to grape and yeast glycosidases, however, and their sensitivity to acid media is unknown (Ribereau-Gayon *et al.* 2001).

Another group of impact odorants are the C_6 alcohols, which are formed from linolenic and lenoleic acids through the action of lipoxygenase and alcohol dehydrogenase in grape tissue (Eggers 2005). Compounds of primary importance include 1-hexanol, (*Z*)-3-hexenol, 2-hexanol, hexanal, and (E)-2-hexenal. Along with their aldehydes, they may produce herbaceous, green off-notes in wines, though the latter two are known to be reduced by yeast during alcoholic fermentation (Kotseridis & Baumes 2000).

Several esters are synthesized in the fruit, arising from fatty acid, amino acid, or cinnamic acid metabolism (Reineccius 2006). In most cultivars, grape-derived esters are of little sensory importance, with two possible exceptions. Isoamyl acetate, an ester produced during fermentation, has also been tentatively identified in the South African *V. vinifera* cultivar Pinotage (Marais *et al.* 1979). In selected cultivars of *V. labrusca*, the phenolic ester methyl anthranilate is one of several compounds producing the 'foxy' note characteristic of the species (Jackson 2000, Schure & Acree 1994).

1.3.3. Volatiles arising from pre-fermentation events

Prefermentation processes like destemming, crushing and pressing involve physical disruption of grape berry structure, allowing interaction between compounds previously separated by cell structure. C_6 aldehydes and alcohols, like trans-2-hexenal, cis-2-hexanol, and 2,4-hexadienal arise during crushing, likely formed by the interaction of

indigenous lipoxygenases and small amounts of linoleic and linolenic acids released from the grapes and leaves (Schreier *et al.* 1976, Iglesais *et al.* 1991). Unlike grape-derived aldehydes, these compounds do not appear to be reduced to alcohols during fermentation, and may lend grassy or herbaceous odors to Sauvignon blanc, Grenache, and wines made from immature grapes (Jackson 2000).

1.3.4. Fermentation-derived volatiles

Primary or Alcoholic Fermentation: While the catabolism of hexoses into ethanol is the primary objective of fermentation, this process is also key to the production of several volatile compounds, produced as by-products of metabolic processes of yeast. While these yeast-derived compounds frequently represent the largest quantity of wine volatiles, most individual compounds exist in small concentrations below flavor thresholds, and have little impact on wine flavor (Schreier 1974, Ebeler 2001). Those that can be perceived are often common to all fermentation products, and are not useful for distinguishing between wines. Still, volatile composition changes measurably during the conversion of juice to wine, suggesting that the formation of new compounds, in addition to the loss of grape volatiles described above, an important role in the character of the final product.

During fermentation, yeasts form fusel alcohols either through sugar catabolism or the decarboxylation and deamination of branched-chain amino acids, primarily via the Ehrlich pathway (ter Schure *et al.* 1998, Boulton *et al.* 1995). Along with their esters, fusel alcohols have the potential to contribute intense aromas to wine (Jackson 2000). 1-Propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, phenethyl

alcohol contribute subtle complexity at levels <3000 mg/L, but become penetrating and disruptive at higher concentrations (Ebeler 2001, Ribereau-Gayon *et al.* 2001).

Several acidic compounds are known to be produced or modified during the course of yeast fermentation. These include volatile fatty acids of up to 12 carbons, primarily hexanoic, decanoic, and octanoic acids, which are thought to be produced via both catabolic and anabolic pathways (Schreier 1974). The absence in wine of fatty acids with greater then a 14 carbon backbone suggests the catabolic degradation of long-chain compounds by ß-oxidation (Schreier 1974). In addition, the anabolic production of fatty acids may proceed via the fatty acid synthetase complex, where the carbon chain lengthens by two carbon atoms through the binding of malonyl-CoA with acyl-CoA in the enzyme complex. This reaction produces free fatty acids when water is available, and the corresponding ester in the presence of alcohol (Nykänen 1986, Rapp 1998).

A variety of fermentation-derived esters contribute to wine aroma, producing a range of fruity, floral, and occasionally grassy notes. Aliphatic esters, especially monocarboxylic acid esters, make up a large portion of those found in wine; of these, the most important are those resulting from the reaction of ethanol and saturated fatty acids, and of acetic acid and fusel alcohols (Jackson 2000). The latter group, the acetates, is the mostly widely studied, and contributes much to general vinous fragrance rather than varietal character (Salinas *et al.* 2004). The shorter chain, low molecular weight acetates are responsible for distinctly fruity aromas, and are particularly important in the aroma of young wines (Vernin *et al.* 1986). The hydrocarbon chain length of the parent acid indicates acetate aroma, which moves from fruity (isoamyl acetate, benzyl acetate) to soapy and, finally, unpleasantly lard-like with C_{16} and C_{18} fatty acids (Jackson 2000).

While terpenes are generally undiminished by fermentation, they may participate in acid-catalyzed rearrangements, resulting in the formation of new compounds and decreased concentrations of original odorants. Linalool, for instance, is found in grapes and young wines, and can be transformed into α-terpineol, nerol, geraniol, and hydroxy linalool during processing and aging (Ebeler 2001). A related compound, wine lactone (3a,4,5,7a-tetrahydro-3,6-dimethylbenzofuran2(3H)-one) is found in some white wines, where it contributes coconut, woody, or sweet notes (Ebeler 2001). Wine lactone is presumed to arise from a monoterpenoid acid precursor in an acid-catalyzed reaction (Winterhalter *et al.* 1998).

Sulfur compounds are often responsible for off-notes in wine, but selected compounds are key to varietal character in some cultivars. These sulfur compounds are almost completely absent in the grape, but must be released from their S-cysteine conjugate precursors by yeast cysteine β-lyase in fermentation (Tominaga *et al.* 2000a). The final concentration of aroma compounds therefore hinges on the pool of available precursors generated in the grape (Tominaga *et al.* 1998). 4-Mercapto-4-methyl-pentan-2-one (MMP), 3-mercaptohexan-1-ol acetate, 4-mercapto-4-methylpentan-1-ol, 3-mercaptohexan-1-ol, and 3-mercapto-3-methylbutan-1-ol have all been identified as impact odorants in Sauvignon blanc, and have been identified in wines made from Gewürztraminer, Riesling, Colombard, Petit Manseng, and Semillon. (Tominaga *et al.* 2000a). An additional compound, 2-furanmethanethiol, has been found to contribute a roast coffee aroma to selected wine varietals (Tominaga *et al.* 2000b). Some of these compounds have been identified in wines made from Cabernet Sauvignon and Merlot, but their sensory impact has not been assessed (Derriot *et al.* 1995). At the concentrations

found in white wines, this group of volatile thiols contributes notes of boxwood, broom, grapefruit zest, passion fruit, and guava to the sensory profile (Peyrot des Gachons *et al.* 2002).

Secondary or Malolactic Fermentation (MLF): In addition to alcoholic fermentation by *Saccharomyces cerevisiae,* some wines undergo a secondary fermentation, or malolactic fermentation (MLF), catalyzed by lactic acid bacteria. The primary aim of this process is to convert malic acid to lactic acid, resulting in decreased wine acidity. Like yeast, the metabolic processes of lactic acid bacteria also produce a range of volatile compounds, which impact wine flavor to varying degrees, depending on bacterial strain and fermentation conditions (Liu 2002). An important compound characterizing wines that have undergone MLF is the ketone 2,3-butanedione (diacetyl), which generally is present in concentrations of 4 mg/L or less, and is perceived as buttery or creamy (Ebeler 2001). Henick-Kling (1993) reported that MLF enhances fruity and buttery notes while reducing green, grassy aromas, the latter perhaps as a result of aldehyde catabolism (Liu 2002).

1.3.5. Volatile evolution during aging

In inert storage, like stainless steel tanks or glass bottles, changes in wine aroma during aging reflect changes in ratios of specific volatile compounds over time. Rapp (1990) classifies wine reactions during bottle maturation into four basic types:

1. Changes in ester content, as acetates decrease and mono- and dicarboxylic acid ethyl esters increase;
2. The formation of carotene degradation products;

3. The formation of carbohydrate breakdown products; and
4. Acid-catalyzed reactions of monoterpene compounds.

Fruity, low molecular weight acetic acid esters usually exceed their equilibrium constants when fermentation ceases, and hydrolyze back to their parent alcohols and acetic acid, resulting in a loss of fruit bouquet (Ramey & Ough 1980, Jackson 2000). Equilibrium concentrations of acetic acid, acetates, and alcohols are reported after six years of bottle maturation (Rapp 1990). In contrast, the concentration of fatty acid and other mono- and dicarboxylic acid esters is often below equilibrium constant at the end of fermentation, and gradually increases during aging due to slow synthesis (Rapp & Gûntert 1986). Equilibria for some of these compounds is still not complete after 10 years of aging (Rapp 1990).

The carotene-derived non-megastigmane C_{13}-norisoprenoids are generally not present in grapes or young wines, but participate in the age bouquet of some cultivars. Vitispirane and TDN (1,1,6-trimethyl-1,2-dihydronaphthalene) variously contribute camphor and kerosene or petroleum notes to aged whites (Ribereau-Gayon *et al.* 2001, Ebeler 2001, Rapp 1990). Research suggests that both non-megastigmanes and b-damascenone can evolve in wine through a variety of transformations, though the extent of this activity is unknown (Winterhalter *et al.* 1990).

Monoterpenes, derived from the grape, undergo acid-catalyzed rearrangements resulting in changes of volatile concentration and the formation of new compounds, such as cis- and trans-1,8-terpine (Ebeler 2001, Rapp 1998, Jackson 2000). Linalool may also be degraded into geraniol, nerol, and α-terpineol, which contribute rose, floral, and musty, pine-like aromas, respectively (Rapp 1998, Jackson 2000)

In addition to internal reactions associated with aging, wines aged in barrels may extract volatile compounds from the oak, lose compounds to oak absorption, or participate in the reactions listed above. In the first case, the oak barrel can contribute vanillin, derived from the wood itself, as well as so-called 'oak lactones,' isomers of β-methyl-γ-octalactone, which contribute oaky and coconut-like notes to the wine (Spillman *et al.* 1997, Chatonnet *et al.* 1990). Extractable phenolic aldehydes developed during barrel toasting contribute roasted vanilla notes, while sugar derived compounds like maltol and 2-hydroxy-3-methyl-cyclopentane are perceived as toasty. Heating also results in the development of lignin-derived volatile phenols, including dimethoxy-2,6-phenol and eugenol, which are perceived as smoky or spicy notes when present in wine, and guaiacol, 4-methyl guaiacol, and vinyl-4-guaiacol, which may be perceived as smoky or tobacco-like. Other compounds produced during toasting include aromatic aldehydes, furfurals, furans, pyrazines, pyridines, and pyrans (Jackson 2000, Diez *et al.* 2004, Chatonnet *et al.* 1990).

Although the transfer of volatiles from wine into cooperage has garnered less attention than that of wood compounds into wine, recent work suggests that sorption may play a role in overall wine flavor. The composition of both the wine and wood affect the rate of volatile sorption. Most wines contain 12-16% ethanol, which acts as a co-solvent with water. At these levels, the solubility of non-polar compounds is increased, subsequently decreasing the rate of wood sorption (Ramirez-Ramirez *et al.* 2004). Solubility of ethyl hexanoate and ethyl octanoate has been found to increase approximately 50% as ethanol concentrations rise from 0-12.5%, and sorption decreased 70% and 40%, respectively, at concentrations rising from 10 to 15% (Ramirez-Ramirez *et*

al. 2004, Barrera-Garcia *et al.* 2006) . The sorption of benzaldehyde decreased 25% between 10 and 15% ethanol, and 2-phenylethanol sorption seemed to stop completely at ethanol levels reaching 15% (Barrera-Garcia *et al.* 2006).

Sorption of several key wine aroma compounds show a linear correlation between total sorption and wood surface-to-wine volume ratio (Ramirez-Ramirez *et al.* 2001). In a standard 228L wine barrel, the ratio is about 140 cm^2/L, but the addition of wood chips, staves, or other oak products may be added to increase surface area. For most aroma compounds, 80% of total sorption is achieved within the first 15 days of wine/wood exposure (Ramirez-Ramirez *et al.* 2001). In a model wine, isoamyl acetate, linalool, ethyl hexanoate, ethyl octanoate, ethyl decanoate, 2-phenylethanol and benzaldehyde equilibrated variously with 3-50% wood sorption by wood, but ß-ionone sorption proved to be highly variable (Ramirez-Ramirez *et al.* 2004). Yeast lees have also been found to sorb selected volatile phenols (Chassagne *et al.* 2005). The sensory impact of volatile sorption is exhibited as a decrease in fruity and floral notes; some studies suggest that the green or stemmy aromas derived from underripe grapes are also reduced (Jackson 2002).

1.3.6. Sensory characteristics of wines produced from hybrid grapes

Wines produced from non-*vinifera* species are often characterized by volatile compounds not found in traditional wine cultivars. The aroma of wines made from *V. muscadinia* cv. Noble is partially dictated by methanolic and ethanolic esters of succinic acid, compounds not found in *V. vinifera* wines (Lamikanra *et al.* 1996). Furaneol, 2,3-dibutanedione, ethyl butanoate, ethyl 2-methylbutanoate, 2-phenylethanol, and o-aminoacetophenone were found to contribute to the characteristic aroma of muscadine

juice, but the presence of all but 2-phenylethanol in muscadine wine has not been investigated (Baek *et al.* 1997). Methyl anthranilate, o-aminoacetophenone, furaneol and its methoxy derivative 2,5-dimethyl-4-methoxy-2,3-dihydro-3(2*H)*-furanone are thought to produce the foxy or candy-like aromas that characterize *V. labrusca* cultivars, as well as juices and wines produced from them (Schure & Acree 1994). It is interesting to note that these characteristic compounds developed differently during grape ripening than did the common norisoprenoid β-damascenone and its glycosidic precursor (Schure & Acree 1995). In total, these differences in grape composition suggest that optimal flavor development in wine produced from non-*vinifera* cultivars may require different cultivation and processing methods than traditional, *V. vinifera* wine grapes.

1.3.7. Sensory characteristics of V. riparia and V. riparia hybrids

Due to limited commercial interest, literature referencing the sensory characteristics of *V. riparia* fruit or products is scant. Early reports suggest that *V. riparia* fruit and wine are both free from foxiness or "any disagreeable wild taste" (Hedrick 1908). Schreier and Paroschy (1980) cataloged the volatile components of unidentified *V. riparia* fruit collected in Ontario after noting that Elvira, a *V. labrusca x V. riparia* hybrid used for white wine production, contained very little of the 'foxy'-smelling methyl anthranilate found in *V. labrusca* cultivars (Nelson *et al.* 1977). While compounds in the acid and carbonyl fraction, as well as the isomer alkylbenzenes found in the hydrocarbon fraction, were largely the same as those previously identified in *V. vinifera* cultivars, the quantity and type of other components varied. Unlike *V. vinifera* grapes, *V. riparia* fruit generated a large number of detectable esters, of which hexyl-2-methylbutanoate, hexyl hexanoate,

methyl salicylate, and ethyl hexanoate were present in the largest quantity. Further, the terpene derivatives linalool, α-terpineol, 4-terpineol, citronellol, nerol, geraniol and 3,7-dimethyl-1,5,7-octa-trien-3-ol, common in *V. vinifera* grapes and press fractions, were present in trace amounts or entirely undetectable in *V. riparia* fruit. It was also noted that the composition of sesquiterpene hydrocarbons differed from that in *V. vinifera*, but compound identifications were not noted in the work. One must emphasize, however, that the *riparia* species shows great interspecific variation in several phenotype charcteristics (Hedrick 1908, Pierquet 1982), and subsequently has potential for vastly different volatile composition.

In addition to the white wine cultivar Elvira, several *riparia*-based hybrids have been used for wine production, of which Baco noir and Maréchal Foch are found throughout the Midwest, and the lesser-known St. Croix, Edelweiss, Kay Gray, St. Pepin, and La Crosse are important in limited cold-climate regions (Pierquet 1982, Hemstad & Luby 2000). Due to their limited commercial use, however, quantification of volatile compounds of these cultivars has not been documented. There is no published data on Frontenac volatile compounds, but Lopez *et al.* (2004) presented preliminary data on 19 Frontenac compounds identified by GC-O and quantified by H-SPME-GC-MS, stating that 3-methyl-1-butanol, 2-phenylethanol, and several unnamed esters were most prevalent.

1.4. Sensory evaluation of wine flavor

As with any food product, the quality of wine is based to a great degree on consumer perception. In wine, the perception of quality hinges on the product's perceived aroma; a

perusal of popular wine literature readily indicates that the examination or ortho- and retronasal sensations are the primary focus of both professional and casual wine evaluation. While the sensitivity and selectivity of modern instruments allows ever-increasing precision in volatile analysis, it is as yet impossible to fully understand human perception of the wine matrix (Noble & Ebeler 2002). Given the considerable variation in human sensitivity to volatile compounds, and the large range of external factors influencing perception (Reineccius 2006), a full understanding of a wine's volatile impact requires evaluation by human subjects in addition to instrumental analysis.

1.4.1. Descriptive analysis

The technique of descriptive analysis involves the detection and description of a product's sensory attributes, both qualitative and quantitative, by a trained sensory panel (Meilgaard *et al.* 1999, Andrews *et al.* 1990). First conceived as the Flavour Profile Method (FPM) in the late 1940's, the family of descriptive analysis tests has grown to include a range of methods for specific sensory applications, including the Texture Profile Method (TPM), Quantitative Descriptive Analysis® (QDA®), Quantitative Flavour Profiling (QFP), and the Spectrum Method (Murray *et al.* 2001). In practice, a generic descriptive analysis that allows the practitioner to modify or combine elements of various methods is most often used to provide targeted solutions (Murray *et al.* 2001).

Because descriptive analysis can be used as a basis to relate descriptive sensory evaluations with instrumental or consumer preference measurements, it is often used for quality control, product comparison, and consumer response as well as sensory characterization (Gacula 1997, Murray *et al.* 2001). In practice, panelists are asked to

generate a descriptor set or lexicon to describe a product's sensory attributes; the wine aroma wheel (Noble *et al.* 1987) is often used as a starting point for wine evaluation. A panel leader generally guides this process, aiding panelists in reaching a consensus and, if necessary, helping to clarify descriptor meaning and eliminate redundant descriptors. For the analysis of wine aroma, a set of 5-10 terms is typically sufficient (Noble & Ebeler 2002). Descriptors may be linked to reference standards, which are ideally simple, reproducible, and linked to only one descriptive term, though terms can be referenced by more than one standard (Rainey 1982). Reference standards may also be used as scale anchors in intensity rating; for odor intensity, a series of 1-butanol concentrations in air, nitrogen, or water is often used (ASTM 1993). In either case, the use of reference standards can shorten panel training time, though choice of descriptor reference standards should be guided by the panel, rather than the panel leader, to avoid bias (Rainey 1982). In addition, panelist performance should be evaluated and assessed in the course of training, as well as during final data analysis (King *et al.* 2001).

After a descriptor set or lexicon has been developed and panelists adequately trained on reference and intensity standards, attribute intensity may be assessed. Line-marking scales, on which a panelist places a mark on a line to indicate intensity, are a popular and fairly simple means of recording perceived intensity. Line-marking scales generally include stimulus end-anchors to define the frame of reference, and may include other intermediate points; the end-anchors may also be indented to avoid bias from panelists reluctant to use the ends of the scale (Lawless & Heymann 1998). When coupled with the use of intensity reference standards, end-anchors and intermediate points are considered equivalent to the reference scale used. Panelist responses are

converted into numerical measurements, allowing analysis of results with such statistical tools as ANOVA and PCA (Stone *et al.* 1974). Sensory software, like SIMS2000 (Sensory Computer Systems, Morristown, NJ), can greatly facilitate both the recording and analysis of panelist response.

For wine service in both descriptor generation and intensity rating, the principles of sound experimental design should be considered- e.g., wines should be served blind and in a randomized complete block, or similar, to prevent panelist bias. If color is not an attribute under consideration, 250 mL, non-colored tulip-shaped wine tasting glasses should be used (ISO 1977), and covered with watchglasses if necessary.

1.4.2. Weaknesses of descriptive analysis for complex odors

Despite its flexibility and frequent use, some sensory scientists question the use of descriptive analysis for descripting and representing complex aroma attributes (Murray *et al.* 2001). In addition, the various works of Laing *et al.* have asserted that humans are unlikely to identify more than 3-4 components in complex odor mixtures, and that most identify complex odor mixtures as a single entity. As descriptive analysis assumes that attributes are independent and are perceived separately, panelists' inability to tease out individual odors may result in a deceptively simple, and inaccurate, report of a products' volatile characteristics (Lawless 1999). Various methods, including time-intensity analysis and specialized training, have been proposed to address this question, which is still under investigation (Murray *et al.* 2001).

1.4.3. Descriptive analysis of wines

Generic descriptive analysis has been used to characterize several single-cultivar or varietal wines, including Cabernet Sauvignon (Heymann & Noble 1987, Heymann & Noble 1989), Chardonnay (Francis *et al.* 1992, Heymann & Noble 1989), Macabeo (de la Presa-Owens & Noble 1995), Parellada (de la Presa-Owens & Noble 1995), Pinot noir (Guinard & Cliff 1987), Sauvignon blanc (Francis *et al.* 1992), Seyval blanc (Andrews *et al.* 1990), Semillon (Francis *et al.* 1992), Shiraz (Abbott *et al.* 1991), Zinfandel (Noble & Shannon 1987), Tannat (Varela & Gámbaro 2006), Touriga Nacional (Falque *et al.* 2004), Mencía (Vilanova & Soto 2005), Albariño (Vilanova & Vilariño 2006) and Xarello (de la Presa-Owens & Noble 1995). To date, this technique has not been applied to wines produced from the University of Minnesota's cold-hardy cultivars.

1.5. Instrumental analysis of wine volatiles

Total aromatic volatile content accounts for less than 0.1% of the wine matrix, and individual compounds are often present at concentrations lower than 1µg/L (Pollnitz 1996, Allen 1991, Kotseridis 1998, Aubrey 1997). Theoretically, the human nose can perceive compounds at concentrations as small as 10^{-19} moles, or 10^{-12} g/L (Feng & Acree 1999, Frederich & Acree 2000, Reineccius 2006). While instrumental analysis has yet to reach this level of sensitivity (Feng & Acree 1999, Reineccius 2006), the most sensitive methods should preferentially be used to detect perceived impact odorants, and preliminary extraction and concentration methods may be required. Over the past 50 years, advanced techniques in liquid chromatography, gas chromatography, infrared

spectroscopy (IR), and nuclear magnetic resonance (NMR) have all been applied to wine volatile analysis, but the combination of gas chromatography with mass spectrometry (GC-MS) has become the accepted standard (Nykänen 1986, Versini *et al.* 2008). Gas chromatography-olfactometry (GCO), in which a human subject is used to detect perceivable volatiles in the GC effluent stream, has been used alone or in combination with GC-MS to identify wine impact odorants (Kotseridis *et al.* 2000b, Lee & Noble 2003, Aznar *et al.* 2001, Campo *et al.* 2005, Culleré *et al.* 2004, Guth 1997a, Guth 1997b).

1.5.1.Gas Chromatography -Mass Spectrometry (GC-MS)

The separation method of gas chromatography was first used for wine analysis in 1956, when Ernest Bayer developed methods for determining wine volatile compounds at the Government Research Institute at Geilweilerhof (Gehrke 2001). Early technology allowed only the separation and identification of fusel alcohols and some esters, but the development of high-resolution columns and sensitive detectors has expanded this range to over 1300 wine aroma compounds (Ebeler 2001). When coupled with a hydrogen flame ionization detector (FID), compounds can be detected in concentrations as low as one nanogram (Pomeranz & Meloan 1994). Compound identifications can then be made through the use of an internal standard and comparison to the Kovats Index (KI) (Kovats 1965). GC-FID and KI comparison are still used extensively for routine identification of a small numbers of clearly separated single peaks (Versini *et al.* 2008).

Inarguably, the mass spectrometer (MS) has become the most important detector for wine volatile analysis via GC (Ebeler 2001, Versini *et al.* 2008). In GC-MS methods,

compounds separated by GC enter the MS in the effluent stream and are bombarded with electrons, producing ions that can be separated by mass or velocity, detected, and measured (Pomeranz & Meloan 1994). GC/MS can detect volatiles at concentrations of 10^{-5} g/L and greater (Frederich & Acree 2000) and is unique in its ability to simultaneously obtain retention time and mass spectra (Reineccius 2006, Ebeler 2001). Comparison of ion fractions to data libraries allows unambiguous identification of compounds present at even trace levels (Versini *et al.* 2008). In wine volatile analysis, both apolar and polar capillary columns have been used, depending on the compounds of interest (Versini *et al.* 2008). In both GC-FID and GC-MS analysis, internal standards of known concentration can be used to quantify compounds of interest.

1.5.2.Gas Chromatography/Olfactometry (GC/O)

In contrast to the purely instrumental GC/MS, gas chromatography/olfactometry is a bioassay that uses human response to identify compounds with perceptible odor, allowing odorless volatiles to be disregarded (Friedrich & Acree, 2000). Informal, unstructured GC-O is probably as old as the GC itself, though the description of the first GC instrument modified for GC/O use was not published until 1964 (Mayol & Acree 2001). At roughly the same time, odor activity values (OAVs) were defined as the concentration of an odorant divided by the odor threshold (Mayol & Acree 2001). While OAVs provide a rough means of assessing olfactory importance, they erroneously assume a linear relationship between odor intensity and perceived intensity values and are poor predictors of compound odor intensity in mixtures (Chaintreau 2002, Mayol & Acree 2001, Audouin *et al.* 2001). Two screening methods for significant odorants, the

combined hedonic aroma response measurement (CHARM) and aroma extraction dilution analysis (AEDA) or aroma extract concentration analysis (AECA) evolved from the use of OAVs, and were developed by Acree and Grosch, respectively (Audouin *et al.* 2001, Chaintreau 2002, Grosch 1994). Both are dilution methods, but CHARM measures dilution value throughout the elution of a compound, while AEDA measures the maximum detected dilution value (Acree 1993). OSME, a screening technique based on continuous tracking of odor intensity throughout a GC run, was proposed in 1989 (Chaintreau 2002, Pollien *et al.* 1999). While appropriate for sample screening, all three techniques are imperfect for compound quantitation, as reviewed by Chaintreau (2002). More recently, Pollien *et al.* (1999) have proposed a method quantifying aroma compounds via detection frequency. Termed GC-SNIF or Detection Frequency Analysis (DFA), it involves panelists depressing a response button for the duration of a perceived odor. Panelist responses are then compiled to produce an averaged olfactogram, with peak heights indicating the detection frequency of each compound; calculation of peak height, or nasal impact frequency (NIF) and peak area, or surface of nasal impact frequency (SNIF) can be used to quantify perceived compounds (Chaintreau 2002, Pollien *et al.* 1997, Pollien *et al.* 1999). GC-SNIF has been found to be comparable in sensitivity to GC-MS/MS for simple volatile solutions, and somewhat less so for complex matrices (Pollien *et al.* 1999). Further, its suitability for quantitative volatile analysis implies utility for qualitative impact odorant analysis, and reduces the inherent risk of missing odor compounds when one or a few panelists are used (Chaintreau 2002). GC-SNIF has been found to be effective with a panel of 6-10 assessors, with 8-10 ideal (Pollien *et al.* 1997). The increased sensitivity, coupled with smaller sample size and

shorter prep time (since serial dilutions aren't required) makes GC-SNIF an attractive option for impact odor analysis, even in complex matrices. Recently, GC-SNIF was applied to the detection of impact odorants in Brazilian Cabernet Sauvignon (Falcão *et al.* 2008).

1.5.3.Combined Gas Chromatography-Olfactometry/Mass Spectrometry (GCO/MS)

One elegant means of determining key odor-active compounds couples GC/MS with GCO. In this method, the end of the GC capillary is routed through a glass or metal splitter, directing effluent to both the MS mass selective detector and a sniff port (Reineccius 2006). This technology allows easy comparison of perceived aromas with volatile compounds, reducing the time necessary for peak identification and comparison, and decreasing risk of improper compound/odor correlation by eliminating the need for separate GC/MS and GC/O runs. GCO-MS has been used successfully for several applications, including identification of odor active wine volatiles (Lee & Noble 2003).

1.5.4. Sample extraction methods

While key impact odorants may occur in concentrations as low as 10^{-11} g/L (ppt), and GCMS identification requires concentrations higher than 10^{-5}, concentration and extraction of volatiles is often necessary for appropriate odor characterization (Feng & Acree 1999). The classic method of isolation and concentration of wine volatiles is continuous liquid-liquid extraction with organic solvents, usually dichloromethane/pentane (2:1 v/v), Freon 11, or Freon 11/dichloromethane (9:1 v/v) (Versini *et al.* 2008). While effective, liquid-liquid extraction presents obvious waste

disposal problems, can be time-consuming (10-24 hours), and may cause skewed results due to excessive sample handling (Versini *et al.* 2008, Reineccius 2006). Methods that improve one or more of these handicaps for wine sampling include solid-phase extraction with polystyrenic resins (Aznar *et al.* 2001), purge-and-trap (Campo *et al.* 2008), supercritical fluid extraction (Karásek *et al.* 2003) and dynamic (Tsachaki *et al.* 2005) and static (Ortega-Heras *et al.* 2002) headspace analysis. Most recently, various forms of micro-scale, solid-phase sorptive extraction have been used for volatile extraction in both headspace and submerged modes.

1.5.5. Sorptive extraction methods: SPME and SBSE

Sorptive extraction involves the partitioning of compounds into a sorptive layer on a fiber or stir bar, according to their partitioning coefficients (Harmon 2000, Salinas *et al.* 2004). The first sorptive extraction method to come into common use is solid-phase micro-extraction (SPME), which has been widely used in wine component analysis (Whiton & Zoecklein 2000, Steffen & Pawliszyn 1996, Mestres *et al.* 1999, da la Calle Garcia *et al.* 1998, Vas *et al.* 1998). SPME utilizes a fused silica fiber coated with one to three different adsorbent phases, including polydimethylsiloxane (PDMS), polyacrylate (PA), carbowax (CW), or the combination of polydimethylsiloxane with divinylbenzene (PDMS-DVB), carboxen (CAR-PDMS), or carboxen and divinylbenzene (CAR-PDMS-DVB) (Versini *et al.* 2008). Of these, PDMS and CAR-PDMS-DVB are most common in wine volatile analysis (Versini *et al.* 2008).

The coated fiber is either submerged into the wine sample or suspended above it in the headspace of a closed system, allowing analytes to partition into, and be held

within, the sorbent phase rather than adsorbed onto an active surface. This creates a weaker interaction with the compounds of interest, allowing desorption at lower temperatures and a subsequent decrease in incidence of analyte degradation (Alves *et al.*2005, Baltussen *et al.* 1999). Finally, the silicone mass fragments of adsorbent degradation products are easily identified by in mass spectrometry, reducing the number of unidentifiable unknowns (Baltussen *et al.* 1999). Use of SPME is relatively rapid, and the absence of organic solvents eliminates the concern of waste disposal and environmental regulation (Fredrich & Acree 2000).

A recent development in sorbent methodology employs a magnetic stir bar coated with polydimethylsiloxane (PDMS). Termed stir bar sorptive extraction (SBSE), this method uses the same theory as SPME, but the stir bar has a PDMS phase with a volume of 25-125 µL, compared to the significantly smaller 0.6 µL of a 100µm SPME fiber (Bicci *et al.* 2002). This larger surface area increases sensitivity as much as a factor of 50-250 (Alves *et al.*2005, Baltussen *et al.* 1999, Salinas *et al.* 2004). Working under the assumption that the partitioning coefficients between PDMS and water are proportional to those for octanol and water (Meylan & Howard 2000), Baltussen *et al.* (1999) extrapolated that the parameter governing analyte recovery from a sample is the ratio of the partitioning constant and the phase ratio between the stir bar PDMS and the aqueous sample. This suggests that compounds with a $K_{O/W} > 500$ are extracted quantitatively onto the physically large PDMS phase on the stir bar, while the smaller PDMS phase on a SPME fiber allows quantitative extraction only for compounds with $K_{O/W} > 10^5$ (Baltussen *et al.* 1999). This theory was illustrated in a comparative study of the two extraction methods, in which SBSE extracted a range of polycyclic aromatic

hydrocarbons from an aqueous solution at similar amounts, while SPME showed a significantly higher sorption of apolar compounds compared to those more polar (Baltussen *et al.* 1999). SBSE has also been shown to be more sensitive than simultaneous steam distillation-extraction (SDE) for the analysis of aroma compounds in grape juice, though reproducibility was somewhat lower (Caven-Quantrill & Buglass 2006).

As with SPME, stir bars used for SBSE are desorbed in the injection port of the gas chromatograph in a rapid, high-temperature step under inert gas flow; while liquid desorption with an organic solvent is possible, thermal desorption ensures the highest sensitivity and eliminates the need for solvent use and disposal (Baltussen *et al.* 1999). SBSE has been successfully coupled with gas chromatography/mass spectrometry (GC/MS) to analyze various volatile compounds in wine (Alves *et al.* 2005, Diez *et al.* 2004, Hayasaka *et al.* 2002, Sandra *et al.* 2001, Zalacain *et al.* 2004). Caven-Quantrill *et al.* (2006) found that peak areas for compounds extracted from a synthetic grape juice solution could be optimized through a 2h extraction at ambient temperature with a stir speed of 1000rpm. However, the proportional sorption of most compounds, discussed above, means that full equilibration of volatile compounds is not required for quantitative analysis.

SBSE has proven to be especially useful in the analysis and identification of trace wine compounds with low sensorial thresholds, like C_{13} norisoprenoids and Maillard reaction products (Alves *et al.* 2005). A comparison of SPME-GC/MS and SBSE-GC/MS suggested that the latter was also more useful in the identification of oak-derived aroma compounds (Alves *et al.* 2005).

1.6. Method weaknesses and considerations

In any analytical process, it is important to consider the shortcomings of chosen procedures and any effect they may have on research findings. In the case of GC analysis, the weaknesses of the sampling or extraction method, the GC separation method, and detection methods must be considered.

1.6.1. Weaknesses of sorptive extraction methods

There are some drawbacks to using SBSE for wine analysis. As the PDMS coating is, in effect, an immobilized liquid phase, it is easily understood that the factors affecting the efficiency of any extraction method- variables such as contact time, efficiency in mixing, pH, and temperature- must also be considered for sorptive extraction (Harmon 2000). Immersion methods present the additional challenge of some non-volatile compounds, such as sugar or polymeric phenols, which may coat the stir bar adsorbent and interfere with desorption; this problem has been identified with SPME fibers (Demyttenaere *et al.* 2003). Rinsing the adsorbent phase in DI water prior to GC analysis generally mitigates the effect. Proteins and other compounds, however, may bind with the adsorbent permanently, and reduce adsorption capacity over time (Prosen & Zupancic-Kralj 1999).

The 12-14% ethanol present in table wines may also hamper analysis (Ebeler 2001); though PDMS selects for non-polar compounds, preliminary work suggests that it is capable of adsorbing ethanol. Ethanol concentrations on the stir bar are high enough to overload the instrument during MS analysis; fortunately, this compound elutes early in

the GC/MS run, allowing practitioners to program a delay and avoid instrument malfunction. Any odorants that co-elute with ethanol, however, are lost.

1.6.2. Weaknesses of GCO/MS

As a combination method, inherent weaknesses of GCO/MS include those found in both component methods. GC/O has been criticized for its subjectivity, even in well-trained panelists; Hanaoka *et al*. (2001) described it as "…a combination of two discontinuous phenomena: the aperiodic and unpredictable elution of odorous compounds from the chromatographic column and the breathing process," and discovered that subject breathing rate influenced both odor detection frequency and intensity. Frederich and Acree (2000) suggest that control of sample preparation, temperature of room and samples, time of day, length and repetition of analyses, repeated reference standardization for panelists, and odor lexicon are all key to reducing experimental error in GC/O work. Panelists have been found to experience fatigue and become less consistent towards the end of GC-O runs lasting longer than 25 minutes; for this reason, long runs should be split into two or more sniffing sessions (Pollien *et al.* 1997, Chaintreau 2002). Other problems include disagreement between panelists, specific anosmias, concentration effects of compounds, and persistent background odors resulting from in-line condensation (Reineccius 2006).

Lacking the uncertainty introduced by human detectors, GC/MS has fewer inherent problems, but care should be taken in interpreting MS results. Ease of operation, and the availability of comprehensive spectra libraries, may lead to compound misidentification by untrained or careless operators; it is therefore essential for all

identifications to be verified with additional data (Reineccius 2006). In addition, when run in full-scan mode for general analysis, quadrupole MS are limited by the amount of time required to make a complete scan, and are therefore less sensitive than in more focused, selected-ion scans (Reineccius 2006).

Finally, the methods employed to combine GC/O and GC/MS raise additional problems. Since the effluent stream must be split to both the MS and the sniff port, the volume of effluent routed to each is reduced; this may result in a panelist perceiving fewer odorants (Reineccius 2006). Splitting a column also requires careful measurement to ensure that odors are perceived by the panelist at approximately the same time a peak is recorded on the MS chromatogram.

1.6.3. General considerations

In addition to the technical or physical limitations listed above, it is key that researchers acknowledge the flaws in the experimental system- in this case, the degree to which the volatiles identified reflect the interactions between wine and consumer that would occur during normal gustation. As indicated previously, the detection limit of instrumental analysis is about 6 times less than that of the human nose, so technological limitations alone restrict unambiguous volatile identification to compounds present in higher concentrations (Friedreich & Acree, 2000). Adding to this handicap, each step of an analytical method modifies the volatile pool in some way, leading to continued debate of optimal protocol.

For solid-phase extraction of odor compounds, the question of submerged vs. headspace sampling of wine volatile analysis has raised some debate. Method choice

hinges largely on the compounds of interest, as both have drawbacks. Headspace sampling has a shorter equilibration time, but lacks the sensitivity of submersion, and generally can be used only to identify main components (Alves *et al.* 2005, Feng & Acree 1999). In contrast, submerged sampling may adsorb some compounds missed in the headspace method, but the high content of ethanol may decrease sorption of minor compounds, and may shorten the lifetime of the solid phase (Alves *et al.* 2005). More to the point, immersion techniques select for odorants in the liquid phase, rather than the headspace (Nongonierma *et al.* 2006). It could be argued that odorants responsible for characteristic wine flavor are those volatile compounds found above the surface of the wine, which are more likely to be sensed by the consumer.

Beyond the submerged vs. headspace issue, the choice of phase coating has a large impact on compounds detected, though the development of combined-phase coatings allows greater choice (Versini *et al.* 2008). The polar/apolar selectivity seen in absorbent phase is magnified by the choice of GC capillary columns, which also exhibit a range of polarity, and may necessitate multiple GC separations on different columns to identify a full range of compounds. As a separation technique, GC inherently hinders a 'true' representation of product aroma; by breaking a wine aroma into component parts and perceiving them individually, compounds are being evaluated "out of context," giving no direct indication of their actual contribution to overall product flavor (Mistry 1997). Further, the elution order of aroma compounds in characterization separations defies randomization, which may result in the contrast effect for closely-spaced volatiles (Mistry 1997, Reineccius 2006).

In short, the same manipulations required to make volatile identification possible change the pool of compounds measured, making an assessment of 'true' aroma profile impossible. While research capabilities constantly improve, at present it must be assumed that the most perceived odorants are often present in measurable quantities, allowing a reasonable approximation of key compounds. Under this assumption, coupled SBSE and GCO/MS represent a combination of precision, sensitivity, durability and ease of use well suited for rapid, reproducible wine volatile analysis.

CHAPTER TWO

Aroma Characterization of Frontenac Wines by Descriptive Analysis

2.1. Acknowledgments

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Wines were donated by Alexis Bailly Vineyards, Hastings, MN, Fieldstone Vineyards, Morgan, MN, Saint Croix Vineyards, Stillwater, MN, and other regional vineyards who wish to remain anonymous.

2.2. Abstract

Frontenac (*Vitis spp.* MN 1047) is a recently introduced, cold-hardy red wine grape that is currently the most-planted grape cultivar in much of the Upper Midwest. Frontenac wines are typically described as having dominant notes of cherry, black currant, plum and spice, but to date, no structured evaluation of common sensory characteristics has been performed. To develop a standard set of descriptors for describing red Frontenac table wines, generic descriptive analysis was performed on six commercially-produced wines. In an effort to identify characteristics common to the grape, wines with different production protocols were sourced from various commercial wineries in Minnesota. Thirteen sensory descriptors were developed and defined with reference standards; correlation plots indicated that attributes were discrete and not

redundant. All 13 attribute descriptors were useful for describing and/or distinguishing between red Frontenac table wines.

2.3. Introduction

The recent expansion of the wine industry into non-traditional northern growing areas is due, in large part, to the increased availability of cold-hardy grape cultivars exhibiting juice chemistry suitable for quality wine production. Frontenac, a cold-hardy red wine grape released by the University of Minnesota's Grape Breeding Program in 1996, is currently the most widely-planted wine grape in Minnesota (Tordsen 2007). An F^1 offspring of Landot 4511 and *Vitis riparia* #89, Frontenac is an interspecific hybrid used to produce rosé, red, and port-style wines. As a relatively new grape, the defining sensory profile, or varietal character, of Frontenac has yet to be determined, though it is popularly described as exhibiting characteristic notes of cherry, black currant, plum and spice. For this work, generic descriptive analysis was performed on six commercially-produced Frontenac table wines, with the objective of determining the varietal character of Frontenac wines and developing a set of descriptors to describe their common aroma characteristics. This sensory vocabulary is the first step towards a common language for describing and discussing the aroma characteristics key to this new variety, and may assist industry members in cold-hardy regions explain their product to consumers. If coupled with volatile analysis and consumer preference testing, these attributes may ultimately allow producers to change processing parameters to better fit their stylistic goals and consumer demand.

Descriptive analysis involves the quantitative characterization of perceived sensory attributes (Stone 1974). Panelists develop a set of descriptors to describe sensory attributes that they are subsequently trained to recognize and rate for intensity. This technique has been used to characterize several single-cultivar or varietal wines, including Cabernet Sauvignon (Heymann & Noble 1987, Heymann & Noble 1989), Chardonnay (Francis *et al.* 1992, Heymann & Noble 1989), Macabeo (de la Presa-Owens & Noble 1995), Parellada (de la Presa-Owens & Noble 1995), Pinot noir (Guinard & Cliff 1987), Sauvignon blanc (Francis *et al.* 1992), Seyval blanc (Andrews *et al.* 1990), Semillon (Francis *et al.* 1992), Shiraz (Abbott *et al.* 1991), Zinfandel (Noble & Shannon 1987), Tannat (Varela & Gámbaro 06), Touriga Nacional (Falque *et al.* 2004), Mencía (Vilanova & Soto 2005), Albariño (Vilanova & Vilariño 2006) and Xarello (de la Presa-Owens & Noble 1995). To date, this descriptive analysis has not been applied to wines produced from the University of Minnesota's cold-hardy cultivars.

2.4. Materials and Methods

2.4.1. Wines

Six commercially produced Frontenac wines were donated by Minnesota wineries, and represent the entire range of commercial red Frontenac table wines available at the time of analysis. Wines were produced in a variety of styles, with fruit from different parts of the state and different processing parameters. Due to the small size of the regional industry, many winemakers requested that wine processing parameters be kept confidential. All wines, however, were at least 85% Frontenac, were processed as red wines, and were two to eight years old at the time of evaluation.

Wines were stored in their original 750mL bottles in a walk-in cooler at 12.8°C for approximately two months after being received from the wineries. Prior to analysis, they were stored in a refrigerator at 4°C for 1 to 4 weeks. All wines were removed and allowed to equilibrate to room temperature (23°C) prior to serving.

2.4.2. Panelists

An eleven-member panel, composed of 7 females and 4 males between 25 and 50 years of age, were selected from a list of volunteer subjects maintained by the University of Minnesota's Sensory Center. Panelists were selected based on availability, frequency of red wine consumption (all consumed red wine at least twice monthly), and lack of familiarity with Frontenac, and were thus thought to be free of the biases and expectations of those familiar with the cultivar. Panelists were screened for the ability to discriminate between two different red wines with a series of triangle tests using a non-vintage Tempranillo, labeled as Silentium (13% alcohol), produced by Bodegas Castillejo de Robledo, Ribera de Duero, Spain, and a 2004 Malbec (13.4% alcohol), produced by Doña Paula los Cardos, Mendoza, Argentina. Wines were chosen by researchers because they had similar levels of perceptible acidity and astringency but different flavor profiles. Candidates were required to successfully identify the different sample in three out of five triangle tests to be included in the analysis panel. IRB approval for use of human subjects was granted for all sensory analyses.

2.4.3. Panelist training

Panelists participated in five training sessions: one session of descriptor generation, three sessions of descriptor training, and one session of odor intensity training. Training sessions lasted one hour, and were held on Tuesday and Wednesday for three consecutive weeks during June and July of 2006. During the first training session, panelists were presented with 60mL of each the six wine samples, blind, in coded, 250 mL ISO tasting glasses (model C66, Libbey Inc, Toledo, OH) topped with watchglasses (ISO 1977). After panelists described each wine using their own terms, a group discussion was held to reach consensus on the descriptors determined to be common to most wine samples, and to group descriptors into families (eg, 'fruity.') As the objective was to determine which attributes were common to all red table wines produced from Frontenac, only descriptors that were found, by group consensus, to exist in at least 60% of the wines were retained.

Table 2.1: Attributes and reference standard formulas for red Frontenac table wine.

Attribute	Reference Standard Formula for 500 mL red wine
Blackberry	30 frozen blackberries, thawed and crushed
Black Currant	75g black currant preserves
Cherry	Unsweetened tart cherry juice (not in wine)
Jammy	50g strawberry jam
Cooked Vegetable	5 mL ea juice from canned asparagus & green beans
Fresh Green	Fresh green beans and asparagus (not in wine)
Cedar	16.5 g cedar shavings soaked in wine 30 min & removed
Spice	Whole Jamaican allspice berries (not in wine)
Black Pepper	5 black peppercorns, crushed, stirred for 5 min
Floral	Rose petals and/or violet pastilles, not in wine
Geranium	50 mL wine with suspected 'geranium taint'
Earthy	50 mL liq from reconstituted dried mushrooms; potting soil (not in wine)
Tamari	San-J brand organic tamari (not in wine)

In a second session, panelists were presented with a subset of the same wines, along with sensory references representing the terms generated in the first session, and additional, related references selected by researchers. Reference compositions found in Noble *et al*. (1987) were used whenever possible; several aroma descriptors, however, varied from those found on the Aroma Wheel. For these aromas, several possible references, as suggested by developed terminology, were offered. After evaluating the wine samples and references individually, a group discussion was held to select the most appropriate reference for each descriptor, and to recommend changes in reference intensity or character. Panelists evaluated the amended references and a subset of Frontenac wines in a third and forth session, and through moderated discussion came to a group consensus on the final set of references required (Table 1).

In a final training session, panelists were asked to familiarize themselves with a 12-point butanol intensity scale (ASTM 1999). They were then given five samples of unknown intensity (12, 6, 4, 8 and 10 on the scale) and asked to identify them. All panelists were able to identify butanol intensity within +/- one scale point.

2.4.4. Descriptive Analysis

In two, 1.5-hour sessions, panelists were asked to rate the intensity of each of the 13 aroma descriptors in the six commercial wines. Panelists were seated in individual sensory booths, and were provided with a full set of sensory references and an abbreviated set of butanol aroma anchors (#3,6,9,12) to reference as needed. Wines were portioned in 60 mL aliquots into 250 mL ISO wine glasses covered with watch glasses, and presented in a Williams Latin Square, balanced for order and carryover effects. Panelists were asked to evaluate wines by sniffing first, then tasting and evaluating

combined retronasal and orthonasal perception. All 13 attributes were evaluated by each panelist in each of the six wines in duplicate, with each panelist receiving all six wines in each of the two evaluation sessions. A 12cm line marking scale, end-anchored and with gradations representing intensity on the butanol scale, on SIMS2000 for Windows software (Sensory Computer Systems, Morristown, NJ) was used to record the intensity rating of each of the 13 sensory attributes.

2.4.5. Statistical analysis

As the goal of the experiment was to determine attributes common to all wines, the binomial response of aroma perceived or not perceived in each wine was calculated for each attribute; a response was considered positive for perception if intensity was rated at level one or above. Attributes were considered to be common to Frontenac wines if a positive response was returned in more than 50% of samples. Descriptor redundancy was assessed with correlation tests performed using R shareware (R Foundation for Statistical Computing, Vienna, Austria), designed to test the correlation of the intensity measures of each attribute with every other attribute. Analysis of Variance (ANOVA) was performed in R for each attribute to determine whether intensity ratings of individual attributes could be used to explain variance between wines. To this end, a split plot model ANOVA was used (Appendix I, p102), with panelists serving as the whole plot and samples as subplots. This model averaged the replicate intensity ratings made for each wine by each panelist, and compared them between each panelist, allowing researchers to disregard panelist variance.

2.5. Results

The DA panel identified 13 aroma attributes common to Frontenac wine character, including four fruity notes, two each floral, spice and vegetative, and one each in the earthy, woody, and caramel categories (Table 2.2). All 13 attributes were perceived more than 70% of the time (Figure 2.1). Correlation analysis showed no significant correlation between any of the attributes, suggesting that all attributes were discrete and non-redundant.

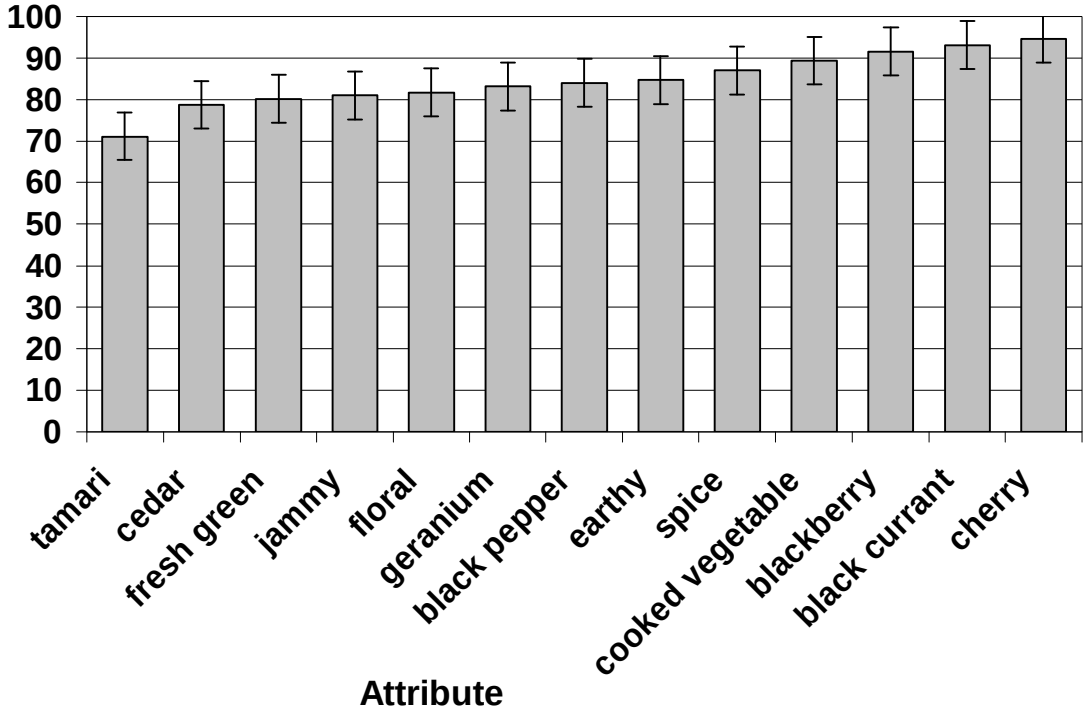
Table 2.2: Aroma attribute descriptors generated by descriptive analysis and Aroma Wheel† categories for red Frontenac wine.

Aroma Wheel† Category	Attribute Descriptor	Attributes Retained in Final Lexicon
Fruity	Blackberry Black Currant Black Cherry Blackberry Jam* Black Currant Jam* Black Cherry Jam*	Blackberry Black Currant Cherry Jammy*
Floral	Rose Violet Geranium	Floral** Geranium
Spicy	Black Pepper Clove Allspice*	Black Pepper Spice**
Vegetative	Canned Green Beans Steamed Artichoke Fresh Green Beans* Fresh Asparagus*	Cooked Vegetable** Fresh Green**
Earthy	Mushroom Humus/Potting Soil*	Earthy**
Woody	Cedar	Cedar
Caramelized	Tamari*	Tamari*
Chemical	Sulfur	--

†*Noble et al., 1987*
**Descriptors not found on wine aroma wheel*
***Second-tier aroma wheel terms; no reference standard formula*

Average intensity ratings for all six wines showed that the 'black currant,' 'cherry,' and 'cooked vegetable' notes were the most intense, with scores of 5.9, 5.5, and 5.7 out of 12 intensity units, respectively (Figure 2.2). Examination of average intensity scores for individual wines suggests that black currant and cherry were perceived as the most intense attributes in four of the wines, and cooked vegetable in the other two (Figure 2.3).

Figure 2.1: Percent perception of sensory attributes in red Frontenac table wines.*

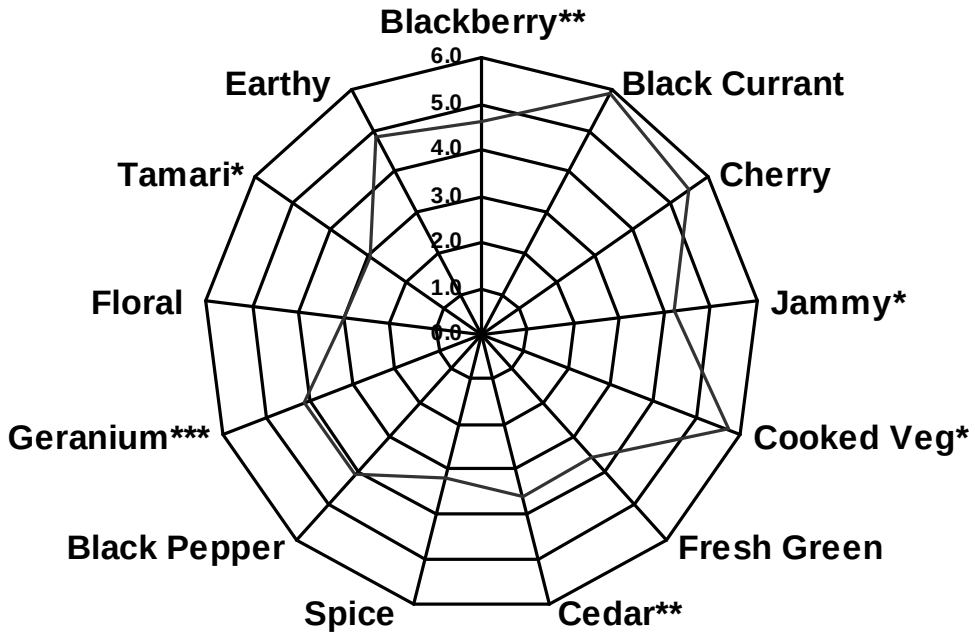


**Error bars indicate 95% confidence interval.*

The split-plot ANOVA indicated that the attributes geranium, cedar, blackberry, jammy, cooked vegetable and tamari all showed significant intensity variation between wine samples, at levels of at least $p<0.05$. Attributes 'black currant,' 'fresh green,' 'black

pepper,' 'spice,' 'cherry,' 'earthy,' and 'floral' showed no significant difference between wines (Figure 2.2).

Figure 2.2: Average intensity ratings on a 12-point scale of attributes across six red Frontenac table wines analyzed by Descriptive Analysis.



*** *Indicates p<0.001in split-plot ANOVA*
** *Indicates p<0.01*
* *Indicates p<0.05*

2.6. Discussion

2.6.1. Attribute development

The sensory panel originally generated a list of 21 attributes, all of which fit into the primary aroma categories in the Wine Aroma Wheel (Noble *et al.*, 1987) (Table 2). In the first session, there was some panelist disagreement on the appropriate identification of the red fruit notes perceived in the wines, so several standard fruity references beyond those proposed by the panel were offered for consideration during session two. Eight

descriptors in the original list (blackberry jam, black currant jam, black cherry jam, allspice, fresh green beans, fresh asparagus, potting soil, and tamari) were not found on the aroma wheel, requiring the formulation of original reference standards. Panelists were encouraged to comment on standard strength and character, and minor changes were subsequently made to several formulas. In the final version, formulas for blackberry and geranium were made stronger, and the Aroma Wheel rose standard of 2-phenylalcohol in wine was rejected for fresh rose petals without wine. In addition, several standards were evaluated and rejected before consensus was reached on an acceptable 'earthy' standard.

After panel discussion, redundant and synonymous terms were discarded or redefined. Ultimately, blackberry jam, black currant jam, and black cherry jam were determined to be one attribute ('jammy') and the cooked/canned vegetable descriptors were combined to produce a the more generic 'cooked vegetable' attribute. Three sets of specific terms that caused contention among panelists were combined to create more general attributes, allowing greater panel agreement. This group included fresh green beans and fresh asparagus, which were paired as 'fresh green', the rose and violet descriptors ('floral') and mushroom and potting soil ('earthy'). The use of two references is sometimes necessary with attributes that cannot be easily represented with a single aroma (Rainey 1986), and may be especially necessary in an effort to develop a list of common characteristics, rather than those representing product differences. In addition, the clove descriptor was determined to be synonymous with spice, and allspice berries were deemed to better represent this character in the wine. Sulfur was discarded when it

was not perceived in any further sensory sessions, as it was presumed to be an isolated fault in one sample wine.

Of the 13 aroma attributes the panel ultimately accepted, seven required the formulation of unique sensory reference standards (Table 2). Five attributes-'spice,' 'cooked vegetable,' 'fresh green,' 'floral,' and 'earthy'- are second-tier Aroma Wheel terms, and as such do not have standard reference formulas. Two additional attributes, 'jammy' and 'tamari,' do not appear on the aroma wheel, but standard formulas were extrapolated from the similar notes of 'strawberry jam' and 'soy sauce' (Noble *et al.*, 1987). While only specific, third-tier terms are represented by reference formulas by Noble *et al.* (1987), it is understood within the industry that more general (i.e., second-tier) terms, and even combined terms, may be required to appropriately describe some wine aromas (Noble *et al.* 1984).

2.6.2. Validation of attributes

During intensity rating, all 13 aroma attributes were perceived in wines more than 70% of the time, well above the designated significance level of 50%. In correlation plots constructed for all attributes, correlation values were less than 0.5 and were not statistically significant, indicating that panelists were using each descriptor independently. The attributes 'black pepper' and 'fresh green' had the highest correlation, at 0.46; this may indicate that these two attributes tend to occur at higher intensities in the same types of wine, perhaps as a result of harvest or processing parameters. This relationship was difficult to determine, however, as no significant difference in intensity was shown for either attribute in the six wines evaluated.

2.6.3. Describing and distinguishing between Frontenac wines

The relatively high intensity ratings and frequency of detection evinced for the 'cherry,' 'black currant' and 'cooked green' notes suggest that these three attributes may be important in defining Frontenac typicity. The 'cherry' and 'black currant' attributes coincide well with empirical consumer data, where Frontenac is generally described as having notes of cherry, berry, and plum (Reisch and Henick-Kling 1997, Leahy 2007). The plum note, being less distinct than the other two, may be used in consumer literature to indicate a general fruitiness. While the 'cooked vegetable' note is not mentioned in winery descriptions of Frontenac wines, related notes like 'green bean' and 'grass' have been cited in industry tasting panels (Mansfield 2004). As herbaceous notes are often seen as a flaw, the result of either the 'hybrid' character of non-*vinfera* wines or of processing underripe fruit, it is likely that advertising and consumer groups would not promote this aspect of the sensory profile, even if commonly found in Frontenac wines. As a means of distinguishing between wines, the 'cooked vegetable' attribute was the only one of these three characteristics to be useful, showing significant, if slight, intensity differences between samples. This variability may be caused by differences in harvest and processing parameters, which are thought to impact compounds that can contribute green notes to wines, as discussed below.

The lack of significant variance between wines in the intensity of more than half of the attributes means that this descriptor list, while useful in describing the commonalities of Frontenac wines, is not particularly useful in distinguishing between individual wine samples. This is not surprising, as attributes that were not common to all wines- i.e., those that may have been very intense in some wines but completely absent in

others- were discarded early in the panel discussion. By eliminating attributes that explained obvious differences, the list of descriptors necessarily became less useful for distinguishing between wines. Of the attributes that did show significant intensity variation between wines, three notes showed fairly little variation, at $p< 0.05$. Only three attributes varied significantly at $p<0.01$, with the 'geranium' note showing the most significant variance between wines. The lack of variation in seven of the 13 attributes seems to suggest that these notes occur at similar intensity in all wine samples regardless of processing methods. Attributes showing greater variation may be contributed by volatile compounds impacted by various growing conditions or processing parameters.

2.6.4. Sources of wine aroma

In a complex matrix such as wine, an aroma perceived and described by a panel may be the result of a unique combination of volatile compounds, rather than denoting individual compounds; this can make the identification of characterizing volatile compounds difficult. While such analysis was beyond the goals of the present study, some general observations about possible aroma origins can be made.

The cherry, blackberry, black currant and jammy notes found in red Frontenac wine are predictable; fruity-smelling esters are very common in wines, especially young wines, where short-chain, low molecular weight acetates are numerous (Vernin ***et al.*** 1986). Analysis of eight red Frontenac table wines, including five used for descriptive analysis, by GCO-MS identified fifteen esters, many of which were described as having fruity or sweet aromas (Chap. 3, p108). As all but two of the wines were less than two years old at the time of evaluation, it is probable that fruity esters impacted wine aroma to

a large degree, as few of the degradation reactions occurring during wine aging would have been in effect. ß-Damascenone, a norisoprenoid derived from the grape, may also contribute fruity or floral notes to wine (Ebeler, 2001), though this compound was not identified as odor-active in the GCO-MS analysis. Both the fermentation-derived acetate esters and grape-derived ß-damascenone are common to all wine types, so neither of these compounds adequately explain the dominance of cherry as a varietal note of Frontenac wines. The current understanding of wine ester formation is limited, however, and grape-derived precursors may strongly influence the final composition and concentration of these compounds.

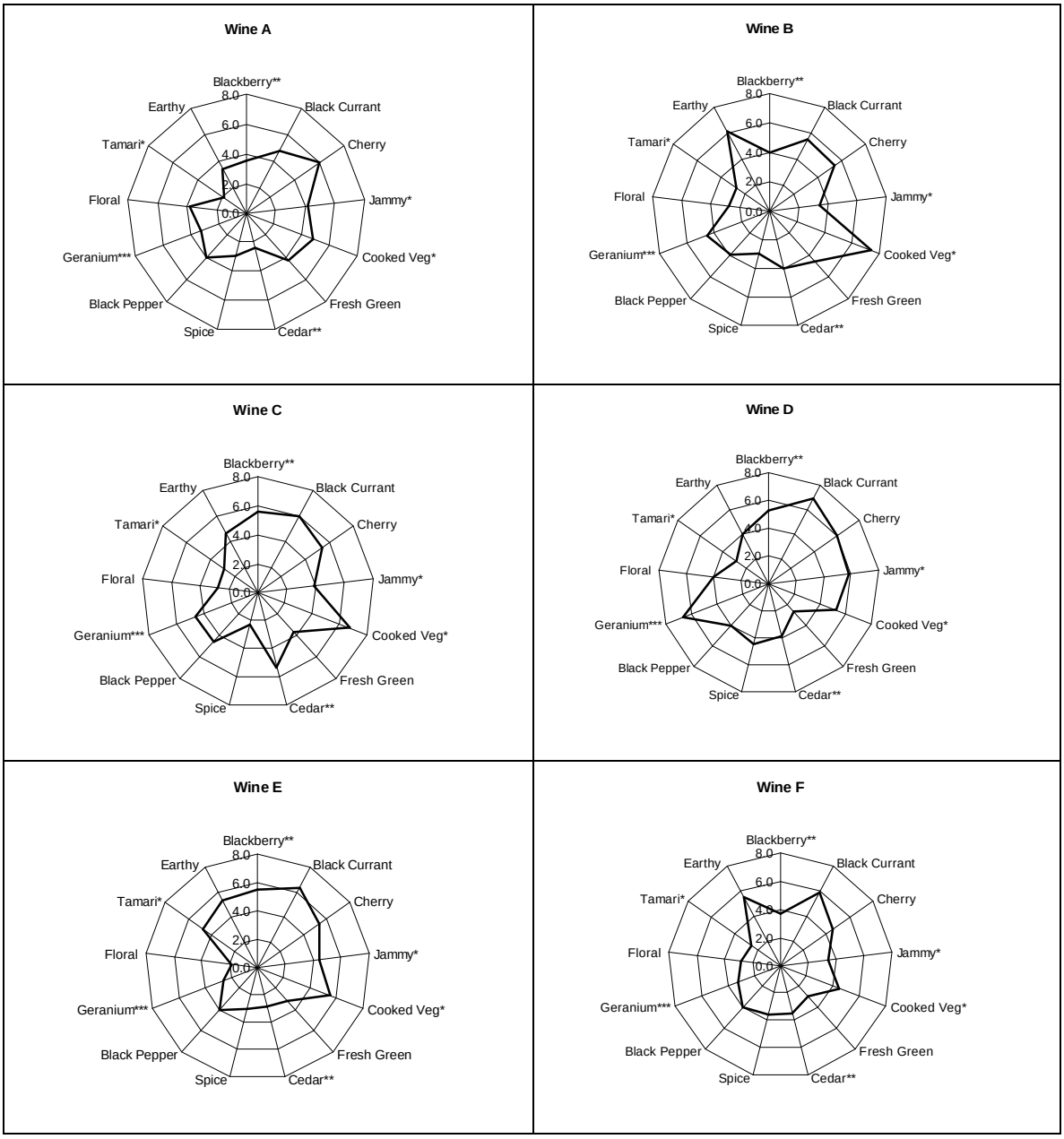
The black currant note common in Frontenac wines has also been described in wines produced from some *V. vinifera* species. In some cases, it has been shown to result from the presence of a family of volatile thiols, which are released from a grape-derived precursor at varying concentrations dictated by fermentation conditions and yeast strain (Delfini *et al.*, 2001). It is unknown whether these precursors are found in *V. riparia*-based winegrape hybrids, and volatile thiols were not identified as impact odorants in GCO-MS analysis.

Both the relatively intense cooked green note and the slightly less pronounced fresh green attribute may be components of the herbaceous 'hybrid character' commonly described in wines produced from non-*vinifera* grapes. Green or herbaceous notes in wines may arise from a variety of mechanisms. Grape-derived C_6 alcohols 1-hexanol, (*Z*)-3-hexenol, 2-hexanol and the related aldehydes hexanal and (E)-2-hexenal may contribute herbaceous notes to wines, though they are somewhat degraded by yeast during fermentation (Kotseridis & Baumes 2000). Another class of grape-derived

compounds, the methoxypyrazines, are associated with the characteristic herbaceous notes found in such *V. vinifera* cultivars as Cabernet Sauvignon and Sauvignon blanc, and may contribute green notes in other grapes if ripening is insufficient (Ebeler 2001, Sala *et al.* 2005). GCO-MS analysis identified three compounds that may be related to these green attributes. These include 1-hexanol, which can be grape- or fermentation derived, the fermentation-derived ethyl ester of 2-methylbutanoic acid, and methyl salicylate. The latter is of particular interest, as it is fairly uncommon in *V. vinifera* wines, but has been identified in *V. riparia* grapes (Schreier and Paroschy, 1980). Methyl salicylate is generally described as having an aroma of mint or wintergreen, and if present above threshold levels, may contribute to the unique green notes found in Frontenac wines.

Of the less intensely perceived attributes, the 'blackberry,' 'jammy,' and 'floral' notes may also be characteristic of esters. Ester type and concentration may vary widely based on precursors and yeast strain, making variation among wines with different production protocols inevitable. An increased perception of jammy fruit has also been reported in wines produced from Cabernet Sauvignon grapes allowed extended hang time prior to harvest (Bisson, 2001), suggesting that the extent of grape maturation may influence the fruit character in the final wine. Perception of fruity and floral notes produced by esters may also decrease as wines age, though there was little evidence of this effect in this study. Wines B and D were 4 and 8 years past vintage date at the time of sensory evaluation, and all other wines 2 years or less, but wine D showed the highest average intensity scores in 'cherry,' 'black currant' and 'jammy' attributes (Figure 3).

Figure 2.3: Average intensity ratings of attributes for six red Frontenac table wines analyzed by Descriptive Analysis.



*** *Indicates p<0.001*
** *Indicates p<0.01*
* *Indicates p<0.05*

The 'cedar' and 'spice' attributes are generally attributed to volatile phenols extracted during oak aging (Chatonnet, 1990), though similar notes may exist in wines without oak contact (Ferriera 2000). The lack of significant variation in intensity of the

'spice' attribute may imply that it originates from the grape rather than from oak extraction. In either case, eugenol, a volatile phenol with a smoky, spicy or clove aroma and extracted from oak products, was identified via GCO-MS analysis in Frontenac wines. The cedar notes do show higher intensity in wines B, C, and D at 4.0, 5.3, and 3.9, respectively. Wines B and D are the oldest sample wines used, and B,C, and D were known to be oak aged to some extent. 'Black pepper,' the other attribute in this family, has been described in some *V. vinifera* species, and the sesquiterpene rotundone was recently identified as a key impact odorant in both black peppercorns and Shiraz, where it contributed a black pepper or spicy note (Wood *et al.*, 2008). The presence of rotundone in Frontenac grapes or wines is currently unknown.

One attribute of particular interest is the 'geranium' note, which showed the most significant intensity variation between wines. In wine, this aroma usually implies the presence of 2-ethoxyhexa-3,5-diene, an intense aroma flaw produced by the metabolism of the antimicrobial additive potassium sorbate by lactic acid bacteria (Crowell and Guymon, 1975). As this compound has not previously been identified in wines without sorbate additions, it is widely accepted as an indicator of microbial spoilage in sorbate-treated wines, and is considered a technical flaw. While analysis with GC-MS did not indicate the presence of 2-ethoxyhexa-3,5-diene in the sample wines, this ether has a very low odor threshold and may be detected by the human nose at concentrations too low for instrumental analysis. It is, however, interesting to note that neither sorbic acid nor ethyl sorbate, an ester found in all wines treated with sorbate, was found in four of the wines, but were present in wines C and D, with intensity ratings of 4.6 and 6.6, respectively. The presence of ethyl sorbate clearly indicates that these two wines were treated with

sorbate, and as such had the potential to develop 2-ethoxyhexa-3,5-diene and the subsequent 'geranium taint.' It must also be noted, however, that wine B, which showed no evidence of sorbate addition, also earned a 'geranium' intensity rating of 4.5. Further investigation is needed to determine whether other precursors to 2-ethoxyhexa-3,5-diene exist in Frontenac wines, if this compound is indeed responsible for the aroma described, or if a geranium note may be inherent to the cultivar and stem from other volatile constituents.

2.7. Conclusions

A sensory lexicon was developed to describe aroma attributes common to red table wines produced from the new, cold-hardy winegrape cultivar Frontenac. Thirteen attributes were defined with sensory references standards, seven of which required the development of new reference formulae. Correlation plots indicated that attributes were not redundant. Seven attributes were not found to vary significantly in intensity among the wines evaluated, and subsequently were not useful for distinguishing between samples in this study. Intensity of the 'geranium' attribute showed the most significant variation between wines, but it is unknown whether this note indicated the presence of the wine flaw 2-ethoxyhexa-3,5-diene, or was contributed by other volatile compounds.

CHAPTER THREE

Identification of Odor-Impact Compounds in Red Table Wines Produced from Frontenac Grapes

3.1. Abstract

Frontenac (*Vitis spp.*) is a recently introduced, cold-hardy red wine grape that is currently the most-planted cultivar in much of the Upper Midwest. Though typically described as having notes of cherry, black currant and spice, the volatile compounds responsible for the characteristic sensory notes of Frontenac wine have not been investigated. In order to identify these odor active compounds, eight Frontenac table wines were evaluated using stir bar sorptive extraction (SBSE) combined with concurrent gas chromatography-olfactometry/mass spectrometry (GCO/MS). Eight panelists evaluated GCO effluent using qualitative detection frequency analysis. Twenty-four volatiles perceived by panelists were identified, including five alcohols, fourteen esters, one lactone, two acids and two volatile phenols. Twenty-three of these were confirmed by Linear Retention Index (LRI) data in separate GC/MS analyses, and 23 were quantified in runs using a known concentration of internal standard. Similar analyses of wines produced from *V. riparia* clone #89, a parent of Frontenac, found 16 volatiles common to Frontenac wines. A brief study of Frontenac juice with two days of skin contact suggested that four volatiles found in the wine may originate in the fruit.

3.2. Introduction

In 1996, the University of Minnesota breeding program released Frontenac (*Vitis spp.*), a red wine grape arising from a cross of the direct producer cultivar Landot 4511 (Landal L.244 X Villard blanc) and *Vitis riparia* clone #89, found growing wild near Jordan, MN (Luby & Hemstad 2006.) Due to its extreme cold-hardiness and suitability for wine production, Frontenac is used to produce rosés, port-style dessert wines, and red table wines in the Upper Midwest and other cold-climate growing areas. Frontenac is currently the most-planted grape cultivar in Minnesota, with a reported 34,260 vines making up 20% of the total vineyard plantings in 2007 (Tordsen *et al.* 2007).

As an interspecific hybrid and F_1 progeny of *V. riparia*, Frontenac produces wines with unique sensory characteristics. While key volatile compounds in fruit from one *V. riparia* cultivar have been identified (Schreier & Paroschy 1980), the vast number and variation among *V. riparia* cultivars (Pierquet 1977) make any comparisons tenuous. Wines produced from Frontenac are commonly described as having notes of cherry, black currant, and a spicy or herbaceous character, and similar attributes were used during descriptive analysis (Ch. 2). Little work has been done to characterize the volatile compounds responsible for these characteristics.

From a quantitative standpoint, fermentation-derived volatiles such as fusel alcohols, fatty acids and esters constitute the largest part of wine aroma (Montedoro & Bertuccioli 1986, Scharpf *et al.* 1986). As a means of expressing varietal character or cultivar typicity, however, this group of odor-active compounds has little impact, as these components are similar to all fermented beverages and contribute little to differentiate between different wine types (Ebeler, 2001). While representing a significantly smaller

proportion of the overall pool of volatiles, volatiles and volatile precursors derived from the grape are thought to produce distinctive differences among wines produced from different grape cultivars (Noble, 1986; Nykänen, 1986). In a few cases, varietal character is defined by a single compound, or a small collection of them; the concentration of a handful of monoterpenes in aromatic grapes, for instance, have been found to correlate directly with flavor intensity in wine, and can be used to distinguish between six aromatic winegrape cultivars (Strauss *et al.*, 1986). For many cultivars, however, varietal character is more elusive, dictated by larger groups of compounds occurring in ratios unique to each grape. Delineating these interactions is more difficult than identifying one or a few distinctive compounds, and subsequently much less is known about the relationships between volatile compounds that define the so-called 'neutral' cultivars. Further, processing parameters such as harvest date, skin contact time, yeast strain, secondary fermentation and aging may alter or mask grape-derived components (Noble 1986; Ebeler 2001). For these reasons, defining varietal character in such cultivars is a difficult task.

As volatile constituents account for only about 0.1% of the total wine matrix, and individual compounds are often present at concentrations lower than 1µg/L (Rapp 1998), identification of perceived impact odorants in wine requires use of the most sensitive analytical methods. In addition, preliminary extraction and concentration of volatiles may be required. Stir bar sorptive extraction (SBSE), a recent development in sorbent methodology, employs a magnetic stir bar coated with polydimethylsiloxane (PDMS) to adsorb and release volatile compounds from complex matrices. SBSE has been successfully coupled with gas chromatography/mass spectrometry (GC/MS) to analyze

various volatile compounds in wine (Alves *et al.* 2005, Diez *et al.* 2004, Hayasaka *et al.* 2002, Sandra *et al.* 2001, Zalacain *et al.* 2004), and has proven to be especially useful in the analysis and identification of trace wine compounds with low sensory thresholds, like C_{13} norisoprenoids and Maillard reaction products (Alves *et al.* 2005). It is hypothesized that the pool of perceptible volatile compounds in red Frontenac table wines will be qualitatively and quantitatively similar to those identified in other monovarietal wine types. For this work, volatile extraction with SBSE has been coupled with concurrent GC/MS and gas chromatography/olfactometry (GCO) to identify perceived odorants in red Frontenac table wines. This combined approach allows easy comparison of perceived aromas with volatile compounds, reducing the time necessary for peak identification and comparison, and decreasing risk of improper compound/odor correlation by eliminating the need for separate GC/MS and GC/O runs.

3.3. Materials and Methods

3.3.1. Wines

To determine volatile compounds common to Frontenac, a selection of eight wines, from various years and producers, were obtained from commercial vineyards and from the University of Minnesota Research Winery (Table 3.1). Since the object of this study was to determine volatile compounds and sensory descriptors that are common to wines produced from regionally-grown Frontenac grapes, an effort was made to acquire wines grown and produced by different wineries and using different production methods, rather than controlling processing variables.

In addition to the Frontenac table wines, two wines produced from *V. riparia* clone #89 (2004 & 2005), and one Frontenac juice sample (2006) were sourced from the University of Minnesota Research Winery. To produce the *V. riparia* wines, grapes were harvested, crushed and destemmed, and the must inoculated with Pasteur Red yeast (Red Star, Cedar Rapids, IA) and fermented on the skins for five days at ambient temperature before pressing. Wine production then proceeded as in Luby *et al* (2006). For the Frontenac juice sample, grapes were crushed and destemmed, then underwent cold soak on the skins for 48 hours at 2°C prior to pressing and sampling.

Table 3.1: Chemical parameters of red Frontenac wines evaluated by gas chromatography-olfactometry/mass spectrometry.

ID	Vintage	pH	TA (g/L)	% Ethanol	Malolactic fermentation	Oak Aging
A†	2004*	3.63	8.02	13.4	yes	yes
B†	2005*	3.10	7.05	13.2	unknown	unknown
C†	1998	3.66	7.40	13.1	yes	yes
D†	2004*	3.52	7.67	12.0	unknown	unknown
E†	2004*	3.53	6.81	12.2	yes	yes
F†	2002	3.70	8.42	12.0	yes	yes
G	2004	3.23	10.57	13.7	yes	no
H	2004	3.29	11.17	13.2	yes	no

† *All analyses performed after storage at -20°C*
** Labeled as non-vintage but harvested during the season reported*

Commercial wines were purchased in 750 mL glass bottles sealed with natural corks. Research *V. riparia* wines were procured in 375 mL glass bottles sealed with natural corks. Prior to analysis, wines were divided into 20mL aliquots, placed into clean, nitrogen-sparged screw-cap 20mL vials and held at -29°C until needed. Frontenac juice was divided into 20 mL screw-cap vials directly after pressing and stored under the same conditions.

3.3.2. Extraction method

Wines or juice samples were removed from cold storage and allowed to rest in sealed storage vials until reaching ambient temperature, approx. 21°C. A 10mm magnetic stir bar coated with polydimethylsiloxane (PDMS) (Gerstel, Baltimore, MD), termed a Twister™, was added. The vial was capped to exclude additional air, and placed on a magnetic stir plate. The sample was stirred at 200 rpm for 90 min, allowing volatiles to reach equilibrium with the Twister™ absorbent. The Twister™ was then rinsed with distilled water to remove any wine, patted dry with lint-free tissue, and used for volatile analysis.

3.3.3. Gas Chromatography Olfactometry/Mass Spectrometry

GCO/MS analysis was performed on an HP 5890 Series II gas chromatograph (Hewlett-Packard, Palo Alto, CA) modified to allow concurrent olfactometric and mass spectrometric analysis on an HP 5970 MS (Hewlett-Packard, Palo Alto, CA). A Twister™, loaded with wine volatiles as described above, was inserted into a split/splitless GC liner plugged with glass wool and sealed with a flip-top injection port lid (Agilent, Palo Alto, CA; part # 5188-2717). The Twister™ was desorbed at 250°C for five min with cryofocussing via a loop of column submerged in a Dewar of liquid nitrogen. Volatiles were then loaded onto a DB5-MS column, 30m × 0.25 mm × 25 mm (J&W, Folsom, CA) with a 30:1 split flow. The oven temperature started at 50°C, was held for two min, and ramped at 10°C/min to 250°C and held five min.

Via a splitter at the end of the column, equivalent portions of the GC effluent were routed to an MS detector and a sniff port, held at 270°C and 250°C, respectively.

The mass spectrometer was operated at 70eV in scan mode, scanning the range *m/z* 29 to 300 at 2.9 scans/second. In the sniff port, the GC column effluent was combined with humidified air to increase olfactory perception by reducing nasal passage dryness and fatigue. Panelists were seated in front of the sniff port while evaluating, and were asked to write descriptors of aromas perceived while simultaneously pressing a button to record the presence of an odor.

3.3.4. Panelist selection and GCO analysis

Eight panelists, 5 females and 3 males, between 22 and 30 years of age, were selected from the students and staff of the University of Minnesota Flavor Laboratory. Panelists were selected based on availability, previous experience with GCO analysis, and lack of known anosmia. During each GC run, panelists were asked to breathe normally, noting the time that they perceived an aroma, and giving a description of the aroma if possible. Panelists sniffed continuously throughout the first 22 minutes of the GCO run, as preliminary trials suggested that no odors were perceived after the first 20 minutes.

Each GCO sniff run was considered an experimental unit, and a positive or negative binary response (i.e., each compound was perceived or not perceived at a certain time) was returned for each compound of interest. Eight panelists evaluated eight wines in a Randomized Complete Block (RCB) design, resulting in a sample size of 64. Panelists did not perform wine analyses in duplicate because the discontinuous nature of human respiration makes true replication of GCO analysis improbable, at best (Hanaoka *et al.*, 2001).

3.3.5. Identification of odor-active compounds

Compounds perceived in at least 50% of the GCO evaluations were identified when possible. Tentative identifications were made through comparison of MS fragmentation patterns with the Wiley 275 Mass Spectra Library. To verify identification, Frontenac wine samples A and G were spiked with a hydrocarbon ladder (C_5-C_{16} in pentane) at 1ppm, and extracted and analyzed via GC-MS in the same manner as the wine samples above (Figure 3.1). Linear Retention Indices (LRIs) were calculated and compared with previously reported Kovats index (KI) values (Kovats 1965).

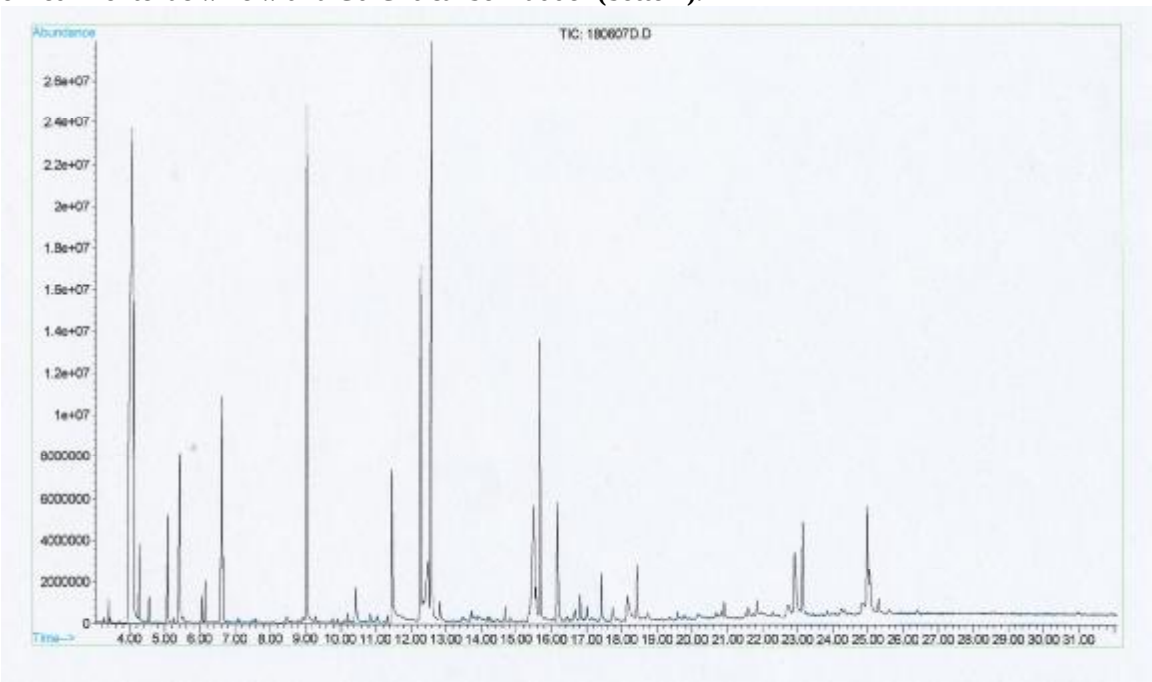
3.3.6. Quantification of odor-active compounds

Approximate concentrations of volatile compounds were determined by making peak area comparisons with the area of a known internal standard. A solution of 2-heptanone in ethanol was added to each of the eight sample wines at a concentration of 6ppm, and the samples were analyzed via GC/MS in the manner described above.

3.4. Results

Eighteen odor regions, i.e. those perceived in more than 50% of the GCO runs, were found in Frontenac wines (Table 3.2). In 16 of these regions, 24 volatile compounds were tentatively identified by comparing MS-derived spectral data with spectra libraries. These included five alcohols, fourteen esters, one lactone, two acids

Figure 3.1: Chromatogram of red Frontenac table wine analyzed via SBSE and GCO/MS (top) and of red Frontenac wine with a C6-C16 carbon ladder (bottom).



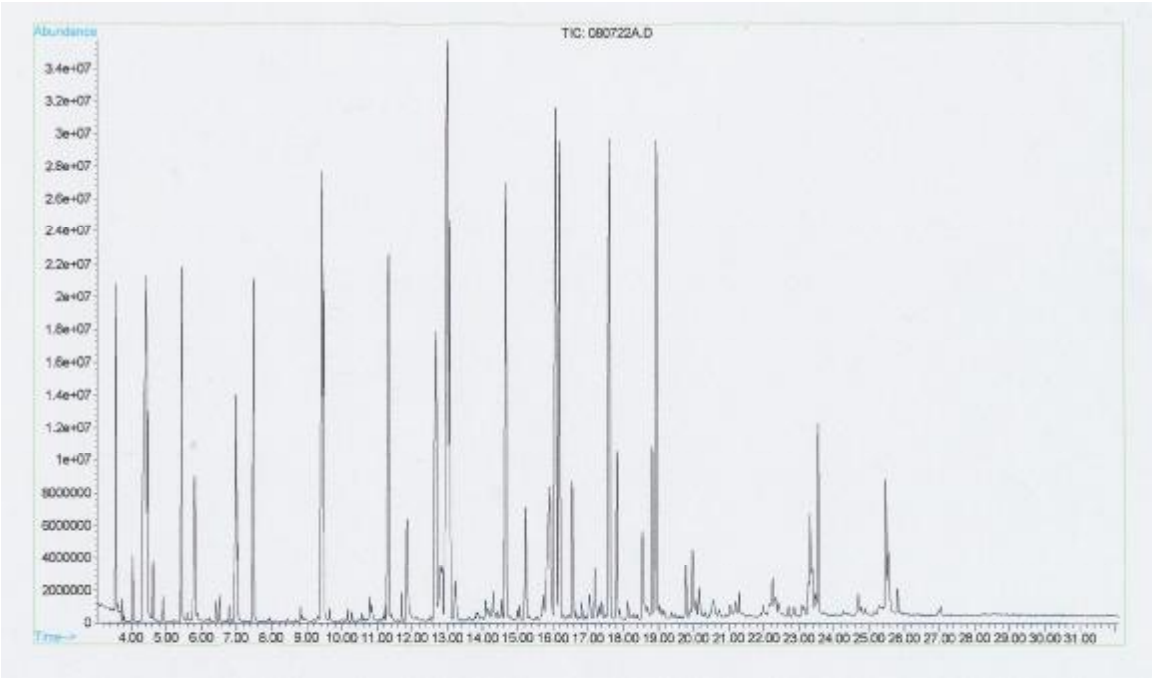


Table 3.2: Odor regions and the associated descriptors for GCO analysis of red Frontenac table wines.

Odor region LRI range	% Frequency of Detection (n=64)	Odor Description
746-754	64	Chemical, solvent, toasted, brothy, green
755-766	95	Citrus, fruity, tropical fruit
800-810	74	Strawberry, artificial fruit, candy-like
822-825	86	Solvent, plastic, nutty, fresh
849-856	92	Green, earthy, fruity, jammy, apple
878-885	84	Grainy, vitamins, fresh nuts, banana, candy
921-929	53	Tropical fruit, fresh, solvent, nutty, cream
991-1002	83	Tropical fruit, bubble gum, nail polish
1080-1089	56	Caramel, fresh bread, toasted
1101-1107	69	Oxidized, very green, leafy, bitter
1107-1118	58	Strong floral, earthy, smoky, burnt, green
1131-1141	81	Floral, wine-like, sweet
1182-1191	78	Tropical fruit, floral, medicinal, waxy
1199-1210	50	Tropical fruit, strong floral, green, minty, fresh
1361-1371	53	Tropical fruit, artificial fruit, spicy, clove
1380-1392	64	Waxy, earthy, weak fruit, barnlike
1393-1406	77	Fruit juice, spice
1471-1482	64	Tea, fresh floral, perfume

and two phenols (Table 3.3). LRI comparisons confirmed the identity of 23 of the 24 volatile compounds tentatively identified via MS. Estimated volatile concentrations were calculated and are presented in Table 3.4.

Nine odor regions contained single aroma compounds. An additional three regions were comprised of structurally similar pairs of closely eluting peaks, namely 2- and 3-methyl-1-butanol (LRI 746 to 754), ethyl-2- and ethyl-3-methylbutyrate (LRI 849 to 856), and 2- and 3-methylbutyl acetate (LRI 878 to 885). Four additional regions encompassed non-related but closely eluting compounds pairs with differing aroma characteristics. Aromas described in two odor regions could not be assigned to volatile compounds. In the region between LRI 1080 to 1089, no identifiable peaks were found.

Two odor regions, those occurring between LRI 1101 to 1107 and between 1107 to 1118, were found to actually be one large region, consisting of three to four large, overlapping peaks, which made separation and identification of individual compounds difficult.

Fifteen volatiles identified in Frontenac wines were also found during GCO/MS analysis in wines produced from two vintages of *V. riparia* #89 (Table 3.4); compound identification was confirmed with LRI calculations. Four volatile compounds identified in Frontenac wines were tentatively identified in the Frontenac juice sample via GC/MS screening (Table 3.4).

3.5. Discussion

The primary objective of this work was to identify the chemical compounds responsible for the aroma of red Frontenac table wines. This goal is complex, as the aroma profile in the finished wine is dictated not only by the quantity and concentration of individual volatiles, but also the synergistic relationships that exist between them, and between volatiles and non-volatile compounds in the wine matrix (Ferreira *et al.* 2000). Regardless, determining which volatile compounds can be perceived by panelists is a first step in developing a clear picture of key impact odorants in Frontenac.

3.5.1. Identifying volatile compounds in odor-active regions

In practical terms, the compounds of interest to Frontenac varietal character are those which can be perceived by the general population. By definition, those volatiles which can be perceived by the 'average' consumer are those perceived by approximately 50% of the population. Assuming that the panelists used are unfamiliar with the product

Table 3.3: Qualitative listing of volatiles identified in GCO odor regions of red Frontenac table wines.

Odor Region LRI range	RI (DB5)	Compound Name	Panelist Descriptors
746-754	745	Isoamyl alcohol	Chemical, solvent
	750	2-Methyl-1-butanol	Toasted, brothy, green
755-766	757	Ethyl Isobutyrate	Citrus, fruity, tropical fruit
800-810	800	Ethylbutanoate	Strawberry, artificial fruit, candylike
822-825	818	Ethyl lactate	Solvent, plastic, nutty, fresh
849-856	848	Ethyl-2-methylbutyrate	Green, earthy
	852	Ethyl-3-methylbutyrate	Fruity, jammy, apple
878-885	875	1-Hexanol	Grainy, vitamins, fresh nuts
	876	Isoamyl acetate	Banana
	878	2-methylbutyl acetate	Banana candy
921-929	921	γ-Butyrolactone*	Solvent, nutty, creamy
	923	Methyl hexanoate	Tropical fruit, fresh
991-1002	998	Ethyl hexanoate	Tropical fruit, bubble gum, nail polish
1080-1089	--	Unknown compound	Caramel, fresh bread, toasted
1101-1118	1106	Linalool	Strong floral
	--	2-3 Unknown compounds	Earthy, smoky, green, leafy, oxidized
1131-1141	1131	Phenethyl alcohol	Floral, wine-like, sweet
1182-1191	1178	Diethyl succinate	Tropical fruit, floral
	1187	Octanoic acid	Medicinal, waxy
1199-1210	1196	Ethyl octanoate	Tropical fruit, strong floral
	1210	Methyl salicylate	Green, minty, fresh
1361-1371	1360	Ethyl dihydrocinnamate	Tropical fruit, artificial fruit
	1370	Eugenol	Spicy, clove
1380-1392	1382	Decanoic acid	Waxy, earthy, weak fruit, barnlike
1393-1406	1393	Ethyl decanoate	Fruit juice, spice
1471-1482	1476	Ethyl cinnamate	Tea, floral, perfume

* *Tentatively identified via GC/MS; not confirmed with LRI comparison.*

and represent the normal range of sensory ability, those volatiles sensed by the 'average' consumer will also perceived by 50% of the GCO panelists. As explained above, panel size was calculated to ensure that these results were significant at a P' of 0.90.

One difficulty lay in determining how to tabulate the number of responses for a given odor; slight variability in compound elution time and panelist response meant that

the recorded time of individual odor perception was not perfectly aligned. To compensate for these differences, response times for each wine were normalized to the odor region around isoamyl acetate, which eluted consistently around 6.60 min and was the compound most uniformly described by panelists as some variant of 'banana.' This realignment revealed that panelists were signaling perception of what appeared to be the same odor at times which varied as much as 0.15 min, while examination of chromatograms showed that peak elution time varied by as much as 0.10 min. Subsequently, it was decided that percent odor perception would be tabulated based on a span of around 0.20 min, as long as compound descriptors could be grouped in one, or at most two, distinctive general character families, such as 'fruity' or 'herbaceous.' In cases where significant odor regions were later determined to encompass two or three aroma compounds, all were retained as significant.

Ultimately, 23 volatile compounds could be positively identified in 17 odor regions. Of these, six potentially originated from the grape, surviving fermentation unaltered to exist in the finished wine. An additional eight compounds may be derived from grape-synthesized precursors, the final concentrations of which may be dictated, in part, by cultivar.

3.5.2. Grape-derived volatiles

Assuming that grape-derived volatiles are of greatest importance to varietal character, one part of defining typicity is identifying odor-active compounds which originate in the fruit and survive fermentation intact. A comparison of odor-active volatiles identified in Frontenac wines and those tentatively identified in Frontenac juice

indicates that four alcohols, 2- and 3-methyl-1-butanol, 1-hexanol, and phenethyl alcohol, may originate in the grape (Table 4). Fusel alcohols 2- and 3-methyl-1-butanol have been identified in the wines of several different *V. vinifera* cultivars, where they are thought to arise largely through fermentation mechanisms (Schreier 1979), but have also been identified in grape berries (Schreier 1976). Both compounds have been shown to develop in fruit through amino acid conversions (Drawert 1975), though this mechanism has not been identified specifically in grape berries.

Phenethyl alcohol is commonly found in wines. This compound can be formed in grapes from the precursor phenylethyl-α-D-glucopyranoside, but is usually only present in small quantities (Winterhalter *et al.* 1999, Garcia *et al.*, 2003). The greater part of the phenylethyl alcohol in wine forms during yeast fermentation, where 2-phenylalanine serves as a precursor (Laminkanra *et al.*, 1996). As the phenylethyl alcohol content was not quantified in the Frontenac juice, it is not known whether phenethyl alcohol exists in concentrations comparable to those found in the finished wine, or if the bulk of the final concentration was formed during fermentation.

The six-carbon alcohol, 1-hexanol, is produced during crushing, when linolenic and linoleic acids are released from the grape skin and enter reactions catalyzed with lipoxygenase, peroxidase and alcohol dehydrogenase (Iglesias *et al.* 1991, Garcia *et al.* 2003). The concentration of 1-hexanol in the finished wine is thus dependent on must aeration (Rocha 2003) and the length of juice-skin contact. Both 1-hexanol and phenethyl alcohol are known to survive fermentation (Schrier 1979).

Table 3.4: Volatile compounds identified in red Frontenac table wines, wines produced from *V. riparia* clone #89, and Frontenac grape juice with two days skin contact.

	Compound	Concentration Range	*V. riparia* clone #89	Frontenac Juice
Alcohols				
	Isoamyl alcohol	4.2-7.5 ppm	+	+
	2-methyl-1-butanol	0.7-1.2 ppm	+	+
	1-hexanol	0 – 0.8 ppm	-	+
	Phenethyl alcohol	0.8 – 2.1 ppm	+	+
	Linalool	--[*]	-	-
Esters				
Isoacid ethyl esters	Ethyl isobutyrate	0.1- 0.5 ppm	+	-
	Ethyl-2-methylbutyrate	50 - 200 ppb	+	-
	Ethyl-3-methylbutyrate	30 - 1600 ppb	+	-
Fatty acid ethyl esters	Ethyl butanoate	0.1 – 0.4 ppm	+	-
	Ethyl hexanoate	1.1 – 3.6 ppm	-	-
	Ethyl octanoate	1.8 – 7.1 ppm	+	-
	Ethyl decanoate	1.1 – 3.7 ppm	+	-
Fusel acetates	Isoamyl acetate	80 – 2000 ppb	+	-
	2-Methylbutyl acetate	200-490 ppb	+	-
Cinnamic esters	Ethyl dihydrocinnamate	3 – 30 ppt[**]	-	-
	Ethyl cinnamate	50 – 160 ppt	-	-
Misc. esters	Ethyl lactate	0.2 – 1.5 ppm	+	-
	Methyl hexanoate	0 – 20 ppb	+	-
	Diethyl succinate	1.3 – 3.8 ppm	+	-
Fatty Acids				
	Octanoic acid	0.7 – 5.2 ppm	-	-
	Decanoic acid	0.3 – 4.9 ppm	-	-
Volatile Phenols				
	Eugenol	10 – 90 ppt	+	-
	Methyl salicylate	60 – 500 ppt	+	-
Lactones				
	ϒ-Butyrolactone	20-40 ppb	+	-

[*] *Coelution of other compounds in this odor region prevented calculation of compound concentration*
[**] *Parts per trillion.*

In a finished wine, 2-methyl-1-butanol, 3-methyl-1-butanol, and phenethyl alcohol contribute subtle complexity at levels <3000 mg/L, but become penetrating and unpleasant at higher concentrations (Ebeler 2001, Ribereau-Gayon *et al.* 2001). In the six commercial Frontenacs examined, the combined concentrations of the closely-spaced 2-

and 3-methyl-1-butanol ranged from 4.8 to 8.4 mg/L, and phenethyl alcohol from 0.7 to 2.1 mg/L, well below the concentration at which the wine's sensory profile would be negatively impacted. Panelists described these compounds as chemical, green, or oxidized. 1-Hexanol eluted very close to isoamyl acetate, and is likely to be the origin of fainter notes of fresh nuts, vitamin and grainy aromas perceived just prior to the strong banana note typical of the latter compound. At levels above the sensory threshold, 1-hexanol has been found to contribute green, herbaceous off-odors in wines (Kotseridis & Baumes 2000).

The monoterpene alcohol linalool was not identified in the Frontenac juice, but is thought to originate in the grape. The importance of this compound is difficult to determine in the wines studied. In the odor region between LRI 1101 and 1118, the co-elution of three or four compounds made unambiguous identification of all volatiles impossible. In wines A, F, and H, however, peaks were slightly separated in this area, allowing identification of linalool. Linalool may contribute floral notes to a wine, being responsible for the panelist descriptors of 'floral,' 'jasmine,' and 'rose perfume' perceived in this odor region. Linalool has been identified in several *V. vinifera* grapes and juices, in higher concentrations in aromatic whites, but also at lower levels in 'neutral' varieties (Strauss *et al.* 1986). Concentrations of linalool in Frontenac could not be calculated due to the multitude of coeluting peaks.

It is worth noting that the volatile phenol, methyl salicylate, which was positively identified in all eight Frontenac wines, may also originate in the grape. While this compound was not identified in the Frontenac juice, it was identified in the *V. riparia* #89 wine, and has been identified in earlier studies of *V. riparia* grapes (Schreier 1980).

Methyl salicylate is rarely seen as a component of wine, although it has been identified in the *V. vinifera sp.* Emir grape of Turkey (Cabaroglu *et al.* 1997) and Huxelrebe (Caven-Quantrill *et al.* 2006). The sensory impact of this compound was not determined for either cultivar. Methyl salicylate is described in the literature as having an odor of wintergreen, mint, or a fresh green character; this correlates well with the waxy, minty, and fresh leaf descriptors assigned by panelists. If present at perceivable levels, this compound may contribute the 'fresh green' descriptor identified by a descriptive analysis of Frontenac table wines (Ch. 2, p 77)

3.5.2. Volatiles evolving from grape-derived precursors

As in most wines, a majority of odor-active compounds in Frontenac were generated during fermentation. While not directly derived from the grape, many so-called 'fermentation products' are produced from precursors found in the fruit, such that varietal differences in precursor concentration result in varietal variation in wine aroma profile. Of these precursor-dependent volatiles, the compounds produced from amino acid metabolism, namely the isoacids and their ethyl esters, and fusel alcohols and their acetate esters, have the greatest sensory impact (Ferreira 2000). This suggests that the ratios of five of Frontenac's odor active compounds - the isoacid esters (ethyl isobutyrate, ethyl-2-methylbutyrate and ethyl-3-methylbutyrate) and the fusel alcohol esters (isoamyl acetate and 2-methylbutyl acetate) may potentially be unique to the cultivar, and could serve as characterizing volatile components.

Three other identified compounds are of uncertain origin. Eugenol is a volatile phenol often found in barrel aged wines. It is formed in oak staves as a result of lignan

breakdown during stave toasting, and can be transferred into the wine with other oak products (Chatonnet 1990). Glycosidically bound precursors of eugenol are also found in the grape, and varietal differences have been observed in the finished wine (Ferreira *et al.* 2000). Two related compounds, the cinnamic esters ethyl dihydrocinnamate and ethyl cinnamate, arise from various precursors found in the grape or in oak (Jackson 2000). In this work, Frontenac samples with and without oak aging were used, so the effect of grape precursors on final eugenol and cinnamic ester concentration is unknown. As all wines showed some amount of these compounds, however, it can be deduced that precursors exist in the Frontenac grape.

3.5.3. Volatiles evolving from fermentation

The two acids with sensory importance, octanoic and decanoic acid, are fermentation derived, and may be produced via anabolic or catabolic mechanisms (Nykänen 1986, Schreier 1974). These compounds have been reported to contribute fruity, fatty, or rancid notes to wine (Gómez-Miguez *et al.*, 2007), which agrees with panelist descriptors (Table 3.3). Octanoic and decanoic acids were found to be present at concentrations above their reported sensory threshold levels (0.5 ppm and 1 ppm, respectively) in some of the Frontenac wines (Table 3.4).

Fatty acid esters, including the ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate identified in Frontenac, are also yeast-produced, synthesized through a process very similar to the anabolic production of fatty acids. The relative concentration ranges of these compounds found in Frontenac generally agreed with those found in studies of both red and white wines, with concentrations in descending order as

follows: ethyl octanoate, ethyl hexanoate, and ethyl butanoate (Baumes *et al*, 1986). These apolar esters have been found, as a group, to be present in smaller concentrations than polar esters like ethyl acetate, but to contribute more to overall wine aroma (Baumes *et al*, 1986). The fatty acid methyl ester, methyl hexanoate, likely developed during fermentation as well; although this compound has been found in grapes (Schreier 1976) it was not identified in *V. riparia* fruit (Schreier 1980) and was not found in Frontenac juice (Table 3.4). As a class, the aroma contribution of fatty acid esters is generally fruity, and is largely considered to be unimportant as a tool for distinguishing between wines (Ebeler 2001).

The remaining volatiles identified in Frontenac contribute little towards distinguishing varietal characteristics. Both ethyl lactate and diethyl succinate are fermentation-derived esters common to wines and other fermented beverages. Both may be synthesized by yeast, and are also produced by strains of lactic acid bacteria, so secondary malolactic fermentation can result in increased concentration in the final wine. γ-Butyrolactone is also commonly found in wines, and is thought to arise from the metabolism of glutamic acid during fermentation (Rocha *et al*., 2004). While it has been identified in the skins of some grape cultivars (Garcia *et al*., 2003), this compound was not found in Frontenac juice. Concentrations of γ-butyrolactone have been found to be higher in red wines, likely as a result of extended skin-wine contact performed in most red wine fermentations, but its sensory impact in overall wine aroma is thought to be minimal (Baumes *et al*. 1986).

3.5.4. Further work

As this work represents only the initial steps in identifying volatile compounds important to Frontenac wine aroma, there are several issues that require further investigation. The odor region from LRI 1080 to 1089, for which no compounds could be identified, may result from a compound present at concentrations below the instrument detection level. Greater sample extraction and concentration may be useful in identifying the compounds responsible for the caramel, fresh bread, and toasted notes described in this region. The co-eluting peaks occurring from LRI 1101 to 1118 also require further analysis, as panelists described several intense and changing aromas throughout this period. In wine H, nonanol was positively identified in this region, eluting right after linalool; if found in all wines, this compound could explain the green, vegetal, and leafy notes panelists described during the same period. Nonanol was also tentatively identified in Frontenac juice. Further investigation, perhaps using MS × MS or similar analysis capable of further compound separation, is necessary to determine which volatiles are co-eluting during this time, and which are important to Frontenac wine aroma.

Additional work is needed to determine whether the volatiles identified are actually responsible for the odors panelists perceived. Enhancing wine samples with each compound, and rerunning GCO analysis, could confirm identifications. Further, a base wine could be enhanced with approximate concentrations of identified compounds, and sensory evaluation of aroma, either as descriptive analysis or triangle difference tests, could be used to determine how closely a collection of these compounds mirrors the original wine.

It would also be of interest to further explore the volatiles present in Frontenac juice. The sample used for this work received only two days of skin contact, less than the 5 to 8 days generally used in Frontenac wine production. Since several compounds discussed earlier may be extracted from fruit skin, it is possible that analysis of Frontenac juice with longer skin contact time would reveal additional compounds of interest. Concentrations of all juice compounds could be compared to red Frontenac wine produced form a specific juice lot, in order to monitor the changes that occur during processing and fermentation, and to determine the source of key volatiles.

3.6. Conclusions

Twenty-four odor-active volatile compounds were identified in red Frontenac table wine, i.e. those perceived in at least 50% of the 64 GCO analyses. All volatiles have been previously reported in other wine types. Six compounds of sensory importance may be derived directly from the grape, and an additional six may develop from grape-derived precursors; these compounds are most likely to be responsible for the varietal character of Frontenac. All other volatiles identified were the product of yeast or bacterial metabolism, and as such are common to most wines and many other fermenting beverages, contributing little to varietal character. Further work is needed to unambiguously identify volatiles important to Frontenac wine typicity.

CHAPTER FOUR

Practical Applications and Further Work

The motivation to begin this work was simple: to fulfill a desire to provide basic information about a largely uncharacterized grape cultivar, so that members of a new and innovative wine industry could use it to produce higher quality products. Ultimately, determining which components are odor-active in Frontenac wines will allow researchers to determine how changes in grape cultivation and processing affect those volatiles, and will subsequently give industry members the tools they need to modify the sensory profile of their final product. In a non-traditional growing region, this type of control is key: consumers are not likely to give local wines a second try if their first experience is unpleasant, so producing a product that consistently exhibits the best attributes of a cultivar is essential to commercial success and industry growth.

The current work, however, is only the first step towards achieving that kind of detailed control. It is certainly a step in the right direction; since Frontenac's introduction in 1996, winemakers had to make processing decisions based on the advice of their fellow industry members or on the results of their own trial-and-error experimentation. Worse, they had no standardized way of discussing their sensory goals. The Wine Aroma Wheel (Noble *et al.*, 1986) was available, with its 101 attributes that can be found in wine, but which of those 101 aromas were found in Frontenac? More importantly, which were positive, and which were flaws? Since the aroma wheel was developed based on traditional, *Vitis vinifera* derived wines, it is possible that aromas that typified Frontenac, an inter-specific hybrid with strong *V. riparia* traits, weren't found in the aroma wheel.

Were these aromas flaws that needed to be suppressed, if possible, during processing, or an expression of the region's newly-found terroir which should be enhanced?

The set of sensory descriptors developed in the first part of this work is designed to give winemakers a starting point. The 13 attributes were selected and defined by panelists free of the biases and expectations of those familiar with the cultivar. As a group, these descriptors give producers their first methodical and standardized definition of Frontenac varietal character, and their first clear view of what consumers unfamiliar with the cultivar may experience. It is interesting that the panel agreed with traditional characterization in some ways; for instance, it's considered 'common knowledge' in the industry that Frontenac wine has dominant notes of cherry and black currant, and this work supports that notion. The other 11 attributes, however, give researchers and industry members a chance to take a fresh look at a varietal they thought they knew. For example, it's often claimed that appropriate winemaking practices can nearly eliminate herbaceousness in Frontenac, but panelists found not one, but two distinct green notes. This leads to several questions: do these characteristics indicate poor production practices? Issues with ripening? Or are they representative of the cultivar, like the 'gooseberry leaf' note in Marlborough Sauvignon blanc? In the same vein, the 'geranium' attribute raises interesting issues, as producers have traditionally believed that 'geranium taint' only develops when wines treated with sorbate are colonized by unwanted malolactic bacteria. Does the presence of this note in all wine samples suggest a low basic level of winemaking skill in the industry? Or is it possible that the geranium note is a part of the varietal character of Frontenac, and subsequently not a flaw? If other compounds that contribute a geranium-like character are consistently identified in

Frontenac samples, it may indicate that 'traditional' thinking is wrong, and force a re-evaluation of this attribute in wines in general. Further, the fact that this panel required the development of some sensory references not found on the aroma wheel suggests that the accepted list of wine aromas may need to be expanded to include additional aromas not found in traditional wines. Defining the varietal character of Frontenac allows the region to understand its own terroir more fully, but also challenges accepted wisdom concerning wine aroma as a whole.

After developing a lexicon for aromas typical to Frontenac wines, the next obvious question is: "Do they like it?" The current work did not include consumer preference trials. A series of consumer trials designed to allow judges to taste and indicate preference for wines with slightly different aroma profiles would tell the industry not only what characteristics can be found in Frontenac, but what characteristics consumers enjoy. Data from these type analyses would help answer some of the questions above- questions like "is the geranium note a flaw, or varietal character?" Such data would then dictate further analytical and processing research, suggesting, for instance, that the origin of the green characters be identified, and means of enhancing or suppressing those attributes through changes in processing should be explored.

In addition to preference testing, further descriptive analysis work may be instructive. As mentioned in Chapter 2, the descriptor set developed by the panel was not very useful for distinguishing between wines, as any attribute not found in at least 60% of the samples was discarded. Expanding that work to include the wider body of Frontenac table wines currently available, and to retain all descriptors generated, would produce an expanded lexicon useful for not only defining the characteristics common to the cultivar,

but also for discussing the differences between them with more accuracy. Similar work should be pursued with Frontenac wines produced in rosé and port styles, as these products are becoming widely popular. As the number of producers working with Frontenac continues to expand, so do the possible variations in winemaking style, which will continue to shape the definition of the cultivar's varietal character.

In addition to sensory analysis, further analytical work is required to improve understanding of the Frontenac wine matrix. The current work sought to identify only those wine components that could be perceived by panelists during GCO analysis, and even this work is incomplete; the first need is to identify the odor-active compounds that remain unknown. This may be accomplished with GC x GC analysis, or similar techniques allowing better peak separation. Once the volatile list is complete, the next logical step is correlating the concentration of odor-active volatiles identified via GCO/MS with attribute intensity measures derived by descriptive analysis. Using multivariate analysis to determine relationships between these two measures may help clarify the role individual volatiles play in the complex matrix that dictates wine aroma.

Once such relationships are established, they offer a means to examine the role that viticultural and processing methods play in developing wine quality. Individual volatile compounds can be tracked from grape ripening through wine aging, to determine how viticultural practices, processing parameters, and aging regimen influence their development and accumulation. These data can then be tied back to consumer preference tests, to see which practices produce wines more attractive to customers. Further, similar tracking studies can be used to chart differences in microclimate within the region,

defining terroir and informing winemakers of processing modifications that may be necessary for grapes grown in their microclimate.

To fully understand the elements that define Frontenac wine, and its relationship to regional terroir, a lengthy chain of analyses remains to be completed. Consumer preference must be gauged, the role of individual volatiles in overall wine aroma established, and extensive analysis of the development and retention of individual components delineated. This program is not unique; similar courses of study are underway around the world for a variety of cultivars of local and global importance. What is unique is the fact that, twelve years after its release, Frontenac is seen to warrant such analysis, and has grown to such importance in the Upper Midwest and other cold-climate growing regions. The current work represents the first steps in understanding a cultivar that is altering the traditionally-held idea of viticulture's geographical limits. Continued research can only improve understanding of quality wine production in the far north, and ensure the positive economic impact of Frontenac and its successors from the breeding program.

APPENDIX I: STATISTICAL TABLES AND RAW DATA

All statistical analyses were performed using R shareware(R Foundation for Statistical Computing, Vienna, Austria).

I.1: R Coding template for ANOVA for descriptive analysis attributes:

```
mydata <- read.csv("Frontenac Data.csv")
attach(mydata)
Sample <- as.factor(Sample)
Panelist <- as.factor(Panelist)
summary(aov(Blackberry ~ Sample + Error(Panelist/Sample)))
```

I.2: R Coding and responses for ANOVA for descriptive analysis attributes:

```
> dat <-read.table("/Users/annakatharinemansfield/Documents/Frontenac data.txt",
head=T)
> attach(dat)
> names(dat)
 [1] "Panelist"       "Sample"         "Rep"
 [4] "Blackberry"     "BlackCurrant"   "Cherry"
 [7] "Jammy"          "CookedVegetable" "FreshGreen"
[10] "Cedar"          "Spice"          "BlackPepper"
[13] "Geranium"       "Floral"         "Tamari"
[16] "Earthy"
> Sample <- as.factor(Sample)
> Panelist <- as.factor(Panelist)

> summary(aov(Blackberry ~ Sample + Error(Panelist/Sample)))
Error: Panelist
                Df      Sum Sq         Mean Sq        F value        Pr(>F)
Residuals       10      455.38         45.54
Error: Panelist:Sample
                Df      Sum Sq         Mean Sq        F value        Pr(>F)
Sample          5       97.38          19.48          2.5311         0.04055 *
Residuals       50      384.71         7.69
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
Error: Within
                Df      Sum Sq         Mean Sq        F value        Pr(>F)
Residuals       66      460.75         6.98

> summary(aov(BlackCurrant ~ Sample + Error(Panelist/Sample)))
Error: Panelist
                Df       Sum Sq         Mean Sq        F value        Pr(>F)
Residuals       10      346.49         34.65
Error: Panelist:Sample
```

```
                Df      Sum Sq      Mean Sq     F value     Pr(>F)
Sample          5       60.63       12.13       1.2157      0.3156
Residuals       50      498.75      9.97

Error: Within
                Df      Sum Sq      Mean Sq     F value     Pr(>F)
Residuals       66      339.94      5.15

> summary(aov(Cherry ~ Sample + Error(Panelist/Sample)))
Error: Panelist
                Df      Sum Sq      Mean Sq     F value     Pr(>F)
Residuals       10      442.81      44.28
Error: Panelist:Sample
                Df      Sum Sq      Mean Sq     F value     Pr(>F)
Sample          5       31.65       6.33        0.7642      0.58
Residuals       50      414.11      8.28
Error: Within
                Df      Sum Sq      Mean Sq     F value     Pr(>F)
Residuals       66      296.170     4.487

> summary(aov(Jammy ~ Sample + Error(Panelist/Sample)))
Error: Panelist
                Df      Sum Sq      Mean Sq     F value     Pr(>F)
Residuals       10      372.17      37.22
Error: Panelist:Sample
                Df      Sum Sq      Mean Sq     F value     Pr(>F)
Sample          5       95.79       19.16       2.2284      0.06585 .
Residuals       50      429.86      8.60
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
Error: Within
                Df      Sum Sq      Mean Sq     F value     Pr(>F)
Residuals       66      447.09      6.77

> summary(aov(CookedVegetable ~ Sample + Error(Panelist/Sample)))
Error: Panelist
                Df      Sum Sq      Mean Sq     F value     Pr(>F)
Residuals       10      955.89      95.59
Error: Panelist:Sample
                Df      Sum Sq      Mean Sq     F value     Pr(>F)
Sample          5       140.94      28.19       2.354       0.05386 .
Residuals       50      598.73      11.97
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
Error: Within
```

```
                Df      Sum Sq      Mean Sq     F value     Pr(>F)
Residuals       66      373.41      5.66


> summary(aov(FreshGreen ~ Sample + Error(Panelist/Sample))
Error: Panelist
                Df      Sum Sq      Mean Sq     F value     Pr(>F)
Residuals       10      675.84       67.58
Error: Panelist:Sample
                Df      Sum Sq      Mean Sq     F value     Pr(>F)
Sample          5       63.07       12.61       1.537   0.1953
Residuals       50      410.34      8.21
Error: Within
                Df      Sum Sq      Mean Sq     F value     Pr(>F)
Residuals       66      290.235     4.397

> summary(aov(Cedar ~ Sample + Error(Panelist/Sample)))
Error: Panelist
                Df      Sum Sq      Mean Sq     F value     Pr(>F)
Residuals       10      220.259     22.026
Error: Panelist:Sample
                Df      Sum Sq      Mean Sq     F value     Pr(>F)
Sample          5       114.61      22.92       2.6382      0.03415 *
Residuals       50      434.42      8.69
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
Error: Within
                Df      Sum Sq      Mean Sq     F value     Pr(>F)
Residuals       66      316.42      4.79

> summary(aov(Spice ~ Sample + Error(Panelist/Sample)))
Error: Panelist
                Df      Sum Sq      Mean Sq     F value     Pr(>F)
Residuals       10      246.852     24.685
Error: Panelist:Sample
                Df      Sum Sq      Mean Sq     F value     Pr(>F)
Sample           5      57.91       11.58       1.2244      0.3116
Residuals       50      472.97      9.46
Error: Within
                Df      Sum Sq      Mean Sq     F value     Pr(>F)
Residuals       66      347.13      5.26

> summary(aov(BlackPepper ~ Sample + Error(Panelist/Sample)))
Error: Panelist
                Df      Sum Sq      Mean Sq     F value     Pr(>F)
```

```
Residuals      10      729.22          72.92
Error: Panelist:Sample
               Df      Sum Sq          Mean Sq         F value         Pr(>F)
Sample          5      5.743           1.149           0.2322          0.9466
Residuals      50      247.371         4.947

Error: Within
               Df      Sum Sq          Mean Sq          F value        Pr(>F)
Residuals      66      287.805         4.361

> summary(aov(Geranium ~ Sample + Error(Panelist/Sample)))
Error: Panelist
               Df      Sum Sq          Mean Sq         F value         Pr(>F)
Residuals      10      397.31          39.73
Error: Panelist:Sample
               Df      Sum Sq          Mean Sq         F value         Pr(>F)
Sample          5      231.05          46.21           5.0999          0.000744 ***
Residuals      50      453.04          9.06
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
Error: Within
               Df      Sum Sq          Mean Sq         F value         Pr(>F)
Residuals      66      452.96          6.86

> summary(aov(Floral ~ Sample + Error(Panelist/Sample)))
Error: Panelist
               Df      Sum Sq          Mean Sq         F value         Pr(>F)
Residuals      10      394.95          39.49
Error: Panelist:Sample
               Df      Sum Sq          Mean Sq         F value         Pr(>F)
Sample          5      66.56           13.31           1.9108          0.1091
Residuals      50      348.35          6.97
Error: Within
                Df      Sum Sq          Mean Sq         F value         Pr(>F)
Residuals      66      353.80          5.36

> summary(aov(Tamari ~ Sample + Error(Panelist/Sample)))
Error: Panelist
               Df      Sum Sq          Mean Sq         F value         Pr(>F)
Residuals      10      470.94          47.09
Error: Panelist:Sample
               Df      Sum Sq          Mean Sq         F value         Pr(>F)
Sample          5      99.72           19.94           2.2507          0.06355 .
Residuals      50      443.05          8.86
---
```

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
Error: Within

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Residuals	66	287.650	4.358		

> summary(aov(Earthy ~ Sample + Error(Panelist/Sample)))
Error: Panelist

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Residuals	10	507.64	50.76		

Error: Panelist:Sample

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Sample	5	117.19	23.44	1.9508	0.1024
Residuals	50	600.72	12.01		

Error: Within

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Residuals	66	419.23	6.35		

I.3: R Coding and results for correlation plots for attributes of descriptive analysis

```
> dat <-read.table("/Users/annakatharinemansfield/Documents/Frontenac data.txt",
head=T)
> attach(dat)
> names(dat)
[1] "Panelist"        "Sample"          "Rep"
 [4] "Blackberry"      "BlackCurrant"    "Cherry"
 [7] "Jammy"           "CookedVegetable" "FreshGreen"
[10] "Cedar"           "Spice"           "BlackPepper"
[13] "Geranium"        "Floral"          "Tamari"
[16] "Earthy"
> plot((dat)[4:16])
> plot(dat$Cherry, dat$Blackberry)
round(cor((dat)[4:16]),3)
```

	Blackberry	BlackCurrant	Cherry	Jammy
Blackberry	1.000	0.327	0.139	0.141
BlackCurrant	0.327	1.000	0.100	0.187
Cherry	0.139	0.100	1.000	-0.002
Jammy	0.141	0.187	-0.002	1.000
CookedVegetable	0.319	0.222	0.042	0.045
FreshGreen	0.153	0.128	0.072	-0.159
Cedar	-0.024	0.052	0.056	0.072
Spice	0.117	0.178	0.035	0.164
BlackPepper	0.373	0.288	0.229	-0.080
Geranium	0.200	0.151	-0.033	0.123
Floral	0.144	-0.106	0.135	-0.090
Tamari	-0.081	-0.142	-0.158	-0.206
Earthy	0.091	0.105	-0.158	-0.047

	CookedVegetable	FreshGreen	Cedar	Spice
Blackberry	0.319	0.153	-0.024	0.117
BlackCurrant	0.222	0.128	0.052	0.178
Cherry	0.042	0.072	0.056	0.035
Jammy	0.045	-0.159	0.072	0.164
CookedVegetable	1.000	0.434	0.132	0.147
FreshGreen	0.434	1.000	0.153	-0.028
Cedar	0.132	0.153	1.000	0.114
Spice	0.147	-0.028	0.114	1.000
BlackPepper	0.395	0.467	0.108	0.139
Geranium	0.245	0.215	0.053	0.213
Floral	-0.056	-0.050	-0.069	0.090
Tamari	0.065	-0.050	0.115	-0.052
Earthy	0.271	0.081	0.145	-0.080

	BlackPepper	Geranium	Floral	Tamari	Earthy
Blackberry	0.373	0.200	0.144	-0.081	0.091
BlackCurrant	0.288	0.151	-0.106	-0.142	0.105
Cherry	0.229	-0.033	0.135	-0.158	-0.158
Jammy	-0.080	0.123	-0.090	-0.206	-0.047
CookedVegetable	0.395	0.245	-0.056	0.065	0.271
FreshGreen	0.467	0.215	-0.050	-0.050	0.081
Cedar	0.108	0.053	-0.069	0.115	0.145
Spice	0.139	0.213	0.090	-0.052	-0.080
BlackPepper	1.000	0.313	0.151	-0.062	0.186
Geranium	0.313	1.000	0.089	-0.033	0.104
Floral	0.151	0.089	1.000	-0.144	-0.073
Tamari	-0.062	-0.033	-0.144	1.000	0.348
Earthy	0.186	0.104	-0.073	0.348	1.000

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