Considerations for the Development and Optimization of Wine made from Partially Dehydrated Grapes in Ontario, Canada

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For my husband. Thank you for everything.

Abstract

The appassimento process for making wine can mitigate climatic challenges associated with cool climate winemaking, as fruit is dried post-harvest, reducing vintage-to-vintage variation due to varying fruit quality. Resultant wines fermented from dried grapes are high in ethanol and described as rich and intensely flavoured. One of the quality challenges facing wine made from partially dehydrated grapes is elevated levels of undesirable oxidation compounds, such as ethyl acetate, acetic acid and acetaldehyde. In this study we aim to characterize wines made from a local yeast isolate, *Saccharomyces bayanus* CN1, which demonstrates limited osmotolerance and may have application to this wine style, as it is a lower producer of such compounds. Wines made with the yeast of interest were compared to wines made with the accepted commercial yeast, *Saccharomyces cerevisiae*, EC1118. Fermentations were established over two vintages at one and three target starting sugar concentrations and a control, respectively. Wines were chemically (enzymatic) and sensorially analyzed. Wines (year two) were subject to volatile organic compound (VOC) and volatile fatty acid (VFA) measurements via Gas Chromatography-Mass Spectrometry.

Another consideration for the development of this wine style is the inclusion of *Botrytis cinerea*, a pathogenic fungus that commonly develops during grape drying, and may impart favourable sensorial characteristics. Grapes were dried to 28.0°Brix and were fermented with EC1118 at 0 and 10% *B. cinerea* infection. A consumer preference test (n=153) that measured liking of wines (CN1 and 0% and

10% *B. cinerea* infection) was conducted. Results indicate that CN1's upper limit for fermentation to dryness is 27.5°Brix. All CN1 wines had significantly lower concentrations (p<0.05) of oxidation compounds than the commercial yeast, and oppositely, higher glycerol levels, along with comparable ethanol concentration to EC1118 wines. Significant differences in the concentrations of VOCs and VFAs, such as 2-phenylethanol and hexanoic acid were observed both within °Brix treatments and amongst yeast strains. Sensorially, the wines differed in intensity for a number of attributes. The consumer study revealed no preference between wines vinified with the different yeast strains.

This work will contribute to the optimization of this wine style in cool climate winemaking regions and beyond.

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Chapter 1 Literature Review

1. Introduction

Winemaking using the appassimento technique is a process by which wine grapes are dried post-harvest (Figure 1.1), resulting in fruit that is dehydrated, and highly concentrated in sugar. This process also concentrates aromas and flavours, giving rise to an intensely flavoured wine, which is high in alcohol and has been fermented to dryness. This process is traditionally used in northern Italy to produce Amarone wine and has recently been utilized as a tool in Ontario, Canada as a way to mitigate wine production risk due to the impact of climate change. This process is relatively new to the Ontario wine industry, thus prompting the necessity for research in this area that is specific to local climate and grape varietals.

The original goal of this study was to characterize a locally isolated yeast and its impact on cool climate appassimento winemaking in Ontario. It was hypothesized that the local yeast, *Saccharomyces bayanus* CN1 (Figure 1.2), would reduce potentially problematic oxidation compounds that can negatively affect appassimento wines when present at high concentrations. Given the positive results this study yielded, and the yeasts' fitness for this wine style, it was prudent to examine this yeast further in terms of its sensorial profile, volatile compound content and consumer acceptance of the wines made with this yeast. The objectives of this study were to 1.) select a suitable drying target for wine grapes that would result in a dry red wine; 2.) compare the chemical profile of wines made with CN1 to the commercially used yeast for this style; 3.) determine the sensory profile of the wines; 4.) identify the abundance of volatile compounds present in the

wines; 5.) measure the impact *of Botrytis cinerea* on appassimento style wines and; 6.) assess consumer acceptance of wines. In order to address these objectives, appassimento style wine was made from barn dried (Figure 1.3) *Vitis vinifera* Cabernet franc grapes over two vintages; year one wines were made with both *S. bayanus* CN1 yeast and *S. cerevisiae* EC1118 yeast at two starting sugar concentrations (23.0 and 28.0°Brix), year two wines were made with both *S. bayanus* CN1 yeast and *S. cerevisiae* EC1118 yeast at four starting sugar concentrations (control 21.5, 24.5, 26.0 and 27.5°Brix). The must and wines were analyzed chemically for the following metabolites and basic physio-chemical characteristics compounds: pH, titratable acidity, residual sugar, nitrogen (amino and ammonia), glycerol, acetic acid, acetaldehyde, ethyl acetate, ethanol and malic acid, lactic acid (year one only). Sensory descriptive analysis was performed using a trained panel, to determine differences wines due to both yeast strain and starting sugar concentration. Head Space-Solid Phase Micro-Extraction-Gas Chromatography- Mass Spectrometry (HS-SPME-GC-MS) was used to quantify the concentration of volatile compounds that are important to wine flavour. Additional fermentations were conducted in year two of winemaking trials; grapes were dried to 28.0°Brix, fermented with *S. cerevisiae* EC1118, and wines were made with 0% *Botrytis cinerea* infection and 10% *B. cinerea* infected grapes by weight (Figure 1.4). These wines were subjected to the same chemical and volatile analysis as the year one and year two appassimento wines and were analyzed sensorially with descriptive analysis to understand the flavour and aroma impact of the fungus on the final wine. Finally, wines made from grapes that were dried to 27.5°Brix and fermented with CN1 were tasted

alongside the wines fermented with EC1118 in the *B. cinerea* trial to assess consumer preference by having participants (n=153) rate the wines with a nine-point hedonic scale (Figure 1.5).

Currently, the only literature on the appassimento wine technique as it applies to the Ontario wine industry that has been published has been by this research group (Kelly et al., 2018). This information can be used to inform industry personnel who are interested in utilizing the appassimento winemaking technique in cool climate regions like Ontario and beyond.

Of the 88 Niagara Peninsula VQA wineries listed in the Wine Marketing Association of Ontario 2017 Guide (includes sub-appellations of Niagara-On-the-Lake, Twenty Valley and Niagara Peninsula), 18 list wines on their websites that utilize the appassimento method. Of the wineries that use this method, some producers use it in almost every wine in their portfolio (both red and white), while others have 1-4 wines made from dried grapes to diversify their portfolio that is mostly comprised of table wines and Icewines. Finally, other producers are using this technique as a blending tool to enhance quality and consistency in table wines. Specifically, Big Head Wines in Niagara-on-the-Lake uses many different varietals such as Cabernet franc, Cabernet sauvignon, Petit Verdot and Merlot in their appassimento style-focused portfolio of wines. Kew Vineyards dries Cabernet franc, Cabernet sauvignon and Merlot, and offers 2-3 appassimento wines (depending on the year) in their portfolio. The capacity of the drying chambers is a limiting factor in terms of production, so the volume of this wine available every year is a function of available space and resources. There isn't a common

variety that is used amongst the wineries that offer this style of wine, and thus there is no regional signature. In the absence of strict regulations for variety that can be used for this method, wineries can experiment with any variety that is well-suited. Drying methods vary throughout the region, thus there is no agreed-upon protocol or facility for grape withering.

The Current Project

This project focuses on considerations for the development and optimization of appassimento-style wine in Ontario. Understanding the impact of post-harvest processing decisions will contribute to improved quality. The yeast central to this project, *S. bayanus* CN1, is an example of an indigenous yeast population that promotes the diversity of style of a specific wine (Dellaglio et al., 2003). This project is the first time this yeast will be fully characterized within the context of a speciality wine style. Previous research with this yeast has trialed it in Icewine fermentation. Positive preliminary results with respect to reduced acetic acid formation in the Icewine along with limited osmotolerance suggest CN1 may be a good fit for appassimento-style wine, as sugar stress is reduced in must from partially dehydrated grapes. Given that commercially-produced appassimento-style wines may be organoleptically impacted by high concentrations of oxidation compounds, yeast choice may help in mitigating potential faults. There is a gap in our understanding of what wines produced with this yeast in this style will taste like, what volatiles are responsible for their profile and how consumers will respond.

Two years of winemaking data at various starting sugar concentrations will elucidate the upper limit of this yeast for fermenting the wine to dryness. An examination of wine metabolites and volatiles will answer some of the basic questions important to this project, such as whether yeast choice and drying targets matter. Given the novelty of this yeast strain, it is prudent to answer other basic questions like what the wines taste like and if consumers will like them to provide valuable insight to the application of this yeast to appassimento-style wine. Further to that, the controlled inclusion of *B. cinerea*, a fungus responsible for sensorial changes in sweet wines and in Amarone, will be investigated. Although this has been characterized in Amarone, there is no literature on the impact of *B. cinerea* in regionally produced Ontario wines.

The production of appassimento-wine is influenced by many factors. Considerations for some of these variables will be implemented in this study to elucidate their impact.

Figure 1.1: Cabernet franc grapes during dehydration in the barn.

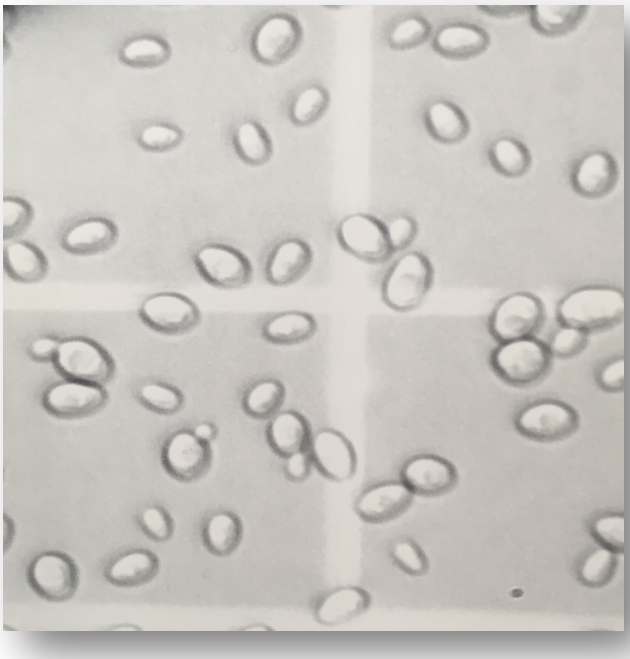

Figure 1.2: Budding S. bayanus CN1 Yeast at 40x objective (400x magnification).

Figure 1.3: Dehydrating Cabernet franc berries on drying racks (top); Drying racks stacked in drying chamber to promote airflow (bottom).

Figure 1.4: Dehydrated Cabernet franc cluster infected with B. cinerea.

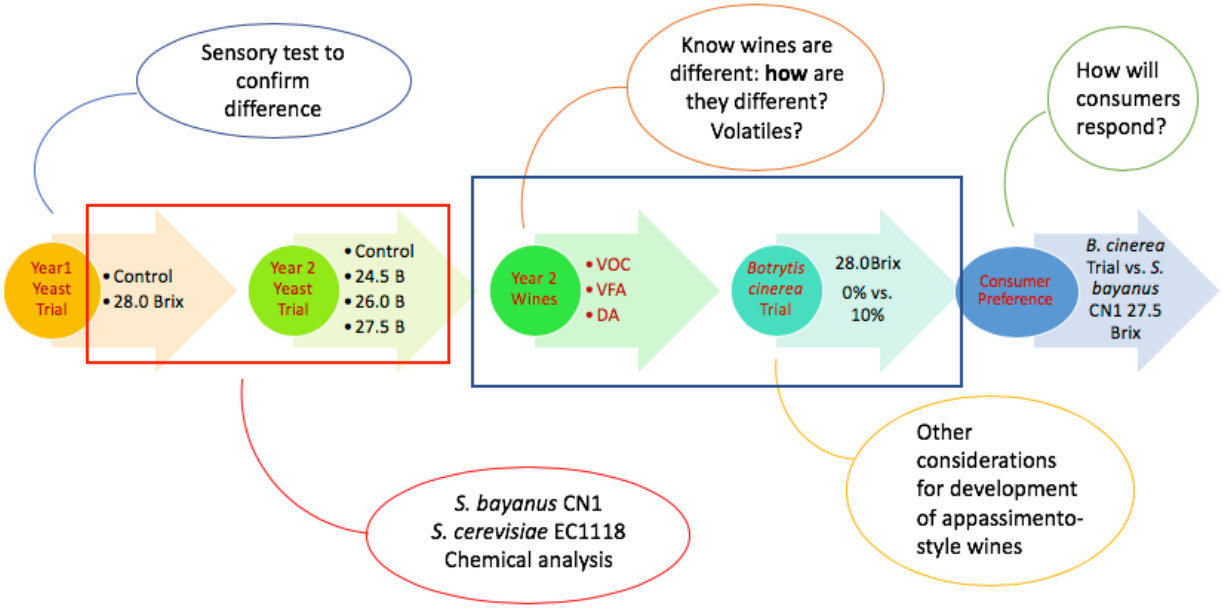

Figure 1.5: Thesis outline and relationship amongst data chapters.

1.1 Wines made from Partially Dehydrated Grapes

Wines made from partially dehydrated grapes are produced using the appassimento technique, where grapes that have been dried post-harvest are then fermented with either selected or indigenous yeast. Diversifying enological products to meet market demand has promoted the use of dehydrated grapes for specialized wine production (Wang et al., 2016). Resultant wines can come in a variety of styles, including sweet, dry, white, rosé or red and can be produced using different techniques. Variations in style depend on not only grape variety and dehydration method, but also on winemaking practices, which can occur before, during or after fermentation (Moio and Piombino, 2013). The dehydrated fruit is high in sugar and subsequently produces a wine that is high in alcohol, along with concentrated flavour and aroma compounds, suggesting positive postharvest flavor development and enrichment can occur as a consequence of the drying process (Bellincontro et al., 2004; Costantini et al., 2006; Moreno et al., 2008). The dehydration process is a key factor in the typical organoleptic characteristics of the wine, giving rise to a specific and unique bouquet (Tosi et al., 2012; Accordini, 2013). The sensory characteristics of appassimento wine are due to biochemical changes from moisture loss, affecting grape compounds such as polyphenols and volatile compounds (Consonni et al., 2011). These wines can be described with aroma attributes such as ripe fruits, prune, cherry jam, toasted almond, licorice, and spicy; and flavour attributes such as velvety, raisiny, high alcohol and concentrated (Iland et al., 2009; Fedrizzi et al., 2011).

1.1.1 History

The appassimento process is used traditionally in Italian wine regions to produce Amarone, Recioto, Valpolicella Ripasso and Sforzato wines. The most renowned and important appassimento style wine is Amarone (Tosi et al., 2012), which differs from its *passito* counterparts in that is a dry wine (Barbanti et al., 2008). Globally, Amarone is considered a benchmark for quality. Amarone is produced from indigenous grape varieties Corvina (the main variety), Rondinella, Molinara and Corvinone in specific proportions (Consonni et al., 2011; Boscaini and Paronetto, 1999; Bellincontro et al., 2016). It is produced in in north-eastern Italy, in the Valpolicella region, a grape growing area that covers 30 000km^2 (Torriani et al., 1999; Accordini, 2013). A rise in demand for this wine has resulted in a ten-fold increase in production since 1995, and as of 2010, more than 410 drying lofts are utilized in Valpolicella (Accordini, 2013). This wine is economically important commercially, as 80% of the quantities are exported to foreign markets, including North America, with an average price of 62 USD/bottle (Accordini, 2013; Bellincontro et al., 2016). While human interest in wine predates all written record (Meinart, 2018), this technique dates back to around 20 BC, when soldiers under the rule of Roman emperor Caesar Augustus brought it to Valpolicella (Pagliarini et al., 2004). The appassimento technique was traditionally employed to produce sweet wines, and written record of its production in the sixth century from Cassiodoro, minister in Ravenna to king Theordoric describes the fruit and wine as follows:

"In the autumn grapes are chosen in the domestic bowers, hung up by the bottom tip, then conserved in jars and in ordinary repositories. They hardened during time, do not

liquefy, unless humours are exuded, and the grapes become sweet. This goes on until December, until winter begins, and wine becomes new when in all the wine cellar is already old" (Paronetto and Dellaglio, 2011).

Other winemaking regions in the world also offer *passito* (raisin wine) wines such as Sauternes from France, Tokaj from Hungary and Xeres from Spain, that utilize the appassimento method to produce (Brenna et al., 2005). In the new world, wine using the appassimento technique is being produced in emerging winemaking regions that are seeking to enhance production of dry red wines and improve desirable characteristics such as flavour, aroma, initial sugar concentration, and expression of tannins. Specifically, in Ontario, Canada, the appassimento technique is emerging as a tool that can be utilized to mitigate the challenges associated with climate change.

1.1.2 Drying

Drying is one of the most frequently used methods for grape processing and food preservation (Ramming, 2009). It can process grapes into raisins to increase shelf-life, and also to dehydrate wine grapes for specialized wine production (Wang et al., 2016). During drying, berries shrink as water is lost, and the skins deteriorate as a consequence (Franco et al., 2004). Usually, the dehydration conditions include temperature between 10 and 20°C and relative humidity between 40-65% to achieve 20-50% berry water loss (considered optimal at 30-40%), which is the main perceptible change in the grapes (De Rosso et al., 2016; Barbanti et al., 2008). Dehydration can be achieved through many methods, including on-vine drying, sun-drying, freeze-drying, oven drying, hot-air drying (thermovinification) and solar drying; all of which have implications for quality and dry

time (Coklar and Akbulut, 2017). Sun drying is the most widely-used method for grape drying, but grapes dried in this way are susceptible to insect attack, solar radiation and rain, which can impact grape structure and intactness (Serratosa et al., 2008). Drying methods that include heating may induce irreversible damage in the cellular structure of the grape skin, which increases the phenolic compounds extracted in the wine during maceration (Machado de Castilhos et al., 2017). Within these drying types, different chambers can be utilized; the main difference is that parameters within the chamber can be controlled, or uncontrolled. Bellincontro et al. (2016) propose three drying categories: 1) dehydration, a controlled method that moderates temperature, relative humidity (RH) and ventilation, 2) drying, including uncontrolled conditions such as sun-drying, and 3) withering, when the process occurs in a naturally ventilated room with or without a partial control of temperature and RH. The impact of controlled versus uncontrolled drying conditions is discussed in detail below.

The grape berry is a living tissue that continues to consume oxygen and eliminate carbon dioxide and heat after harvest (Mencarelli and Bellincontro, 2013). Biochemical and molecular changes continue in the post-ripening berries, processes that are similar to senescence (Zenoni et al., 2016). The berries are sensitive to postharvest water stress, and the concentration of compounds and metabolites impacted by drying are varied depending of variety (De Rosso et al., 2016). Dehydration rate (Bellincontro et al., 2004), along with grape variety, modifies the release of volatiles like ethyl acetate and acetic acid, as well as ethanol, esters and higher alcohols. Accurately controlling the environmental conditions (independent of external climatic conditions outside of the

chamber) under which grapes are dehydrated can play a role in the development of compounds that impact the organoleptic profiles of appassimento wines (Panceri et al., 2017; Chkaiban et al., 2007). The chemical and sensory characteristics of wines fermented with dehydrated grapes is strongly influenced by the dehydration technique, as varying dehydration directly influences the chemical composition (Panceri et al., 2015). When controlled drying conditions are compared to traditional sun drying, resultant wines had an improvement in colour suitability, an increase in phenolic compounds, and sensory profiles were improved. Further, drying time is shorter in controlled conditions, though sun drying conditions are most cost-effective (Marquez et al., 2013; Coklar and Akbulut, 2017). Grapes dried in shared conditions contain higher concentrations of free and glycosylated volatile compounds when compared to sun-dried grapes (Piombino et al., 2010). Other studies (Chkaiban et al., 2007) that compare controlled (tunnel-dried grapes with controlled temperature, RH and air flow) to non-controlled environments (representative of the traditional technique, where open windows are the only source of air flow, and environmental factors are susceptible to external climatic conditions) saw an impact in weight loss, volatile compound development (C6 compounds and isoamyl acetate), aldehydes, ethyl acetate and acetic acid formation. This finding is supported by Constantini et al., (2006), who dehydrated grapes under controlled conditions, and observed C6 compound formation, and increased volatile acidity as a result of the quick drying. Chamber drying results a faster berry dehydration rate than any traditional technique regardless of external climatic conditions (Frangipane et al., 2012). Colour is also impacted by drying method, as

phenolic compound oxidation that is correlated to browning of grapes is increased during sun drying (Figueiredo-González et al., 2013). Temperature plays an important role because it directly affects the water evaporation rate and lower temperature may reduce the oxidation of volatile compounds, which is favorable for increasing quality (Mencarelli et al., 2010; Cirilli et al., 2012). Dehydrating grapes at high temperatures can result in a loss in varietal aroma, and less desirable oxidation aroma becomes the predominating primary aroma (Mencarelli and Bellincontro, 2013). Maintaining a temperature of 10°C or less during dehydration will result in slowing down the water stress response to reduce to formation of oxidation compounds, maintain varietal aroma and delay the formation of volatile acidity, while dehydration at 20°C favours aroma complexity and increases volatile acidity (Mencarelli and Bellincontro, 2013) (Figure 1.6). Sugar concentration at harvest is another important consideration, as dehydrated berries that are riper (higher initial sugar concentration) have higher concentrations of terpenes (linalool and geraniol), outlining the importance of initial berry maturity on flavour and aroma profile (Urcan et al., 2017).

Grape variety is an important consideration for this technique and making appropriate choices will ultimately optimize quality. Bunches should not be densely packed, berries should be moderate in size and have a thick skin (Failla et al., 2013). One study (Rolle et al., 2010) looked exclusively at skin hardness as a factor in the dehydration kinetics of different grape varieties. Different grapevine cultivars (Moscato bianco and Erbaluce) had different drying rates, which could be attributed to, in some part, skin hardness (Rolle et al., 2010). Efficient indicators of varietal suitability for on-

vine withering include berry skin hardness and thickness, as well as peduncle detachment resistance, suggesting these characteristics may be beneficial for off-vine drying, as well (Rolle et al., 2012). Some traditionally utilized varieties are as follows: Corvina (used to produce Recioto and Amarone della Valpolicella), Muscat of Alexandria (*Passito* di Pantelleria), Pinot grigio (Malvoisie di Nus), Trebbioano di Soave (Recioto di Soave), Nebbiolo (Sforzato Valtellina) and Gewürztraminer (Terntino) (Failla et al., 2013). The variation in grape varieties used for the traditional production of wines from partially dehydrated grapes is promising, as it indicates that there are many options for winemakers, which may result in a more diversified wine catalogue.

During drying, tartaric acid may decline in some berries (Rösti et al., 2018). At 28.4°Brix, there was a reported 48% and 35% drop in tartaric acid in Shiraz and Merlot, respectively, likely due to the precipitation of potassium hydrogen tartrate inside the berry. This finding has implications for wine quality and cultivar selection for oenological decisions.

An important consideration for grapes that are selected for drying is their health and susceptibility to rot. During drying, the evaporation of water causes changes in the cellular structure of the skins, which lose elasticity and become susceptible to breakage (Marquez et al., 2013). Correlations to rot include bunch compactness and berry skin thickness (Accordini, 2013). Different berries have different dehydration rates and susceptibility to fungal attack based on skin thickness (La Guerche et al., 2006).

Post-harvest fungal infections may affect dehydrated grapes used for the production of *passito* wines, as they are vulnerable to fungal attack during drying.

Vulnerability comes from skin wounds caused by dehydration, insect presence and handling of grapes. Drying rooms are a source of fungal diversity, causing infections that may impact wine quality (Lorenzini et al., 2016). Grapes in uncontrolled drying rooms are more susceptible to rot than controlled conditions where humidity is controlled (Fedrizzi et al., 2011). In particular, *Botrytis cinerea* (in the form of noble rot) is the most important fungal infection that contributes positively to the aromatic profile to enhance wine quality (Magyar and Soós, 2016; Lorenzini et al., 2013; Paronetto and Dellaglio, 2011). *B. cinerea* manifests in two forms: the desirable noble rot and the devastating grey rot (Negri et al., 2017). To date, a significant production of Amarone wine is still obtained from the traditional withering process (uncontrolled drying chamber), where the mould infection is difficult to control. In the form of grey mould, *B. cinerea* can negatively impact organoleptic quality at infection rates as low at 5% (Ky et al., 2012). The modern approach to this style of wine is to therefore control the drying chamber to limit the development of grey mold. Interestingly, grey mould and noble rot symptoms are caused by the same species, and there is no genetic difference between the isolates causing the different symptoms (Fournier et al., 2013). Global climate change may impact the occurrence of proper conditions needed for natural noble rot development (Vannini and Chilosi, 2013), indicating urgency for control over this factor. In Amarone, botrytized grapes included in the fermentation at in infection rate of 29% results in wines described with attributes such as “muddy”, “sherry-cognac” and “mushroom” (Zappoli et al., 2018). The impact of *B. cinerea* on wines made from partially dehydrated

grapes in Ontario has not been defined in literature. This study includes a chapter on this important wine grape fungus that potentially contributes to wine quality.

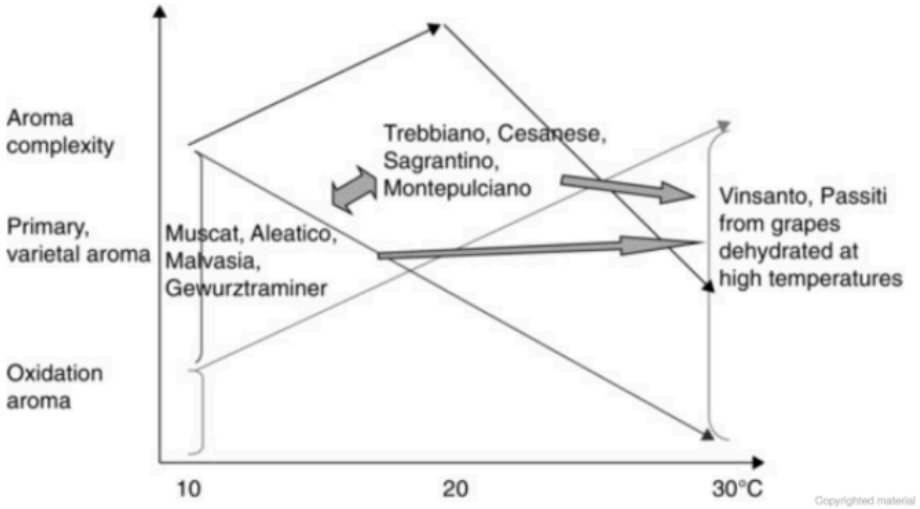

Figure 1.6: Changes of aromatic panorama of different grapes cultivars during dehydration (40% weight loss) at different temperatures based on experimental and commercial data (from Mencarelli and Bellincontro, 2013).

1.1.3 Microbiota of Grape Surface During Drying

There are changes in the microbiota on the surface of the grape during drying (Rantsiou et al., 2013). A recent study by Lorenzini and Zapparoli (2019) observed yeast-like fungi and yeast populations on the surface of withering grapes, which has a considerable impact on the final characteristics of *passito* wines. Most important was the sanitary state of the grapes, as damaged grapes contained a higher concentration of yeast cells on the berry surface. Yeast-like fungi isolates belonged solely to *Aureobasidium pullulans*, which is able to adapt to osmotically stressful environments, and has inhibitory effects on the growth of *B. cinerea*, *Aspergillus* and *Pencillium* (other grape fungi). Rantsiou et al. (2013) observed grape microbiotia during drying. For the first half of the withering process, *Hanseniaspora uvarum* was the most abundant

species, its frequency decreasing towards the end of dehydration. *Candida zemplinina* and *Aureobasidium pullulans* were major components of the microbiota at the end of the process. The largest number of species were present on the grape surface on the last day of dehydration. During fermentation, *S. cerevisiae* was the dominant population, but other species like *C. zemplinina* and *H. uvarum* were present, indicating a presence other than the starter culture for up to 14 days of fermentation. Spontaneous fermentation may avoid the standardisation of aromatic profiles experienced when using commercial strains of *S. cerevisiae.*

Traditionally, fermentation for Amarone is conducted at low temperature (3-5°C) by indigenous yeast. The surface of the grapes contains a large variety of moulds, bacteria and yeasts. Only a small proportion of the yeast can participate in the fermentation (Romano et al., 2003). Dellaglio et al. (2003) endeavored to evaluate the biodiversity of the *Saccharomyces* population that participate in the production of traditional Amarone fermentation; this is, without a selected starter culture. A total of 109 yeast strains were isolated from eight wineries in the Valpolicella area, where wines go through two fermentations: initial fermentation in tank, and re-fermentation in barrel. This study identified both *S. cerevisiae* and *S. bayanus* species throughout the study and indicated different distribution. The majority of S*. bayanus* strains were isolated during initial fermentation, while *S. cerevisiae* was found in highest numbers during re-fermentation. This suggests different benefits of each yeast; *S. cerevisiae* being the more ethanol-tolerant strain, while the various cryophilic *S. bayanus* strains fermented at successfully at low temperatures, even with selective pressure from the

presence of *B. cinerea* infected grapes. Due to its natural association with Amarone, *S. bayanus* is considered a specific and distinctive organism in Amarone fermentation (Paronetto and Dellaglio 2011).

1.2.2 Aroma and Flavour Composition

Characteristic flavour and aroma profiles of food and beverages arise from the composition and concentration of the volatile compounds present. Volatility refers to the ease of evaporation of a compound into the air, which allows for the volatile constituents to enter and move within the nasal or oral cavities where they can bind to olfactory receptors and elicit an olfactory response (Stradwick et al., 2017). Important compounds that contribute to the uniqueness of this wine are volatile organic compounds. Wine flavour is composed by a wide variety of compounds with different aromatic properties. Wine aroma is purely associated with odorous, volatile compounds can be detected at much lower concentrations than taste compounds (typically 10^{-4} to 10^{-12} g/L) and can be identified both ortho- and retro-nasally (while smelling a wine or tasting a sample). Flavour refers to the effects of both odour and taste: the totality of sensations perceived in the mouth, including touch. Contributions to taste are from both volatile and non-volatile compounds. While hundreds of different volatile compounds are present in a given wine, only a subset are likely to be actively contributing to flavour. (Lambrechts and Pretorius, 2000; Francis and Newton, 2005). Volatile components responsible for wine aroma come from a diverse group of chemical classes, mainly alcohols, esters, terpenes, norisoprenoids, volatile thiols, volatile fatty acids lactones, aldehydes, ketones and methoxypyrazines (Giacosa et al, 2019). Generally, wine aromas

come from three sources, and are described according to their origin. Primary aroma compounds originate from the grape, secondary aroma compounds are formed during fermentation, and tertiary aroma compounds are produced during ageing (Loizzo et al., 2013).

1.2.2.1 Grape-Derived

Primary, grape-derived aroma compounds that are responsible for varietal character of wines can be attributed to a few aroma compounds that are directly linked to specific aromas and flavours. The grapevine variety is the most important factor in determining the varietal character (D'Onofrio, 2013). The compounds can exist as volatiles (free form) or as non-volatile, odourless precursors which are released into their odour active form during processing (Rapp and Mandery 1986). These non-volatile constituents are known as aromatic precursors, and contribution to the wine matrix is realized when the flavour compound, the aglycone, is released from its glycosidically bound form (Stradwick et al., 2017). Primary volatile compounds include monoterpenes, norisoprenoids, aliphatics, phenylpropanoids, methoxypyrazines benzene compounds, C6 alcohols and volatile thiol compounds (Geffroy et al., 2018; Ruiz-Bejarano et al., 2016). There are few esters occurring in small quantities in grapes that contribute to the aroma of *Vitis vinifera* varieties, rather, they contribute to the characteristic aroma of indigenous varieties such as *V. labrusca* (Jackson, 2008). When grapes dry, some primary compounds increase. In Moscato bianco and Aleatico varieties (commonly used for *passito* wine production), there is a marked increase in monoterpenes, and above-threshold concentration of free monoterpenes, as well as geraniol. In Sangiovese, there

is a high concentration of monoterpenes, C13-norisoprenoids and benzene derivatives (Giacosa, et al., 2019).

Bellincontro et al. (2004) found that dehydrating Trebbiano, Malvasia and Sangiovese grapes either through controlled dehydration or in an accelerated drying tunnel increased the sugar content, ethanol concentration and the concentration of esters and higher alcohols, along with C6 compounds like hexanal in tunnel-treated grapes.

Ethanol, acetaldehyde, acetic acid and ethyl acetate are compounds which change during grape dehydration (Chkaiban et al., 2007; Constantini et al., 2006). Franco et al. (2004) found differences in ethanol, phenylethanol, ethyl acetate, isoamyl alcohol hexanoic acid, isobutanol, benzyl alcohol, 2-phenylethanol and 5-methylfurfural as a consequence of drying in Perdo Ximenez grapes. Santonico et al. (2010) report that acetic acid, ethyl acetate, ethanol, isoamyl acetate and hexanol are some of the compounds correlated to grape dehydration. Interestingly, this study indicated that the significant biochemical changes occur as a consequence of mass loss, rather than temperature. In particular, the changes are noted most dramatically at 40% weight loss. Urcan et al. (2017) found that in dehydrated berries, alcohols were the predominant volatiles compounds of all compounds measured, particularly 1-hexanol. This is further supported by a recent study (D'Onofrio et al., 2019) where peak concentrations of aroma compounds in different varietals at varied timepoints during withering were measured. In Rondinella grapes, optimal expression of volatiles occurred at 10 and/or 20% weight loss, Corvinone was at 20% weight loss and Corvina at 30% weight loss.

Understanding important varietals and their potential impact on aroma and flavour on appassimento-style wine can optimize quality so to make suitable processing decisions.

1.2.2.2 Wine-Derived

The secondary compounds produced by yeasts and bacteria during alcoholic and malolactic fermentation, known as fermentative flavour, generate the greater part of the aromatic compounds in wine, and are yeast strain specific (Romano et al., 2003; Fleet, 2003). With respect to the formation of aroma and flavour compounds in wine, it is important to consider the variables contributing to variation amongst such compounds. For example, the grapes, the strain of yeast, temperature, maceration, clarification procedures and nutrient medium (Clarke and Bakker, 2004) all contribute to sensorial differences in wines. The volatile compounds synthesised by wine yeasts include higher alcohols, medium- and long-chain volatile acids, acetate esters and ethyl esters (fruity and floral aromas) and aldehydes (buttery, fruity and nutty aromas) (Molina et al., 2007). Quantitively, ethanol, glycerol and acetic acid are the most abundant compounds in the wine matrix (Styger et al., 2011). Ethanol is the dominating alcohol, while diols, higher alcohols and esters (the majority of which are formed during fermentation) account for about 0.4-1.4g/L in red wine (Rapp and Manderey, 1986). Increased ethanol levels were found to change the perception of a wine from fruity to herbaceous and can also increase the perceived astringency of the tannins and the bitterness, roughness, and hotness of wine (Styger et al., 2011), an important consideration for high-alcohol appassimento-style wine.

With respect to post-harvest dehydration of grapes, it is the secondary metabolism of the grapes that is impacted, resulting in changes in phenolic and volatile wine contents (Bellincontro et al., 2004; Constantini et al., 2006; Marquez et al., 2013). As stated earlier, changes caused in grape and wine aroma profile are dependent on the dehydration process utilized; particularly influenced by temperature, RH and airflow (Panceri et al., 2016; Crilli et al 2007). Variation in the concentrations of these compounds can be classified by their drying time (López de Lerma et al., 2012). This study suggests, though, that excessive drying can compromise aromatic quality. When volatiles are monitored over the course of drying, most of the compounds reached maximum concentration at a dehydration rate of 18.8%. Initial conditions such as starting sugar concentrations can have a significant effect on volatile flavour production, where more volatiles are produced in wines generated from high sugar must (Lee et al., 2004). Although postharvest dehydration influences the volatile composition of grapes, the degree and significance of the changes depends on the starting sugar concentration (Moreno et al, 2008), where wine aroma analysis indicated an increase in compounds including guaiacol, cirtonellol and eugenol due to higher starting sugar concentration when compared to control. This study also reported the production of important compounds, norisoprenoids in particular, after harvest, consistent with changes that occur during extended ripening on the vine. This suggests an increase in floral aroma attributes is possible in wines made from partially dehydrated grapes. In a more recent study by Bellincontro et al. (2017) that compared wines made from grapes dried in a controlled airspeed in a drying tunnel (1.2m/s and 2.5m/s) and a non-controlled

environment, they reported a higher percentage of high alcohols in the control berries, and aldehydes and some esters were higher in the tunnel-dried grapes. Panceri et al. (2016) also utilized a controlled drying chamber and indicated higher concentrations of aldehydes and vanillin derivatives such as ethyl vanillate and vanilic acid in wines made with partially dehydrated grapes. Loizzo et al. (2013) reported increased concentrations of ethyl hexanoate, ethyl decanoate and isoamyl alcohol as the major constituents of *passito* wine. Other abundant compounds include acetic acid, and hexadeconaoic acid, and higher alcohols such as iso-butanol, 1-hexanol and 2,3-butanedoil. Terpenes were also indicated, but at much lower concentrations than the other volatiles.

Differences in volatile compounds due to dehydration may be due to sensitivity to water stress and enzymatic activity of lipoxygenase (LOX) during the drying process (Urcan et al., 2017). LOX is an important oxidative enzyme involved in lipid oxidation, which is temperature dependent and also dependent on the level of dehydration. The accumulation of C6 compounds has been indicated as a link to the accumulation of abscisic acid that promotes the activation of LOX (Costantini et al., 2006; Bellincontro et al., 2004).

All of these changes in volatile compounds may induce sensory changes, which can be classified into categories in relation to the kind of aroma they contribute to the matrix. Wines made from partially dehydrated grapes can be described with odours categories such as fruity, solvent, sweet, and roasted (Franco et al., 2004). Further, wines produced from partially dehydrated grapes have been described as higher in viscosity, astringency

and alcoholic sensation as well as higher intensities for terms like coffee, wood, vanilla and alcohol when compared to tables wines made with fresh fruit (Panceri et al., 2015; Panceri et al., 2017). Sensory perceptual differences in wines due to differences in volatile organic compound production is dependent on the sensory detection threshold of such compounds. In an effort to standardize this, odour activity values (OAVs) are calculated by dividing the concentration of an aromatic compound by its odour threshold value (Zhang et al., 2007). Sensory detection threshold values are defined as the lowest concentration of odorant that could be recognised by at least 50% of the individuals as different from that of a blank (Ferreira et al., 2000). An aroma compound found above its threshold (OAV>1) is considered as having an odour impact. The greater OAV above threshold, the more the aroma compound is thought to contribute to overall aroma (Ferreira et al., 2000). Optimizing wine sensory properties requires an understanding of the impact of the odorants that are produced, and how they interact with other components within the wine matrix. One of the long-standing goals of wine research has been to identify the volatile compounds that are central to particular olfactory attributes of wine, whether it be a subtle or dominating aroma note (Francis and Newton, 2005).

1.2 Fermentation Challenges for Wines made from Partially Dehydrated Grapes

Alcoholic fermentation is a redox-inert reaction. Glycolysis, the central metabolic pathway in *S. cerevisiae* yeast (Hohmann, 2002), uses an oxidized NAD^+ cofactor and reduces it to NADH via the conversion of glyceraldehydes-3-phosphate (GAP) to 1,3-bispohosphoglycerate to produce pyruvate. Alcoholic fermentation regenerates the

oxidized cofactor NAD^+ from the oxidation of NADH in the reduction of acetaldehyde to ethanol, which restores the redox balance of the cell. When fermenting partially dehydrated grapes, the management of the fermentation is of upmost importance. In stressful environmental conditions, such as a high sugar matrix, the yeast is placed under extreme stress, and yeast cells alter their metabolism to survive (Erasmus et al., 2004). This stress, known as osmotic stress, is well-understood in *S. cerevisiae* yeast. When transmembrane proteins that act as osmosensors detect extracellular stress, yeast cells respond via activation of the high osmolarity glycerol (HOG) pathway (Hohmann, 2002). Under osmotic stress, yeast exhibit a decreased growth rate during the initial phase of fermentation resulting in reduced biomass, along with cell shrinkage due to loss of cystolic water, and elevated levels of glycerol and acetic acid (Kontkanen et al., 2004). Osmoregulation is the cellular survival response directed at restoring and maintaining cell volume and turgor pressure as to continue normal biological function (Nevoigt and Stahl, 1997). The mechanism by which cells counteract the outflow of water is the intracellular accumulation of compatible solutes, and glycerol is indicated as having a role in *S. cerevisiae* osmoregulation (Hohmann, 2002). Due to the lack of a transhydrogenase in yeast to convert reducing equivalents between the NAD^+/NADH system and the $NADP^+$/NADPH system, yeast must rely on metabolite formation to maintain the intracellular redox balance for the coenzyme systems (van Dijken and Scheffers 1986). Glycerol formation is dependent on the glycerol-3-phosphate dehydrogenase enzyme that converts dihydroxyacetone phosphate to glycerol-3-phosphate, accompanied by an increase in NAD^+. The shift in redox balance

(NADH:NAD^+ratio) caused by the increased formation of glycerol is corrected via acetic acid production, which reduces NAD^+ back to NADH. Acetic acid biosynthesis may occur through the action of cystolic NAD+-dependent oxidation of acetaldehyde to acetic acid by a cytosolic aldehyde dehydrogenase (ALD), resulting in the reduction of NAD^+ to NADH (Pigeau and Inglis 2007). This accumulation occurs intracellularly and is then released into the matrix. There is a direct correlation between the sugar concentration in juice and the amount of glycerol and acetic acid produced by yeast, exemplified in Icewine fermentation where acetic acid increased from 0.17 to 1.24 g/L and glycerol increased from 5.3 to 9.3 g/L as the juice concentration increased from 21.3 to 38.8°Brix respectively (Pigeau and Inglis, 2005). Pigeau and Inglis (2007) also found that increasing the soluble solids concentration of Icewine must from 40 to 46 °Brix decreased yeast growth, sugar consumption rate, the total amount of sugar consumed, and the total concentration of ethanol produced.

1.2.1.2 Oxidation Compounds of Interest

Wine made using the appassimento technique is at risk for off-flavours and odours to become problematic due to high starting sugar concentration of the must. Acetic acid and associated compounds like acetaldehyde and ethyl acetate can accumulate and potentially mask fermentation aroma, resulting in off-odours that can impact wine quality (Moio and Piombino, 2013). When yeast cells are exposed to high sugar environments, they produce higher concentration of glycerol and acetic acid (Erasmus et al., 2004). Acetic acid has been measured in wines made from partially dehydrated grapes across literature with a concentration range of 0.5±0.04 g/L to

1.23±0.01 g/L (Panceri et al. 2015; Loizzo et al., 2013; Torchio et al., 2016; López de Lerma et al., 2012; Giordano et al., 2009), with different varieties and drying methods and times implemented in all studies. As weight loss increased from 20% to 30% in Amarone wine, acetic acid (0.48±0.03 g/L to 0.62±0.08 g/L in Corvina, 0.47±0.03 g/L to 0.68±0.06 g/L in Corvinone and 0.51±0.06 g/L to 0.65±0.08 g/L in Rondinella, respectively) and glycerol levels (8.10± 0.32 mg/L to 9.40±0.40 mg/L in Corvina, 8.70±0.23 mg/L to 9.54±0.65 mg/L in Corvinone and 9.00±0.23 mg/L to 9.48±0.18 mg/L in Rondinella, respectively) increased concurrently (Bellincontro et al., 2016). During Amarone fermentation, the pattern of glycerol and acetic acid production differed between *S. cerevisiae* and *S. uvarum* (closely related to *S. bayanus*) yeasts, with higher glycerol yields and lower acetic acid production in the wines fermented with *S. uvarum* reported (Tosi et al, 2009). Acetic acid is an important component influencing the final quality of wine. At elevated levels, it is associated with spoilage and can reduce varietal character (Nurgel et al., 2004; Macías et al., 2012). In table wine, acetic acid is detectable at 0.6–0.9 g/L and considered problematic at 1.2–1.3 g/L (Macías et al. 2012). Considerations for different wine styles are written in to legislation on limits for these compounds (discussed below). Volatile ester concentrations in wine are generally low, and ethyl acetate has the highest concentration in wines of this low-producing class of volatiles, and it is considered a volatile constituent that has great sensorial impact on wines made from dehydrated grapes (Moio and Piombino 2013). Ethyl acetate concentration was reported in Amarone wines fermented with seven *S. bayanus* yeast strains (range: 0.7-1.7 mg/L, average 1.2 mg/L) and 14 *S. cerevisiae* yeast strains (range:

1.2-7.1 mg/L, average 3.1 mg/L), and *S. bayanus* strains consistently produce less of this metabolite (Torriani et al., 1999). Ethyl acetate is produced in greater concentrations when starting sugar concentration is high (Lee et al., 2004). The presence of ethyl acetate is always accompanied by acetic acid, as it forms from acetic acid and ethanol (Jackson, 2008). Ethyl acetate is considered to negatively affect a wine matrix at 1.0 g/L, as it may potentially mask favourable compounds like fruity ethyl esters (Jackson, 2017), while its detection threshold falls within 0.10 g/L and 0.12 g/L. Acetaldehyde is a major component of fermentation, and an important aroma compound formed from pyruvate early during vinification and constitutes more than 90% of the total aldehyde content of wine. It is the end product of glycolysis in *S. cerevisiae* and is also a precursor metabolite for ethanol synthesis (Styger et al., 2011). Acetaldehyde serves as the electron acceptor used for NADH re-oxidation during fermentative growth and is reduced to ethanol by alcohol dehydrogenase (Hohmann, 2002). Chkaiban et al. (2007) have indicated that this response is higher when dehydration occurs at a faster rate. With respect to drying, both acetaldehyde and ethyl acetate content in grapes significantly increased at 10% of weight loss and at 26% of weight loss, respectively, in uncontrolled and controlled drying environments (Chkaiban et al., 2007), again outlining the importance of drying conditions on quality. Acetaldehyde is considered favourable at concentrations of around 0.70 g/L, imparting fruit characteristics to the wine, yet at higher concentrations (1.0 to 1.2 g/L), it represents a wine fault and is reminiscent of bruised apples and oxidation (Byrne and Howell, 2017). The “marked, oxidized sensory profile” (Jackson,

2008) of appassimento-style wine is tolerant of these compounds, but quality can be compromised when concentrations of these compounds are too high.

1.3 Yeast Selection for High Sugar Fermentation

The chemical composition of musts and wines is dependent on several factors, such as the grape variety, maturation level, rootstock, weather, vineyard conditions, soil type, fertilizer, oenological factors and yeast species used for fermentation (Panceri et al. 2015). Yeast, however, have the dominating influence because of their role in conducting the alcoholic fermentation (Fleet, 2003). There are important considerations for yeast selection for production of wine made from partially dehydrated grapes. Certainly, the yeast will need to be ethanol-tolerant, tolerant of osmotic stress and robust enough to endure the challenges associated with high sugar fermentation. Utilization of yeast strains that enhance varietal wine flavours as a wine to contribute to wine complexity is common practice amongst winemakers (Cordente et al., 2012). When wines made with selected *S. cerevisiae* strains were sensorially compared to 'wild' *S. cerevisiae* strains, organoleptic differentiation was observed, suggesting the 'flavour' phenotype has indeed been a target for wine yeast domestication. Further, domesticated strains have been indicated a having different sensory profiles (Cordente et al., 2012). Yeast aroma production can be classified into general chemical categories: alcohols, esters, carbonyl compounds, sulfur-containing compounds and organic acids, all of which differ based on the strain of yeast used for primary fermentation (Thorngate, 1998). The production of yeast-derived compounds is highly variable amongst selected yeast strains, and therefore appropriate yeast selection can assist in

contributing to the sensorial impact of resultant wines. To this end, management of volatile acidity can be achieved with appropriate yeast selection due to the formation of a great number of by-products. (Lambrechts and Pretorious, 2000). Volatile acidity may exceed legal limits if fermentations are not managed. Icewine juice represents an osmotically stressful matrix due to the high starting sugar content with similar quality challenges to the must of partially fermented grapes. The choice of yeast strain could determine if metabolites concentrations in a wine will fall within legislated limits and therefore be accepted or rejected based on these parameters (Eramasus et al, 2004). Seven commercially available yeast strains were assessed for fermentation rate, acetic acid and glycerol production, along with sensory characteristics in Icewine fermentation (40°Brix starting soluble solids). Recommended yeasts for Icewine production based on these characteristics include SST, N96 and EC1118. Another a widely-used yeast for Icewine is the commercially available *S. cerevisiae* strain K1-V1116 (Kontkanen et al., 2004; Yang et al., 2017; Heit et al, 2018). Literature on wines made from partially dehydrated grapes (particularly Amarone) specify various strains of *S. cerevisiae* as the preferred selected yeast (Azzolini et al., 2013; López de Lerma et al., 2012; Fedrizzi et al., 2011). Considering all of the important characteristics for yeast selection for high sugar fermentation, a locally isolated yeast, *S. bayanus* CN1, has been indicated as a low producer of the oxidation compounds ethyl acetate, acetaldehyde and acetic acid, and may be applicable for the production of appassimento-style wines (Kelly et al., 2018).

1.3.1 Taxonomy of S. bayanus Yeast

Of all the selected yeast available for initiating alcoholic fermentation generally, *S. cerevisiae* is almost universally preferred and is ubiquitously referred to as "wine yeast" (Swiegers et al., 2005; Eglinton et al., 2000). Extensive ecological surveys of the natural variability of *Saccharomyces* populations have indicated a wide polymorphism amongst species and strains (Dellaglio et al., 2003). The *Saccharomyces* genus is composed of eight species: *S. arboricolus*, *S. bayanus*, *S. cariocanus*, *S. cerevisiae*, *S. kudriavzevii*, *S. mikatae*, *S. paradoxus* and S*. pastorianus* (Pérez-Través et al., 2014). The *Saccharomyces bayanus* species complex has been the source of considerable controversy with competing groups arguing that there were two natural subgroups under the same species (*Saccharomyces bayanus* var. *uvarum* and *Saccharomyces bayanus* var. *bayanus*) or two natural species (*S. bayanus* and *S. uvarum*). With the discovery of *S. eubayanus*, *S. bayanus* was more easily classified as an "industrial hybrid", as it is derived from the natural species *S. cerevisiae*, *S. eubayanus* and *S. uvarum* (Pérez-Través et al., 2014). It is closely related to *S. pastorianus*, which is derived from *S. cerevisiae* and *S. eubayanus* (Hittinger, 2013). In agreement with this, Libkind et al. (2011), indicate that all known strains of *S. bayanus* and its typestrain CBS 380^{T} are likely hybrids of *S. eubayanus* and *S. uvarum* with some contribution of *S. cerevisiae*. These authors suggest that both *S. bayanus* and *S. pastorianus* are considered hybrid varieties, whereas *S. uvarum* and *S. eubayanus* are natural species. Due to this debate, and the close relation to the natural varieties, the taxonomy of *S. bayanus* has changed over time.

As previously stated, *S. bayanus* yeast strains have been indicated as naturally-occurring during the dehydration and fermentation of such wine grapes. In general, wine fermentation with cryotolerant *S. bayanus* strains result in greater concentrations of some higher molecular weight alcohols (particularly 2- phenylethanol), and acetate esters (isoamyl acetate, 2-phenylethyl acetate, and ethyl lactate), increased glycerol concentration, and lower acetic acid production than *S. cerevisiae* (Eglinton et al., 2000; Naumov et al., 2011; Swiegers et al., 2005). The yeast central to this study, the indigenous *S. bayanus* CN1 strain, appears to be a good fit for appassimento style winemaking, due to its low production of potentially problematic compounds that arise during grape drying and high sugar winemaking. Considering the osmotic challenges associated with the fermentation of partially dehydrated grapes, a step-wise acclimatization technique (Kontkanen et al., 2004) has been indicated as an appropriate inoculation method for Icewine. This conditioning method resulted in higher cell biomass and viability of the yeast cells allowing more sugar to be consumed in a shorter time and for the must to be fermented to the desired alcohol concentration. This kind of consideration for the fermentation of must from partially dehydrated grapes will increase yeast survival and mitigate potential wine quality problems.

1.4 Ontario Wine Industry

The designated wine appellations in Ontario can be divided into two categories: principle wine regions, which includes the Niagara Peninsula, Lake Erie North Shore and Prince Edward County, and emerging wine regions, which includes Norfolk, Huron, Grey and Durham (Shaw, 2017). Of these areas, the Niagara Peninsula has the most land

under vine and the largest concentration of wineries (Voronov et al., 2013). It is situated at N43° latitude, considered within the regional climate limits of commercial grapewine production, denoted as a cool climate region (Shaw, 1999). As a wine region, Ontario experienced a pivotal moment when the 1988 Free Trade Agreement with the United States of America was implemented, exposing the industry to foreign competition, thus prompting the Ontario wine industry to adapt the fine winemaking standards implemented in international wine regions (Voronov et al., 2013). This included replacing the cold-hearty *Vitis labrusca* varietals with *Vitis vinifera* plantings (Voronov et al., 2013). Since then, Ontario's wine industry has gained international recognition as a legitimate producer. However, the climate is changing, and adaptive strategies to manage extreme weather events associated with climate change are necessary to maintain quality (Pickering et al., 2015). This includes new technologies, exploring new potential areas for wine production, selection of suitable varietals, and diversification (Pickering et al., 2015).

1.4.1 Cool Climate Winemaking

Cool climate wine regions have been characterised based on their ripening capacity, based on growing degree days and monthly temperature averages (Shaw, 1999). In Ontario, the regional climate is well-suited to the growth and production of early maturing varieties like Chardonnay, Pinot noir and Riesling (Ziraldo and Kaiser, 2007). Selection of cold-hardy varieties and suitable mesoclimates have enabled growers to reduce the incidence of freeze damage caused by late spring and early autumn frosts and low winter temperatures (Shaw, 2017). Even with appropriate site

and cultivar selection, a changing climate poses an ongoing threat to the wine industry, particularly in regions where sensitivity and vulnerability to climate change are more pronounced (Cyr et al., 2010). Grape dehydration in a protected environment represents a promising alternative for further ripening in the wine sector, because it can be carried out regardless of the regional climatic conditions. In adverse weather conditions, which can change vintage-to-vintage, this method may mitigate this threat, and potentially stabilize wine quality. However, unpredictable changes such as increased frequencies of extreme weather events and changes in average temperatures during growing season threaten the stability of grape yield, development and composition, as well as wine production and quality (Shaw, 2017; Ashenfelter and Storchmann, 2010; Teixeira et al., 2013). Regionally, these risks manifest as winter injury from frost damage, severe heat, drought, cooler temperatures during growing season and above-average rainfall (Cyr et al., 2010). Damage to the primary buds of *V. vinifera* vines is the most common form of winter damage in the Ontario's wine region (Shaw, 2017). With climate change in mind, agronomic and varietal adaptive methods will be critical to ensure sustainability. Understanding berry physiology and their response to abiotic stress will inform viticultural decisions to enable the breeding of appropriate cultivars moving forward in future climatic conditions (Rösti et al., 2018).

1.4.2 Regulations

Currently there is very little regulation around the production of appassimento style wine in Ontario. In British Columbia and Ontario, wine regulations have been established by Vintners Quality Alliance (VQA), which designates viticultural areas,

regional appellations and sub-appellations of wines produced within these grape growing areas. VQA Ontario requires that wines labelled with VQA symbol and appellation names follow standards and quality regulations for both grapes and wines, with consideration of grape ripeness and variety, fermentation techniques, labelling requirements, chemical criteria and sensory evaluations, based on the wine style, variety or wine category. The only reference to dried grapes within the legislation, falling under the category of "Vin de Curé" (VQA, 2019), states that grapes must be dried to a minimum of 27.0 °Brix at time of transfer to the fermentation vessel. Further, VQA Ontario has outlined permissible limits of volatile acidity (VA) for Vin de Curé, based on starting Brix (Table 1.1). The regulations for Vin de Curé apply to both sweet and dry wines produced from grapes dried post-harvest. Lacking from the regulations is an officially designated name for wines produced in this method, like the trademarked term Icewine that comes with a stringent set of regulations based on production standards in Ontario. In Valpolicella, production rules for Amarone are enforced by DOCG (Denominazione di Origini Controllata e Garantita), giving Amarone designated status as of 2010. These rules are quite rigid, and regulate the authorized grape varieties, number of vines per hectare, grape yield, minimum potential alcohol of grapes before dehydration (11%) and post-dehydration (14%), date of vinification (not before December 1), ageing time, maximum residual sugar (12 g/L), labelling requirements and others (DOCG).

Table 1.1: Permissible limits for VA (mg/L acetic acid) for Vine de Curé in Canada, set by VQA Ontario.

°Brix at beginning of fermentation	Acetic acid (mg/L)

27-28	1500
28-32	1800
Over 32	2100

1.4.3 CCOVI Appassimento Project

In the New World, where regulation is less strictly enforced, there are opportunities to adopt new technologies to optimize the traditional methods utilized in wine regions of the Old World. The Cool Climate Oenology and Viticulture Institute at Brock University launched a research initiative to examine the composition and sensory characteristics of wines produced with grapes dried by five different post-harvest drying techniques. Cabernet franc grapes were dried with the following drying regimes: kiln dried in refurbished kilns previously used in the tobacco industry (representing fast drying, at Reif Estates Winery); dried in a flower greenhouse during the shoulder-season (medium length drying, at European Planters in Niagara-on-the-Lake); dried in a commercial barn that represents the traditional drying method used in Valpolicella (slow drying length, Cave Spring Cellars drying barn); drying in a forced-air chamber that controls temperature and humidity (Vineland Research and Innovation Centre); and finally drying grapes by leaving netted clusters on the wine to wither naturally. The time required to reach the target °Brix (26.0 and 28.0) differed, based on temperature and humidity differences in each method. (Appassimento Wines for Ontario, Research Brief). The appassimento project represents a broad project that investigates drying technologies for the Ontario wine industry. A subset of the appassimento project is this PhD project, that utilizes one of the drying methods (commercial barn) to dry grapes

used to ferment appassimento with a locally isolated yeast wines over a broader starting sugar concentration range.

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Chapter 2 Characterization of *Saccharomyces bayanus* CN1 for Fermenting Partially Dehydrated Grapes Grown in Cool Climate Winemaking Regions

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Abstract: This project aims to characterize and define an autochthonous yeast, *Saccharomyces bayanus* CN1, for wine production from partially dehydrated grapes. The yeast was identified via PCR and Basic Local Alignment Search Tool (BLAST) analysis as *Saccharomyces bayanus*, and then subsequently used in fermentations using partially dehydrated or control grapes. Wine grapes were dried to 28.0°Brix from the control grapes at a regular harvest of 23.0°Brix. Both the partially dehydrated and control grapes were then vinified with each of two yeast strains, *S. bayanus* CN1 and *S. cerevisiae* EC1118, which is a common yeast used for making wine from partially dehydrated grapes. Chemical analysis gas chromatography-flame ionization detector (GC-FID) and enzymatic of wines at each starting sugar level showed that CN1 produced comparable ethanol levels to EC1118, while producing higher levels of glycerol, but lower levels of oxidative compounds (acetic acid, ethyl acetate, and acetaldehyde) compared to EC1118. Yeast choice impacted the wine hue; the degree of red pigment colouration and total red pigment concentration differed between yeasts. A sensory

triangle test (*n* = 40) showed that wines made from different starting sugar concentrations and yeast strains both differed significantly. This newly identified *S. bayanus* strain appears to be well-suited for this style of wine production from partially dehydrated grapes by reducing the oxidative compounds in the wine, with potential commercial application for cool climate wine regions.

Keywords: winemaking; partially dehydrated grapes; appassimento; yeast; *Saccharomyces bayanus*; sensory; Ontario; climate change adaptation

2.1 Introduction

In an increasingly competitive international marketplace, important strategic considerations include a focus on the reliable production of high-value wines, and on styles that help differentiate and brand a wine region. This creates particular opportunities for the emerging wine regions of the New World, to adapt the traditions of the Old World while developing technological advancements in viticulture and oenology to assist in the expression of regionality [1]. In the recent past, winemakers in Ontario, Canada have highlighted their unique regional identity with products such as sparkling Icewine (e.g., Inniskillin Wines). Moving beyond that, there is room for additional signature products that can help define this region. Developing such wine styles and their corresponding production technologies can support the sustainability of established appellations, as well as the development of nascent grape-growing regions.

The Ontario industry is economically important [2], and its success is intrinsically linked to its unique climate, which allows the growth of a range of premium *vinifera* grape

varieties [3]. However, it can be challenging to achieve optimal grape ripeness in the shorter growing season that is associated with Ontario's cool climate [4]. Further, weather volatility is an additional threat to grape-growing in this region, with the most salient risks associated with temperature extremes, rainfall variability, and winter and frost damage [5]. Therefore, it is prudent to adopt innovative strategies in order to mitigate the risks associated with a changing climate and stabilize quality from vintage to vintage.

Postharvest grape-drying (appassimento) followed by vinification is a technique that is traditionally employed in Northern Italy for Amarone wine production [6]. This method consists of ripening grapes off-vine to produce withered or partially dehydrated fruit. The drying process increases the concentration of total soluble solids, phenolic compounds, and odorants in the grapes [7,8]. The wines produced from these grapes have a higher concentration of ethanol, volatile aroma compounds, and anthocyanins [9,10]. In Ontario, Canada, wines made from partially dehydrated grapes are regulated by the Vintners Quality Alliance (VQA) under the term Vin de Curé [11].

Despite these benefits, wines made from partially dehydrated grapes can have increased levels of undesirable oxidation compounds in the wine, most notably acetic acid, ethyl acetate, and acetaldehyde [10,12,13]. At elevated concentrations, these compounds can negatively affect the organoleptic quality of the wine [14], and in the case of acetic acid, exceed legal limits enforced by the VQA [11]. The development of

these compounds is directly related to the high starting sugar concentration in the must that creates an environment of high osmotic stress for yeast.

Glycerol, the major compatible solute in *S. cerevisiae*, accumulates intracellularly as a survival response to hyperosmotic stress [15]. The accumulation of glycerol maintains cell volume and turgor pressure while limiting the efflux of intracellular water [15,16]. Glycerol formation is accompanied by an increase in NAD^+ production [17]. Under these conditions, the shift in redox balance ($NADH:NAD^+$ ratio) caused by the increased formation of glycerol is corrected via acetic acid production, which reduces NAD^+ to NADH [17–20]. Monitoring the development of glycerol and acetic acid during fermentation can therefore provide insights into the yeast's management of redox balance and hyperosmotic stress.

It has been suggested that autochthonous starter cultures have benefits for regional wines, including sparkling wines, in that they may be well-adapted to specific environmental conditions, and prospectively enhance the desired flavor and aroma profiles, which can impact the quality of regional wines [21–26]. We previously conducted a spontaneous fermentation of local Riesling Icewine juice from Ontario, Canada and identified that *Candida dattilla* along with *Kloeckera apiculata* and *Cryptococcus laurentii* dominated the fermentation and were still present at the end (day 30), whereas *S. cerevisiae* was not found [27]. In a later study, this *Candida dattilla* strain, which was initially identified using API Biomedical kits, was further identified as a *Saccharomyces* species by DNA sequencing of the 5.8S-ITS region. It was likely *S.*

bayanus or *S. pastorianus*, but the identification could not be finalized past the genus (unpublished). Since *S. bayanus* is reported as producing lower acetic acid levels during wine fermentation [28], the strain isolated from Icewine grapes in Ontario was further tested on its own in the osmotically stressful Icewine fermentation condition. A pure starter culture of this yeast was built up and inoculated into filter-sterilized 41.6°Brix Riesling Icewine juice, where it produced 7.7% *v*/*v* ethanol compared to 10.8% *v*/*v* from the control *S. cerevisiae* K1-V1116. However, the isolated yeast produced 1.3-fold lower acetic acid/sugar consumed compared to K1-V1116 [29]. Although this yeast did attain the minimum alcohol required for Icewine of 7%, commercial Icewines in Canada have been found to range between 8.4–12.6% *v*/*v* ethanol and for Riesling Icewines, between 9.1–12.2% *v*/*v* [30]. The combined value of autochthonous yeast for the expression of regionality and the positive preliminary results in Icewine led us to characterize this yeast strain during the fermentation of must from partially dehydrated grapes, which provides a less stressful sugar environment than Icewine juice, but still has potentially problematic oxidative quality concerns from this wine style [10,12,13].

In this study, a local yeast isolated from the skin of Riesling Icewine grapes [27] is tested in the fermentation of partially dehydrated grapes. Grapes were dried to 28.0°Brix and vinified with one of two yeast strains, *S. cerevisiae* EC1118, the commonly used yeast for this wine style, and the yeast of interest, CN1. Grapes picked at 23.0°Brix (a sugar level typical for red table wine production) were also fermented with the two yeast strains as a control.

The main objectives of our study are to (i) identify this locally-isolated yeast, (ii) determine its fitness for making wine from partially dehydrated grapes, and (iii) more fully understand the impact of high sugar fermentation on red wine composition, colour, and sensory quality. The results from this study should assist in optimizing winemaking from partially dehydrated grapes in cool climate wine areas such as Ontario, Canada, as well as inform international wine regions that are seeking regional differentiation or further innovation of their wine styles.

2.2 Materials and Methods

2.2.1 Yeast Strains

Two yeast strains were selected to carry out the fermentations. The commercial *S. cerevisiae* strain EC1118, was purchased from Lallemand (Montreal, QC, Canada). The local strain was isolated from Riesling Icewine grapes from the Niagara Region in Ontario, at the Cool Climate Oenology and Viticulture Institute (CCOVI). Four genomic areas were analyzed to identify this yeast: the internal transcribed spacer regions (ITS1 and ITS2), including the *5.8S* gene of the ribosomal DNA (GenBank accession number: MH317189); the D1/D2 domain of a large subunit of the *26S rRNA* gene region (GenBank accession number: MH318011); the mitochondrial *β-tubulin* gene (GenBank accession number: MH339593); and the mitochondrial cytochrome oxidase II gene (*COX*II) (GenBank accession number: MH339594). The ITS1-*5.8S rRNA*-ITS2 gene region was amplified via PCR with the universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG) and ITS4 (5'-TCCTCCGCTTATTGATATGC). The D1/D2 domain was amplified with the primers NL-1 (5'-GCATATCAATAAGCGGAGGAAAAG) and NL-4 (5'-

GGTCCGJGTTTCAAGACGG). The *β-tubulin* gene was amplified with the primer pair βtub3 (5'-TGGGCYAAGGGTYAYTAYAC) and βtub4r (5'-GCCTCAGTRAAYTCCATYTCRTCCAT), and the *COX*II gene was amplified with the primers COII5 (5'-GGTATTTTAGAATTACATGA) and COII3 (5'-ATTTATTGTTCRTTTAATCA). DNA sequencing analysis (Robarts Research Institute, London, ON, Canada) was performed on all four amplified genes, and the results were compared with all of the available sequence databases of DNA using the Basic Local Alignment Search Tool (BLAST).

2.2.2 Grape Harvest, Desiccation and Processing

Vitis vinifera Cabernet franc grapes were hand-harvested at Mazza Vineyards in Niagara-on the-Lake, Ontario, Canada, at approximately 23.0°Brix. First, 209 kg of grapes were picked and placed in perforated drying containers in a single layer. Grapes were divided into two parcels and delivered to two locations. One of the parcels was delivered to CCOVI (Brock University, St Catharines, ON, Canada) and processed on the following day after temperature stabilization overnight at room temperature. The other parcel was delivered to Cave Spring Cellars Barn (4424 Cave Spring Road, Beamsville, ON, Canada), which is dedicated to drying grapes for producing commercial Vin de Curé wines [11]. The drying containers were stacked 14 layers high, with adequate air space between each container to receive natural ventilation in the barn. Fifteen randomly selected clusters were collected weekly. The samples were hand-crushed in a plastic bag and strained through a metal strainer to collect must. Must samples were analyzed for soluble solids, pH, and titratable acidity. Once the target sugar concentration was reached (28.0°Brix), the partially dehydrated grapes were delivered to CCOVI for

processing after temperature stabilization overnight. Grapes were crushed and destemmed (model Gamma 50, Mori-TEM; Florence, Italy) into 30-L steel fermentation vessels with tight-fitting lids. Must was blanketed with CO_2, lids were secured, and vessels were stored at 22°C prior to yeast inoculation. Must volume was estimated by multiplying weight by 0.75 for control must, and 0.60 for partially dehydrated grape must to account for desiccation effects. Then, 500 mg L^{-1} of diammonium phosphate (DAP; Laffort, Bordeaux, France) was added to the must and mixed by punch down. A further 250 mg L^{-1} of DAP was added on the third day of fermentation to reduce yeast stress.

2.2.3 Winemaking

Four sets of triplicate fermentations were carried out: (i) 23.0°Brix must fermented with *S. cerevisiae* EC1118, (ii) 23.0°Brix must fermented with *S. bayanus* CN1, (iii) 28.0°Brix must fermented with EC1118, and (iv) 28.0°Brix must fermented with CN1. Fermentations were conducted using the same microvinification protocols. *S. cerevisiae* EC1118 was rehydrated according to manufacturer's directions and plated out on yeast extract peptone dextrose plates (YPD, 1% yeast extract, 2% peptone, 2% dextrose, 2% agar). CN1 yeast was prepared from a frozen glycerol stock, and also plated out on YPD plates. Both yeasts were grown to appropriate colony size prior to preparing a starter culture in sterile-filtered grape juice. The starter cultures were built up in sterile-filtered Cabernet franc must, and then followed a step-wise acclimatization procedure as outlined in Kontkanen et al. [20]. The yeast strains were inoculated from YPD plates into

750 mL of 10°Brix sterile-filtered must with the addition of 2 g L^{-1} DAP and grown aerobically at 25°C with shaking at 0.605× *g* until cell concentration reached 2 × 10^8 cells mL^{-1}, as determined by haemocytometry. Then, 750 mL of sterile-filtered 23.0°Brix control must was added to each build-up culture and held for 1 h at 25°C with swirling every half hour. Then 1.5 L of control cultures for both EC1118 and CN1 were added to 28.5 L of 23°Brix control must to reach an inoculum of 5.0 × 10^6 cells mL^{-1} in 30L stainless steel fermentation vessels. The 28.0°Brix treatment required one more acclimatization step for both yeast, and 750 mL of sterile-filtered 28.0°Brix dehydrated grape must was added to each starter culture and held for 2 h at 25°C with swirling every half hour, after which the 2.25-L culture was inoculated into 27.75 L of 28.0°Brix dehydrated grape must to reach an inoculum of 5.0 × 10^6cells mL^{-1} in the 30L fermentations.

After inoculation, the fermentations were gently mixed by punch down and moved to a temperature-controlled chamber at 22°C. Fermentations were monitored once daily by recording soluble solids (hydrometer, °Brix) and temperature (thermometer, °C). The caps were punched down twice daily with 20 plunges per vessel using a separate punch-down tool for each yeast trial; this number was gradually reduced to four plunges near the end of the fermentation. As the cap started to fall, fermentations were blanketed with CO_2 to protect them from oxidation. Fermentations were considered complete once the yeast stopped consuming sugar (<5 g L^{-1}) and/or the sugar concentration stayed the same for three consecutive days, as confirmed by a wine scan analysis

conducted by WineScan™ FT120 (FOSS, Hillerød, Denmark). Once complete, fermentation replicates were pressed separately with a small bladder press (Enotecnica Pillan, Vicenza, Italy) at one bar for two minutes into glass carboys. Then, 50 mg L^{-1} of sulfur dioxide (as potassium metabisulfite) was added to each treatment, which were left to settle at room temperature. Wines were then racked and moved to a −2°C chamber for cold stabilization. Wines were subsequently filtered through 0.45 µm filter pads, bottled in 750 mL glass wine bottles, with a manual bottler (Criveller Group; Niagara Falls, ON, Canada), closed with natural cork with an automated corker (model ETSILON-R, Bertolaso; San Vito, Italy), and stored in the CCOVI wine cellar (17.5°C, 74.5% RH).

2.2.4 Grape, Must and Fermentation Analysis

Fermentation temperature was monitored with a thermometer (°C). Soluble solids were determined using an Abbe bench top refractometer (model 10450, American Optical; Buffalo, NY, USA) for grape and must samples, and using a degree Brix hydrometer for fermentation time course samples. pH was determined using a pH meter (SympHony, VWR, SB70P, Mississauga, ON, Canada), and titratable acidity was determined by titration with 0.1 mol L^{-1} of NaOH to an endpoint of pH 8.2 [31]. Glucose, fructose, glycerol, acetaldehyde, ethanol in must, amino acid nitrogen, ammonia nitrogen, acetic acid, lactic acid, and malic acid were determined with Megazyme Kits (K-FRUGL, K-GCROL, K-ACHD, K-ETOH, K-PANOPA, K-AMIAR, K-ACET, K-LATE, K-LMALL; Megazyme International Ireland, Limited, Bray Company, Wicklow, Ireland). Ethyl acetate and ethanol in wine were determined by gas chromatography (GC) using a Hewlett-Packard

6890 series gas chromatograph (Agilent Technologies Incorporated, Santa Clara, CA, USA) equipped with a flame ionization detector (FID), split/split-less injector, and Chemstation software (version E.02.00.493). Separations were carried out with a DB®-WAX (30 m, 0.25 mm, 0.25 µm) GC column (122-7032 model; Agilent Technologies, Santa Clara, CA, USA) with helium as the carrier gas at a flow rate of 1.5 mL min^{-1}.

2.2.5 Colour Evaluation

Measures of colour density, hue, degree of red pigment colouration, and total red pigments were conducted based on the methods of Iland et al. [32] by UV-Vis spectrophotometer (Cary 60, Agilent Technologies, Santa Clara, CA, USA).

2.2.6 Sensory Evaluation

A preliminary bench tasting (n = 4) of the wines established that the winemaking replicates within each treatment were similar enough to blend into representative treatments for difference testing. Therefore, four treatments were presented to the panelists (EC1118, 23.0°Brix; CN1, 23.0°Brix; EC1118, 28.0°Brix; CN1, 28.0°Brix). A balanced and randomized triangle test design composed of six sets of triads was used to compare all of the treatments to each other. Each participant (n = 40) tasted a total of 18 samples over the course of two sessions. The first session consisted of three sets of three wines, separated by forced three-minute breaks between each set to minimize fatigue and carry-over effects. Consumption of water and unsalted crackers was encouraged. The samples were coded with a three-digit randomly assigned code, and the participants were asked to evaluate them in the order presented. Participants were

instructed to assess aroma by sniffing and flavor by tasting and expectorating the samples, and determine differences based on these observations. Their answers were recorded using the Compusense FiveTM computer program (Compusense Inc., Guelph, ON, Canada). The same format was used for the second session, which was completed after a one-hour break. The evaluations took place in individual booths in the sensory evaluation lab at CCOVI, which was equipped with red lighting to mask possible colour differences. Data was analyzed by comparing the number of correct responses to a critical value table for triangle tests [33].

2.2.7 Statistical Analysis

Analysis of variance (ANOVA) with mean separation by Fisher's Protected Least Significant Difference (LSD) test ($p < 0.05$) was conducted on chemical and colour parameters using the XLSTAT statistical software package (Addinsoft, Version 7.1; New York, NY, USA).

2.2.8 Statement of Ethics

All of the subjects gave their informed consent for inclusion before they participated in the study. The protocol for the study was approved by Brock University's Research Ethics Board (file number 14-021-INGLIS).

2.3 Results

2.3.1 Yeast Strain Identification

The sequencing results of the ITS1-*5.8S rRNA*-ITS2 gene region and the D1/D2 domain gene region were only able to identify the isolate at the genus level as a *Saccharomyces*

strain. Therefore, the mitochondrial genes *β-tubulin* [34] and *COX*II [35] were selected as biomarkers for further identification. The amplified sequences of *β-tubulin* showed a 99% similarity in sequence identity with a query coverage of 100% to three *S. bayanus* strains (Table 2.1). The results from the *COX*II mitochondrial gene reported an identical level of similarity to CBS 380^T and CBS 395^T Table 2.1), which are widely accepted type strains (taxonomic standards) of *S. bayanus* and *S. uvarum*, respectively [36,37]. Based on the Genbank sequence comparisons, we have identified this yeast as *S. bayanus*. Recent research reports the nearly identical similarity of the complete mitochondrial genome between these two potential species [38], further raising the question of whether *S. bayanus* and *S. uvarum* should be classified into two separate species (*S. bayanus*, *S. uvarum*) or two varieties under the species *S. bayanus* (*S. bayanus* var. *bayanus*, *S. bayanus* var. *uvarum*) [36].

Table 2.1: Homology of CN1 mitochondrial genes with GenBank sequences.

Gene Region	NCBI Database Strain for Sequence Comparison	GenBank Accession Number	Base Pairs*	Alignment Results		
				Max Score	Query Coverage	Sequence Identity
β-tubulin	*S. bayanus* Strain BCRC 21818	FJ238317.1	849/852	1555	100%	99%
	S. bayanus Strain BCRC 21964	FJ238319.1	848/852	1550	100%	99%
	S. bayanus Strain BCRC 21816	FJ238316.1	847/852	1546	100%	99%
	S. eubayanus Strain N/A	XM 018364800.1	815/852	1367	100%	96%
	S. pastorianus Strain BCRC 21420	FJ238324.1	813/852	1356	100%	95%
*COX*II	*S. bayanus* Strain CBS380^{T}	KX657743.1	632/635	1157	99%	99%
	S. uvarum Strain CBS395^{T}	KX657742.1	632/635	1157	99%	99%
	S. bayanus Strain CBS380	AP014933.1	632/635	1157	99%	99%
	S. bayanus x *S. uvarum* Strain CECT1991	JN676774.1	585/585	1081	91%	100%
	S. *eubayanus* Strain CRUB1975	KF530344.1	608/620	1079	97%	98%

2.3.2 Fermentation Kinetics and Metabolites

The must parameters for all treatments are listed in Table 2.2. The *S. bayanus* CN1 yeast consumed sugars at a higher rate than the control yeast EC1118 at the beginning of both fermentation treatments but left 15.8 g L^{-1}unfermented sugar (mainly fructose) in the 28°Brix treatment wine (Figure 2.1; Table 2.3). Despite CN1 leaving residual sugar in the high Brix ferments, CN1 produced a comparable level of ethanol to EC1118, and significantly less oxidative compounds (acetaldehyde, acetic acid, ethyl acetate) for both the control and wines made from the dehydrated grapes (Table 2.3). Regardless of the winemaking treatment, wines fermented with CN1 contained higher levels of glycerol,

titratable acidity, and malic acid in comparison to wines fermented with EC1118, but lower lactic acid in the 23°Brix fermentation (Table 2.3).

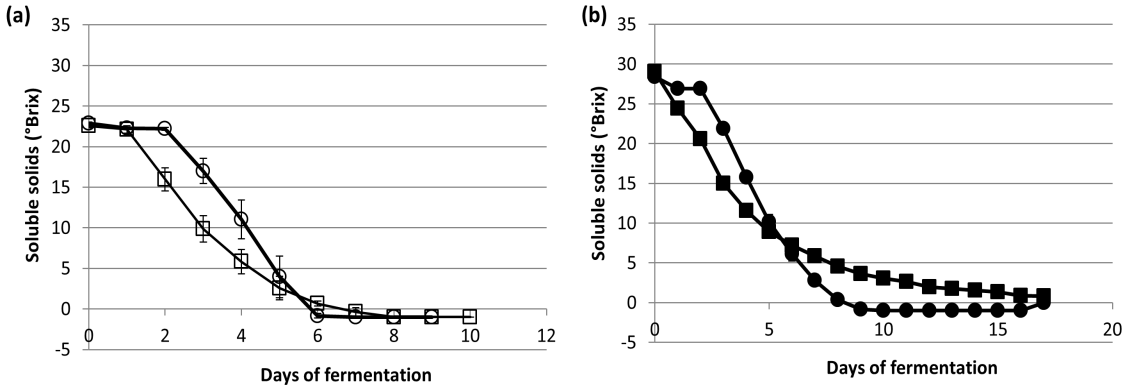

Figure 2.1: Soluble solid levels during fermentation.

(a) The 23°Brix control must was inoculated with EC1118 (○) and CN1 (□); (b) the 28°Brix partially dehydrated grape must was inoculated with EC1118 (●) and CN1 (■). Data represents the mean value ± standard deviation of duplicate measurements per sample (three winemaking replicates per treatment).

Table 2.2: Chemical composition of Cabernet franc control must (23 °Brix) and must from partially dehydrated grapes (28 °Brix).

Data represents the mean value ± standard deviation of duplicate measurements per sample (three winemaking replicates per treatment). Lowercase letters within the same parameter indicate differences between treatments (Fisher's Protected $LSD_{0.05}$).

Parameter	23°Brix EC1118	23°Brix CN1	28°Brix EC1118	28°Brix CN1
Reducing sugar (g L^{-1})	218 ±8^{b}	198 ±12^{a}	300 ±3^{c}	301 ±3^{c}
Glucose (g L^{-1})	108 ±4^{b}	98 ±6^{a}	145 ±2^{c}	145 ±1^{c}
Fructose (g L^{-1})	111 ±5^{b}	100 ±6^{a}	155 ±2^{c}	156 ±2^{c}
pH	3.39 ±0.05^{a}	3.35 ±0.01^{a}	3.34 ±0.03^{a}	3.33 ±0.03^{a}
Titratable acidity (g L^{-1} tartaric acid)	5.8 ±0.2^{b}	6.1 ±0.1^{c}	4.8 ±0.0^{a}	4.9 ±0.0^{a}
Ammonia nitrogen (mg N L^{-1})	17 ±9^{b}	12 ±2^{a,b}	8 ±1^{a}	8 ±2^{a,b}
Primary amino nitrogen (mg N L^{-1})	62 ±13^{b}	47 ±2^{a}	61 ±3^{b}	63 ±5^{b}
Ethanol (% v/v)	0.009 ±0.004^{a}	0.005 ±0.001^{a}	0.030 ±0.006^{b}	0.031 ±0.006^{b}
Glycerol (g L^{-1})	0.0 ±0.0^{a}	0.0 ±0.0^{a}	0.3 ±0.1^{b}	0.3 ±0.0^{b}
Malic acid (g L^{-1})	2.2±0.3^{a}	2.1±0.1^{a}	2.1 ±0.1^{a}	2.0 ±0.1^{a}
Lactic acid (g L^{-1})	0.04±0.00^{a}	0.04±0.11^{a}	0.05 ±0.00^{b}	0.06 ±0.00^{b}
Acetaldehyde (mg L^{-1})	<18^{a}	<18^{a}	<18^{a}	<18^{a}
Acetic acid (g L^{-1})	0.01 ±0.00^{a}	0.00 ±0.00^{a}	0.01 ±0.00^{b}	0.01 ±0.00^{b}
Ethyl acetate (mg L^{-1})	n/d	n/d	n/d	n/d

Table 2.3: Chemical composition of Cabernet franc control wine (23 °Brix) and wine made from partially dehydrated grapes (28 °Brix).

Data represents the mean value ± standard deviation of duplicate measurements per sample (three winemaking replicates per treatment). Lowercase letters within the same parameter indicate differences between treatments (Fisher's Protected $LSD_{0.05}$).

Parameter	23°Brix EC1118	23°Brix CN1	28°Brix EC1118	28°Brix CN1
Reducing sugar (g L^{-1})	<0.07^{a}	0.2 ±0.0^{a}	<0.07^{a}	15.8 ±6.7^{b}
Glucose (g L^{-1})	<0.07^{a}	<0.07^{a}	<0.07^{a}	1.1 ±0.7^{b}
Fructose (g L^{-1})	<0.07^{a}	0.1 ±0.0^{a}	<0.07^{a}	14.7 ±6.0^{b}
pH	3.78 ±0.09^{b}	3.54 ±0.04^{a}	3.74 ±0.00^{b}	3.59 ±0.05^{a}
Titratable acidity (g L^{-1} tartaric acid)	6.4 ±0.3^{a}	9.4 ±0.3^{c}	6.8 ±0.2^{a}	8.1 ±0.3^{b}
Ammonia nitrogen (mg N L^{-1})	<6^{a}	<6^{a}	<6^{a}	<6^{a}
Primary amino nitrogen (mg N L^{-1})	28 ±3^{a}	24 ±3^{a}	40 ±2^{b}	36 ±4^{b}
Ethanol (% v/v)	13.0 ±0.3^{a}	12.6 ±0.4^{a}	15.3 ±0.7^{b}	14.7 ±0.2^{b}
Glycerol (g L^{-1})	8.5 ±0.4^{a}	11.1 ±0.6^{b}	11.2 ±0.1^{b}	13.6 ±0.2^{c}
Malic acid (g L^{-1})	1.6±0.4^{a}	4.2±0.2^{c}	1.9 ±0.1^{a}	2.5 ±0.1^{b}
Lactic acid (g L^{-1})	0.45±0.42^{b}	0.04±0.01^{a}	<0.03^{a}	<0.03^{a}
Acetaldehyde (mg L^{-1})	56 ±7^{b}	38 ±5^{a}	88 ±7^{d}	70 ±9^{c}
Acetic acid (g L^{-1})	0.30 ±0.02^{c}	0.06 ±0.01^{a}	0.36 ±0.02^{d}	0.20 ±0.02^{b}
Ethyl acetate (mg L^{-1})	36 ±3^{b}	21 ±3^{a}	37 ±13^{b}	33 ±2^{a}

2.3.3 Colour and Sensory Evaluation:

There were no significant differences between the wines in colour density, which describes the intensity of wine colour (Figure 2.2a). The hue, a measure of the shade of wine colour, was lower in the 23°Brix CN1 wine (Figure 2.2b). The total red pigments in CN1 wines were lower than that in EC1118 wines for both winemaking treatments (Figure 2.2c). However, the degree of red pigment colouration was higher in CN1 wines than in EC1118 wines, suggesting a higher percentage of red-coloured pigments in wines fermented by CN1 despite the lower concentrations of total red pigments (Figure 2.2c,d). Sensory evaluation also indicated perceptible differences between all wines with different yeast and starting sugar treatment (Table 2.4).

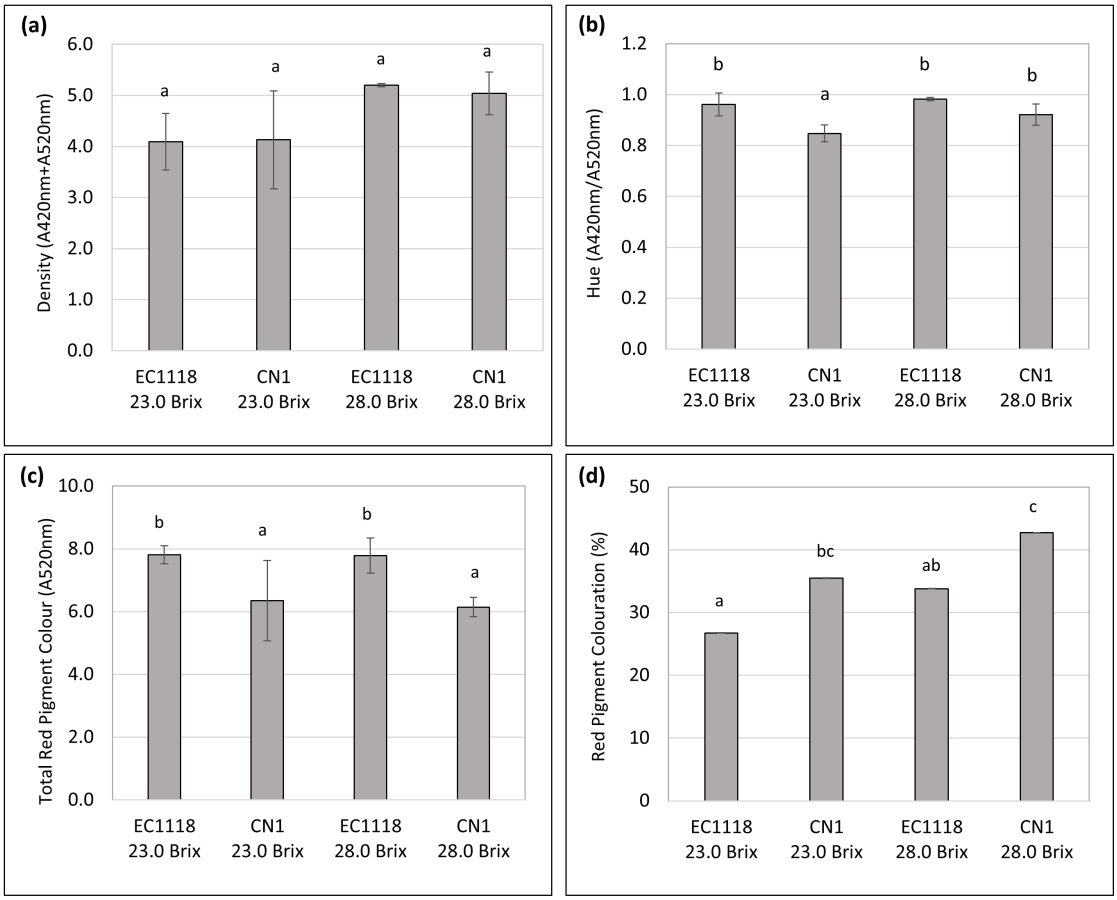

Figure 2.2: (a) Wine colour density (b) wine hue, (c) total red pigment colour (anthocyanins, oligomers and polymers) and (d) degree of red pigment colouration (%) of control wines (23°Brix) and wines made from partially dehydrated grapes (28 °Brix) with either EC1118 or CN1.

Data represents the mean value ± standard deviation of duplicate measurements per sample (three winemaking replicates per treatment). Lowercase letters indicate differences between treatments (Fisher's Protected $LSD_{0.05}$).

Table 2.4: Triangle Test results to determine sensory differences between wines (n=40).

Significance was assessed by comparing proportion of correct responses to critical values [33].

Paired Treatments	Correct	Incorrect	Total	Significance
23°Brix EC1118 vs. 23°Brix CN1	25	15	40	p=0.001
28°Brix EC1118 vs. 28°Brix CN1	34	6	40	p=0.001
23°Brix EC1118 vs. 28°Brix EC1118	25	15	40	p=0.001
23°Brix EC1118 vs. 28°Brix CN1	32	8	40	p=0.001
23°Brix CN1 vs. 28°Brix EC1118	26	14	40	p=0.001
23°Brix CN1 vs. 28°Brix CN1	37	3	40	p=0.001

2.4 Discussion

The main aim of this study is to investigate a low acetic acid-producing yeast, the newly identified yeast *S. bayanus* CN1, within the context of wine production from partially dehydrated grapes, which is a process that involves a high sugar fermentation and is often associated with undesirable oxidation compounds. The results presented in this study are based on chemical and preliminary sensorial analysis that demonstrate lower oxidation compounds produced by CN1 and perceptive differences from EC1118, which is the commonly used yeast for this winemaking style.

In an analysis of Amarone vinified with *S. cerevisiae* EC1118, the authors report concentrations of 0.56 ± 0.02 g L^{-1}acetic acid, 57.20 ± 2.12 mg L^{-1} ethyl acetate, 18.47% ethanol, and 6.41 ± 1.00 gL^{-1} residual sugar [39]. Their study reported a starting sugar concentration of 30°Brix, which is higher than the present study, contributing to the different but proportional results. An analysis of commercial Amarone wines over four vintages (1998–2001) reported similar acetic acid levels of 0.52–0.62 g L^{-1}, ethanol levels of 15.15–15.88%, and residual sugar levels of 0.29–0.8%, equating to 2.9–8 g L^{-1} [6]. The

wines in this current study that were made from partially dehydrated grapes had a starting sugar concentration of 28.0°Brix, resulting in an ethanol range of 14.7–15.3%, which is proportional to the starting sugar concentration of the Amarone wines outlined in the literature. Similarly, the high starting sugar wines fermented in this study with EC1118 had an acetic acid concentration of 0.36 g/L^{-1}; this is lower than the Amarone values reported in the literature, which is likely due to the lower starting sugar concentration, while CN1 produced even lower levels of acetic acid at 0.20 g/L^{-1}. This result suggests the potential commercial application of the CN1 yeast to winemaking using partially dehydrated grapes to assist in mitigating the quality challenges associated with undesirable oxidation compounds in the final wine [10,13]. This wine style in Ontario in commercial production targets starting sugar concentrations of the dried fruit between 27–28°Brix. Although CN1 did not ferment the 28°Brix must to complete dryness, Amarone wines are also found with residual sugar [6,39]. Additionally, Alessandria et al. [40] found that autochthonous yeast yielded incomplete sugar transformation, but the authors suggest that this result should not be considered negative for this type of wine, as residual sugar is typical for some wines made from partially dehydrated grapes, offering an opportunity for stylistic considerations for the winemaker [39–42]. Further, studies are currently underway to evaluate the sugar range over which CN1 does ferment to dryness.

Despite the lower production of acetic acid by CN1 in the wines, this yeast produced higher concentrations of glycerol in comparison to EC1118. It has been well-established

that glycerol is produced as an intracellular osmolyte in *S. cerevisiae* under hyperosmotic stress during wine fermentations accompanied by acetic acid production. The link between these two metabolites in *S. cerevisiae* under hyperosmotic stress is based on a redox balance of the NAD^+/NADH system. The formation of glycerol generates NAD^+ [15,17,43]. Acetic acid production from acetaldehyde reduces NAD^+ to NADH through the activity of a NAD^+-dependent aldehyde dehydrogenase, and corrects the redox shift [17,18,44]. We recently reported a 24-fold higher NAD^+/total NAD(H) ratio in *S. cerevisiae* on fermentation day 2 during fermentation of 39°Brix juice compared to 20°Brix juice, which was correlated with higher glycerol production followed by acetic acid production [17]. In this current study, higher acetic acid production under osmotic stress was also noted in both yeast strains at the 28°Brix treatment compared to the 23°Brix treatment. However, *S. bayanus* CN1 produced more glycerol, but less acetic acid, in comparison to *S. cerevisiae* EC1118 at this higher brix condition. *S. bayanus* CN1 has a different response to osmotic stress than *S. cerevisiae*. Acetic acid may still be produced by *S. bayanus* as a response to glycerol production, but it may be further metabolized within the yeast as opposed to being released from the cell into the wine. Alternatively, a different metabolite may be used to reduce NAD^+ to NADH for redox balance, resulting in the lower acetic acid in the wine. Additional studies investigating the NAD(H) ratios in CN1 and yeast metabolites will provide insight on the mechanism and regulation of acetic acid production in high sugar fermentations in this yeast.

Wine colour provides a quick reference of potential quality for consumers. The consumer can gather information about the wine's age, condition, body, and possible defects simply by looking at the wine as it leaves the bottle [45]. The basis for red wine colour is anthocyanin content, and major secondary factors that are known to affect colour density are pH and sulfur dioxide (SO_2) content. Interestingly, despite the low pigment content present in the wines vinified by *S. bayanus* CN1, at both sugar levels, they displayed a higher percentage of red-coloured pigments than wines produced by EC1118. The CN1 control wine also showed a lower wine colour hue compared to the other treatments. This could be caused by the lower pH in wines vinified by *S. bayanus*. It is accepted in the literature that the structure and colour of anthocyanins are affected by pH, as acidification enhances the colour intensity of red wine via the formation of the flavylium cation [46]. In addition to their direct role on colour, anthocyanins can also contribute to the taste and chemical characteristics of wine because of their interactions with other molecules [47,48]. Therefore, they could have influenced the sensorially perceptible differences in the wines that were detected in this study. This is in agreement with the existing literature that found perceptible sensorial differences between the Amarone wines fermented with commercial *S. cerevisiae* yeast and those fermented with the inclusion of autochthonous yeast and non-*S. cerevisiae* yeast [39,49]. It is also important to note that there are differences in the wines in other categories; namely, orthonasal and/or retronasal sensory differences, as well as discrepancies in ethanol or residual sugar concentrations that could contribute to discriminating among the wines. The desirable higher percentage of red-coloured

pigments associated with CN1 and the established sensory differences amongst the treatments raise further questions about the organoleptic implications of using this yeast for wine production from partially dehydrated grapes. The differences are yet to be fully characterized; approaches such as quantitative sensory profiling and consumer preference testing would be useful in this regard.

2.5 Conclusions

This study lays the groundwork for further investigation of the potential of *S. bayanus* CN1 yeast for winemaking from partially dehydrated grapes in Ontario and other geographic regions that experience cool or marginal climates for grape growing. Although vinifying grapes for Vin de Curé poses risks for winemakers of increased oxidative compounds, the reward is in a high-value product that also adds diversity to the portfolio of a winery as well as its region. The findings on the isolate CN1 reported in this study are positive with respect to the legislated limits on oxidative compounds and desired red colour hue and have established sensory differences from the accepted commercial standard EC1118. Further to that, we recommend an additional sensory evaluation of wine made from *S. bayanus* CN1 in order to more fully understand its market potential. Additionally, understanding the difference between glycerol and acetic acid production of CN1 in comparison to *S. cerevisiae* EC1118 might contribute to the management of high acetic acid frequently associated with high sugar fermentations.

Author Contributions: D.L.I. and G.P. conceived and designed the experiments; L.D. and F.D.P. conducted the fermentations; L.D. performed the chemical analysis; J.K. conducted the colour and sensory analysis; F.Y. prepared the yeast culture for fermentations; A.B., F.Y. and J.K. performed yeast identification; C.N. isolated the local *S. bayanus* strain. J.K., F.Y., G.P. and D.L.I. contributed to the writing of the manuscript.

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Chapter 3 Investigation of *Saccharomyces bayanus* CN1 Yeast Strain for Winemaking from Partially Dehydrated Grapes in Cool Climate Viticultural Areas

Abstract

The aim of this project is to investigate the application of a locally isolated yeast, *Saccharomyces bayanus* CN1, to wine made from partially dehydrated grapes. Appassimento style wines are made from grapes that have been dehydrated post-harvest to concentrate flavours and aromas. In cool climate winemaking regions that experience extreme climatic fluctuation that may impact fruit maturity and subsequent wine quality, drying grapes in a protected environment post-harvest may help optimize quality and help stabilize vintage-to-vintage variation. Previous work with this yeast yielded wines made from partially dehydrated grapes with 15.8 (±6.7) g/L of residual sugar, where often these wines are fermented to dryness. Therefore, drying targets for the partially dehydrated grapes were tested to determine the upper limit of fruit concentration that would allow CN1 to ferment the wines to less than 5 g/L residual sugar. Cabernet franc grapes were partially dehydrated to three different post-harvest sugar targets (24.5°Brix, 26.0°Brix and 27.5°Brix) along with a control of non-dehydrated grapes (21.5°Brix) and inoculated with either *S. bayanus* CN1 or *S. cerevisiae* EC1118, the commonly-used commercial yeast for appassimento-style winemaking. All wines were successfully vinified to dryness (<5 g/L residual sugar). Fermentation kinetics are similar for control, 24.5°Brix wines and 26.0 wines, but at 27.5°Brix, CN1 fermentations took three days longer in comparison to EC1118. Chemical analysis of wines at each starting sugar concentration shows significantly lower levels of oxidative compounds

(acetic acid, acetaldehyde and ethyl acetate) in wines produced with CN1, while CN1 produced higher levels of glycerol when compared to *S. cerevisiae* EC1118. Both yeasts produced comparable ethanol levels at each Brix level tested. This project will contribute to characterization of an indigenous yeast, *S. bayanus* CN1, that has application to this wine style, as it may assist in mitigating some of the quality challenges associated due to high levels of oxidation compounds with wine made from partially dehydrated grapes.

3.1 Introduction

Winemaking using the appassimento technique involves post-harvest dehydration of wine grapes, and subsequent processing when target starting sugar concentrations are reached. Grape dehydration increases sugars, polyphenols and aromatic compounds (Paronetto & Dellaglio, 2011), resulting in a rich wine high in ethanol and with a unique sensory profile (Moreno et al., 2008).

Vinifying partially dehydrated grapes for high quality wine production has been implicated as beneficial to cool climate viticultural regions such as Ontario, Canada, as it can potentially mitigate the challenges of a changing climate, which can threaten the sustainability and ongoing success of cool climate wine industries (Pickering et al., 2015). This strategy represents a way of adapting to vintage-to-vintage variation that may jeopardise the stability of grape yield, development and composition, as well as wine production and quality (Shaw, 2017; Ashenfelter and Storchmann, 2010; Teixeira et al., 2013). Grapes are further ripened postharvest in a protected environment allowing them to achieve high levels of sugar and volatile constituents, despite ambient weather conditions in the vineyard (Paronetto and Dellaglio, 2011).

During postharvest dehydration, berry volatile organic compounds (VOCs) increase as water is lost (Bellincontro et al., 2016). Important for wine quality, oxidation compounds such as acetaldehyde, acetic acid and ethyl acetate also increase during dehydration or during high sugar fermentation, which can negatively impact the organoleptic profile of the final wine when present at elevated concentrations (Bellincontro et al., 2004; López de Lerma et al., 2012). Fermenting must with high starting sugar concentrations can pose quality challenges to winemakers due to overproduction of potentially unfavourable compounds like acetic acid (Pigeau and Inglis, 2005; Nevoigt et al., 1997).

In Ontario, wine made from partially dehydrated grapes is regulated by the Vintners Quality Alliance (VQA), Canada's governing wine authority, under the term Vin de Curé. Grapes must be dried to a minimum of 27.0 °Brix at time of transfer to the fermentation vessel (VQA, 2019). Further, VQA Ontario has outlined permissible limits of volatile acidity (VA) for Vin de Curé, based on starting Brix. Thus, adhering to legally imposed limits of such parameters are important considerations for this wine style.
Of the many tools available to the winemaker in influencing final wine composition and quality, yeast strain choice can be one of those tools. In the case of winemaking with high sugar must such as appassimento style wines, initiating and completing fermentation are important yeast selection factors along with low production of oxidation compounds to consider to tailor yeast choice to wine style. In the present study, an indigenous yeast *S. bayanus* CN1, isolated from the skin of Riesling Icewine grapes (Nurgel et al., 2004; Kelly et al., 2018), has been trialed for winemaking from

partially dehydrated grapes. Its benefit is that it reduces the aforementioned undesirable compounds in the final wine when compared to *S. cerevisiae* EC1118, the commonly used commercial yeast for this wine style. This agrees with other winemaking trials that utilize *S. bayanus* strains for fermentation, resulting in wines with increased glycerol concentration and lower acetic acid production than *S. cerevisiae* (Eglinton et al., 2000).

In a preliminary trial of this yeast in vinifying appassimento must dried to 28.0°Brix (Kelly et al., 2018), residual sugar levels were too high (15.8 (±6.7) g/L) for this typically dry wine. Thus, further work on applying this yeast to the appassimento winemaking method was required. The aim of this study is to further define the starting sugar concentration range of the dehydrated grapes under which CN1 can ferment to dryness. This will be assessed by comparing the fermentation dynamics, sugar consumption, resulting ethanol and additional yeast metabolites in the wines fermented by CN1 in comparison to EC1118 at varying dehydration stages of the starting grapes. In order to examine this, local Cabernet franc grapes were dehydrated to three target starting sugar concentrations: 24.5°Brix, 26.0°Brix and 27.5°Brix and compared to control fruit not dehydrated but processed immediately after picking (21.5°Brix). These grapes were vinified with *S. bayanus* CN1 and a commercial strain, *S. cerevisiae* EC1118, and assessed chemically. This work will further characterize *S. bayanus* CN1 and determine its upper fermentative limit for vinification of wine made from partially dehydrated grapes.

3.2 Materials and Methods

3.2.1 Harvest and Grape Drying

Cabernet franc grapes were selectively hand-picked from Mazza Vineyards (Niagara-on-the-Lake, Ontario, Canada) and placed in perforated picking bins in a single-layer. A total weight of 821 kg was harvested and was divided into four parcels. One represents the control treatment (183 kg, 21.5°Brix), which was delivered to the Cool Climate Oenology and Viticulture Institute (CCOVI, Brock University, St. Catharines, ON, Canada) to be processed on the following day after temperature stabilization overnight at room temperature (18°C). The other three parcels were delivered to a Niagara barn (Cave Spring Winery Barn, 4424 Cave Spring Road, Beamsville, Ontario, Canada) dedicated to commercial appassimento grape drying to dehydrate grapes to three drying targets (24.5, 26.0 and 27.5°Brix). The picking bins were stacked 14-layers high, with adequate air space between each container to receive ventilation in the barn. The drying barn did not have internal temperature and humidity regulation available. The barn was moderately susceptible to external temperature conditions, as the barn was not insulated. Stand up fans were placed strategically throughout the facility to promote airflow. Grapes were sampled weekly (15 randomly selected clusters), and 105 randomly selected berries from the 15 clusters were weighed out for analysis. The clusters and berries were then crushed by hand in a plastic bag and strained through a metal strainer to collect must for immediate determination of soluble solids, pH and titratable acidity. Once the fruit reached the drying target, the parcel was delivered to CCOVI for processing on the following day after temperature stabilization overnight at room

temperature (18°C). The drying time was 31 days to reach 24.5°Brix (original weight 197 kg, post-drying weight 146 kg), 37 days to reach 26.0°Brix (original weight 215 kg, post-drying weight 164 kg), and 61 days to reach 27.5°Brix (original weight 226 kg, post-drying weight 158 kg). During drying, some formation of *Botrytis cinerea* within the grape clusters was observed. Any fruit that was potentially infected with *B. cinerea* was culled by hand after inspection before processing (none for control fruit or 24.5°Brix treatment; 12.6 kg total removed from 26.0°Brix treatment, and 10.2 kg total removed from 27.5°Brix treatment).

3.2.2 Yeast strains

Two yeast strains were used in this study for wine fermentations: the commercial yeast *S. cerevisiae* strain EC1118 was supplied by Lallemand Inc. (Montreal, QC, Canada) and *S. bayanus* CN1, which was isolated at CCOVI (St. Catharines, Ontario, Canada) from the bloom of local Riesling Icewine grapes (Kelly et al., 2018).

3.2.3 Winemaking

A total of eight fermentation treatments were carried out, each in triplicate and examined in this experiment; must vinified at a target of 21.5, 24.5, 26.0 and 27.5°Brix inoculated with *S. cerevisiae* EC1118 or *S. bayanus* CN1 at each sugar level. Before fruit processing, control and dried grapes were divided randomly and equally into three replicates based on weight (approximately 20 kg grapes/replicate), and each replicate was processed separately through the crusher/destemmer (model Gamma 50, Mori-TEM; Florence, Italy) into 20L steel fermentation vessels with lids. Musts were homogenized and 100 mL of sample from each replicate was taken for chemical

analysis. Musts were blanketed with carbon dioxide, the temperature was brought to 22°C and 500 mg/L of diammonium phosphate (DAP) (Laffort; Bordeaux, France) was added to each fermentation prior to inoculation. A further 250 mg/L of DAP was added on the third day of fermentation.

3.2.4 Fermentation

The yeast culture build-up and acclimatization procedure is described in Kelly et al. (2018) with following modifications. Yeast cultures were built up in 0.48 L of sterile 10°Brix must, diluted from sterile filtered Cabernet franc control must used in this study. Diluted 17°Brix must (0.48L) was used for the first step of acclimatization and the must at respective drying targets (0.48L) was used for the second step of acclimatization for fermentations at different sugar levels. This 1.44 L starter culture was inoculated into each fermentation to achieve an inoculum at 0.35 g/L (5.0 x 10^6cells/mL) to a final volume of approximately 20L. All fermentations were kept at 22°C, punched down twice daily and monitored once daily by recording soluble solids (hydrometer, °Brix), specific gravity (hydrometer, specific gravity) and temperature (thermometer, °C). Winemaking replicates received 20 plunges per vessel, and as the fermentation progressed, this number was reduced to four plunges per vessel by the end of fermentation, using a separate punch down tool for each yeast trial to prevent cross-contamination. Samples were collected daily (5 x 1mL aliquot and 1 x 50mL tube) and stored at -30°C until metabolite analysis. As the cap started to fall, fermentations were blanketed with CO_2 to protect from oxidation. Fermentations were considered complete once the sugar level measured <5 g/L as confirmed by FOSS (WineScanTM; Hillerød, Denmark). Once

complete, fermentation replicates were pressed separately with a small bladder press (Enotecnica Pillan; Vicenza, Italy) at one bar for two minutes into 11L glass carboys. Treatments were sulfited at 50 mg/L of sulfur dioxide (as potassium metabisulfite) and settled at room temperature. Wines were then racked and moved to a -2°C chamber for cold stabilization until bottling. Before bottling, an additional 50 mg/L of sulfur dioxide (as potassium metabisulfite) was added to each treatment. Wines were subsequently pad filtered through filter pads and bottled as separate treatments into 750mL glass bottles with a manual bottler (Criveller Group; Niagara Falls, Ontario, Canada), closed with natural cork and automated corker (model ETSILON-R, Bertolaso; San Vito, Italy) then stored in the CCOVI wine cellar (17.5°C and 74.5% humidity).

3.2.5 Grape, Must and Fermentation Analysis

Fermentation temperature was monitored with a thermometer (°Celsius). Soluble solids were determined using an ABBE bench top refractometer (model 10450, American Optical; Buffalo, New York, United States of America) for grape and must samples and using a degree Brix hydrometer for fermentation time course samples. pH was determined by pH meter (model SB70P, SympHony, VWR; Mississauga, Ontario, Canada) and titratable acidity by titration with 0.1 mol/L NaOH to an endpoint of pH 8.2 (Zoecklein, 1995). Glucose and fructose, glycerol, acetaldehyde, amino acid nitrogen, ammonia nitrogen, acetic acid was determined in the starting must and fermentation samples with Megazyme kits (K-FRUGL, K-GCROL, K-ACHD, K-PANOPA, K-AMIAR, K-ACET; Megazyme International Ireland, Limited, Bray Company; Wicklow, Ireland). Yeast assimilable nitrogen content (YANC) was the sum of ammonia nitrogen and amino acid

nitrogen. Ethyl acetate and final ethanol were determined by gas chromatography using a Hewlett-Packard 6890 series gas chromatograph equipped with a flame ionization detector (FID) and a split/split-less injector (Agilent Technologies Incorporated; Palo Alto, California, United States of America). Separations were carried out with a DB®-WAX (30 m, 0.25 mm, 0.25 µm) GC column (model 122-7032, Agilent Technologies Incorporated; Palo Alto, California, United States of America) with helium as the carrier gas at a flow rate of 1.5 mL/min. Analyzing software was Chemstation (version E.02.00.493, Aligent Technologies Incorporated; Mississauga, Ontario, Canada).

3.2.6 Statistical Analysis

Differences between variables were determined by XLSTAT statistical software package released by Addinsoft (Version 7.1; New York, New York, United States of America). Statistical methods used were analysis of variance (ANOVA) with mean separation by Fisher's Least Significant Difference (LSD) test ($p < 0.05$) and Student's *t*-Test ($p<0.05$, $p<0.01$, $p<0.001$).

3.3 Results

3.3.1 Must Analysis for Grapes Dehydrated to Target Levels

Grapes at harvest measured approximately 21.5°Brix (227-236 g/L reducing sugar) and were dehydrated up to a target of 24.5, 26.0 and 27.5°Brix. The grapes required 31 days to reach the 24.5 Brix target, 37 days to reach the 26 Brix target and 61 days to reach the 27.5 Brix target. Must analysis once grapes reached target levels and before any DAP addition is presented in Table 3.1. Starting acetic acid and acetaldehyde concentrations were low, and no measurable ethyl acetate at the limit of detection of

the method was present in grapes at all target Brix levels. Reducing sugars increased as a function of drying.

Table 3.1: Must metabolites and basic physio-chemical characteristics for grapes dehydrated to target levels (mean ± standard deviation).

Control and dehydrated grapes were divided randomly and equally into three replicates based on weight and each sample was tested in duplicate for all metabolites other than soluble solids. Lowercase (EC1118) and uppercase (CN1) letters indicate statistical differences within the same yeast treatment determined by analysis of variance (ANOVA) with mean separation by Fisher's Least Significant Difference (LSD; $p < 0.05$).

Metabolite	Yeast	Control (21.5°Brix) Target	24.5°Brix Target	26.0°Brix Target	27.5°Brix Target
Soluble Solids (°Brix)	*S. cerevisiae* EC1118	21.5±0.5	24.3±0.1	26.0±0.2	27.4±0.1
	S. bayanus CN1	21.7±0.5	24.4±0.1	25.9±0.1	27.5±0.0
Reducing Sugar (g/L)	*S. cerevisiae* EC1118	236±7d	267±8c	293±4b	313±3a
	S. bayanus CN1	227±5D	269±7C	290±4B	315±6A
pH	*S. cerevisiae* EC1118	3.41±0.07d	3.76±0.02b	3.65±0.02c	3.84±0.02a
	S. bayanus CN1	3.43±0.01C	3.71±0.03B	3.69±0.03B	3.85±0.01A
Titratable Acidity (g/L tartaric acid)	*S. cerevisiae* EC1118	5.1±0.3a	4.8±0.1b	4.4±0.1c	4.7±0.0b
	S. bayanus CN1	4.9±0.3A	4.7±0.1A,B	4.5±0.1B	4.6±0.1B
Ammonia (mg N/L)	*S. cerevisiae* EC1118	13.2±5.8a	12.4±1.0a	6.6±0.5b	11.2±0.7a,b
	S. bayanus CN1	15.2±3.8A	11.2±1.0A,B	8.5±0.7B	13.6±2.3A
Amino acid nitrogen (mg N/mL)	*S. cerevisiae* EC1118	82.3±19.3b	98.1±19.6a,b	87.1±5.1b	115.3±8.3a
	S. bayanus CN1	92.7±14.7B	103.1±3.6B	93.0±3.0B	125.6±10.6A
YANC (mg N/L)	*S. cerevisiae* EC1118	95.5±24.9a	110.5±20.5a	93.6±6.4a	125.6±7.5a
	S. bayanus CN1	108.0 ±18.5A	114.3±4.5A	101.5±2.3A	139.2±11.3A
Glycerol (g/L)	*S. cerevisiae* EC1118	0.03±0.00d	0.64±0.09a	0.35+0.01c	0.47±0.03b
	S. bayanus CN1	0.05±0.03B	0.50±0.10A	0.41±0.01A	0.50±0.05A
Acetaldehyde (mg/L)	*S. cerevisiae* EC1118	12±0b	14±1a	12±0b	12±0b
	S. bayanus CN1	12±0B	13±1A	12±0B	12±0B
Acetic acid (g/L)	*S. cerevisiae* EC1118	0.004 ±0.001d	0.017 ±0.001c	0.012 ±0.001b	0.021 ±0.002a
	S. bayanus CN1	0.003 ±0.001C	0.015 ±0.001B	0.015 ±0.003B	0.019 ±0.002A

Ethyl Acetate (mg/L)	*S. cerevisiae* EC1118	<3.0 (LOD)	<3.0 (LOD)	<3.0 (LOD)	<3.0 (LOD)
	S. bayanus CN1	<3.0 (LOD)	<3.0 (LOD)	<3.0 (LOD)	<3.0 (LOD)

3.3.2 Yeast Cell Growth and Fermentation Kinetics for Grapes Dehydrated to Different Levels

Fermentations using CN1 were compared to fermentations using the commercially available EC1118 at four starting sugar concentrations, with each treatment fermented in triplicate, thus establishing 24 fermentations for analysis.

Viable cell concentrations differed between fermentations inoculated with EC1118 and CN1 in all treatments, with fermentations inoculated with CN1 showing approximately half the peak cell concentration in comparison to those inoculated with EC1118 under all fermentation conditions tested (Figure 3.1, A-D). Despite these lower viable cell concentrations for fermentations inoculated with CN1, these fermentations consumed sugar at a comparable rate to those inoculated with EC1118 at 21.5, 24.5 and 26.0°Brix treatments, fermenting to dryness. At the 27.5°Brix treatment, fermentations inoculated with CN1 showed a slower sugar consumption rate, requiring three additional days to complete the fermentations and left 2.35 g/L residual sugar in the wine (Figure 3.2, Table 3.2).

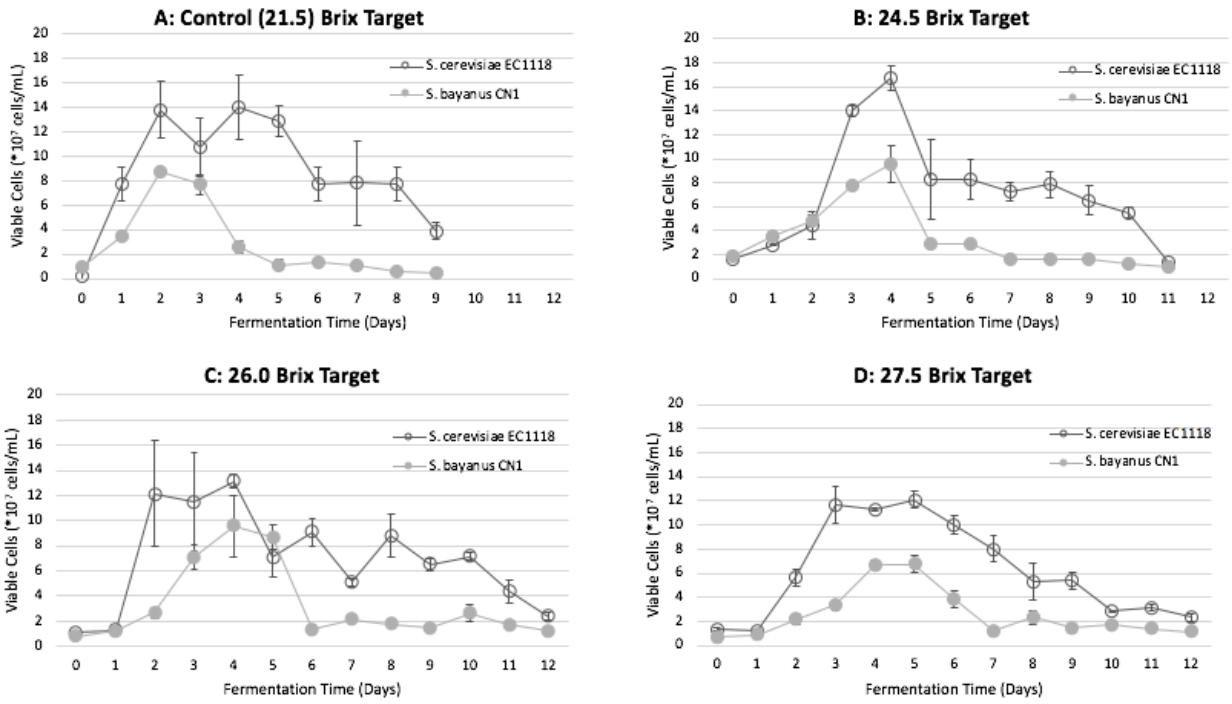

Figure 3.1: Total viable cell counts of (A.) 21.5 °Brix (control), (B.) 24.5 °Brix, (C.) 26.0 °Brix and (D.) 27.5 °Brix for S. cerevisiae EC1118 and S. bayanus CN1.

Each data point is the average of three winemaking replicates, with each replicate counted in duplicate with standard deviation shown as error bars.

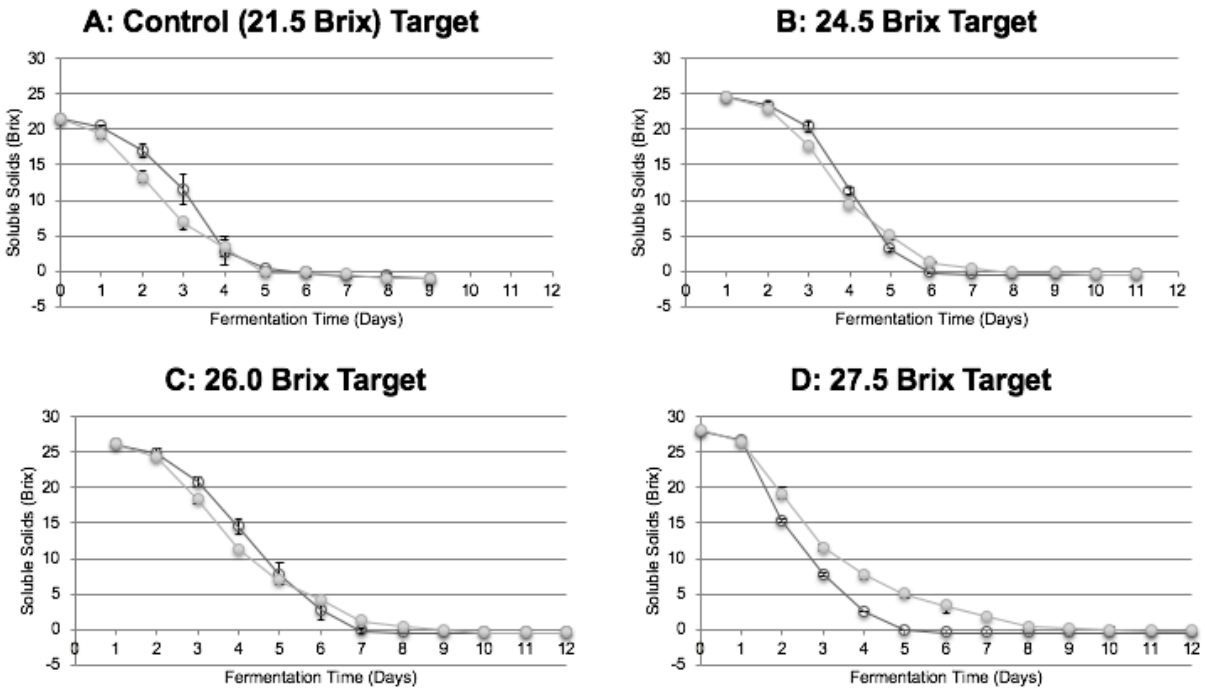

Figure 3.2: Fermentation kinetics of EC1118 and CN1 at (A) 21.5 °Brix (control) target, (B) 24.5 °Brix target, (C) 26.0 °Brix target and (D) 27.5 °Brix target.

Each data point represents the mean from triplicate fermentations with standard deviation shown as error bars.

3.3.3 Metabolites

CN1 inoculated fermentations for the control must consumed more YANC and produced lower titratable acidity in comparison to the control treatment inoculated with EC1118. For all treatments using dried grapes (24.5, 26 and 27.5 Brix), fermentations inoculated with CN1 produced more titratable acidity relative to those inoculated with EC1118 (Table *3.2*). For each sugar level tested, except 24.5 Brix, fermentations inoculated with CN1 had the same ethanol concentration in the wines as that found in wines resulting from EC1118 (Figure 3.3A). CN1 inoculated fermentations using the dehydrated grape treatments produced higher concentrations of glycerol in comparison to wines from the EC1118 inoculations (Figure 3.3 B). However, all CN1 fermentations produced

significantly less acetic acid and ethyl acetate at each sugar level tested in comparison to EC1118 (Figure 3.3D,E) and significantly less acetaldehyde at the 26 and 27.5Brix treatments

Table 3.2: Basic physio-chemical characteristics in control wine and wine made from partially dehydrated grapes (mean ± standard deviation).

Fermentations were conducted in triplicate and each sample was tested in duplicate for all metabolites. Lowercase (EC1118) and uppercase (CN1) letters indicate statistical differences within the same yeast treatment determined by analysis of variance (ANOVA) with mean separation by Fisher's Least Significant Difference (LSD; $p < 0.05$).

Metabolite	Yeast	Control (21.5°Brix) Target	24.5 °Brix Target	26.0 °Brix Target	27.5°Brix Target
Reducing Sugar (g/L)	*S. cerevisiae* EC1118	0.07±0.00a	0.11±0.07a	0.07±0.00a	0.09±0.03a
	S. bayanus CN1	0.07±0.00b	0.26±0.15b	0.07±0.00b	2.35±1.23a
pH	*S. cerevisiae* EC1118	3.82±0.05b	3.87±0.01b	3.84±0.02b	4.02±0.01a
	S. bayanus CN1	3.78±0.07b	3.77±0.03b	3.72±0.04b	3.9±0.03a
Titratable Acidity (g/L tartaric acid)	*S. cerevisiae* EC1118	8.5±0.3a	6.5±0.1b	6.7±0.1b	6.4±0.1b
	S. bayanus CN1	6.7±0.1c	7.7±0.1b	7.8±0.1a	6.8±0.1c
Ammonia (mg N/L)	*S. cerevisiae* EC1118	8.8±4.9a	6.3±0.6a	9.3±4.6a	6.5±1.5a
	S. bayanus CN1	6.0±0.0a	6.0±0.0a	10.3±3.8a	12.5±4.6a
Amino acid nitrogen (mg N/mL)	*S. cerevisiae* EC1118	39.8±11.4c	52.3±0.7b,c	61.5±2.9b	81.0±5.2a
	S. bayanus CN1	65.8±1.3b	62.6±4.5b	66.9±3.9cb	75.4±1.4a
YANC (mg N/L)	*S. cerevisiae* EC1118	48.7±18.9c	58.6±0.7b,c	70.8±6.2a,b	87.5±7.8a
	S. bayanus CN1	71.8±1.6b	68.6±5.5b	77.2±4.3b	87.9±6.0a

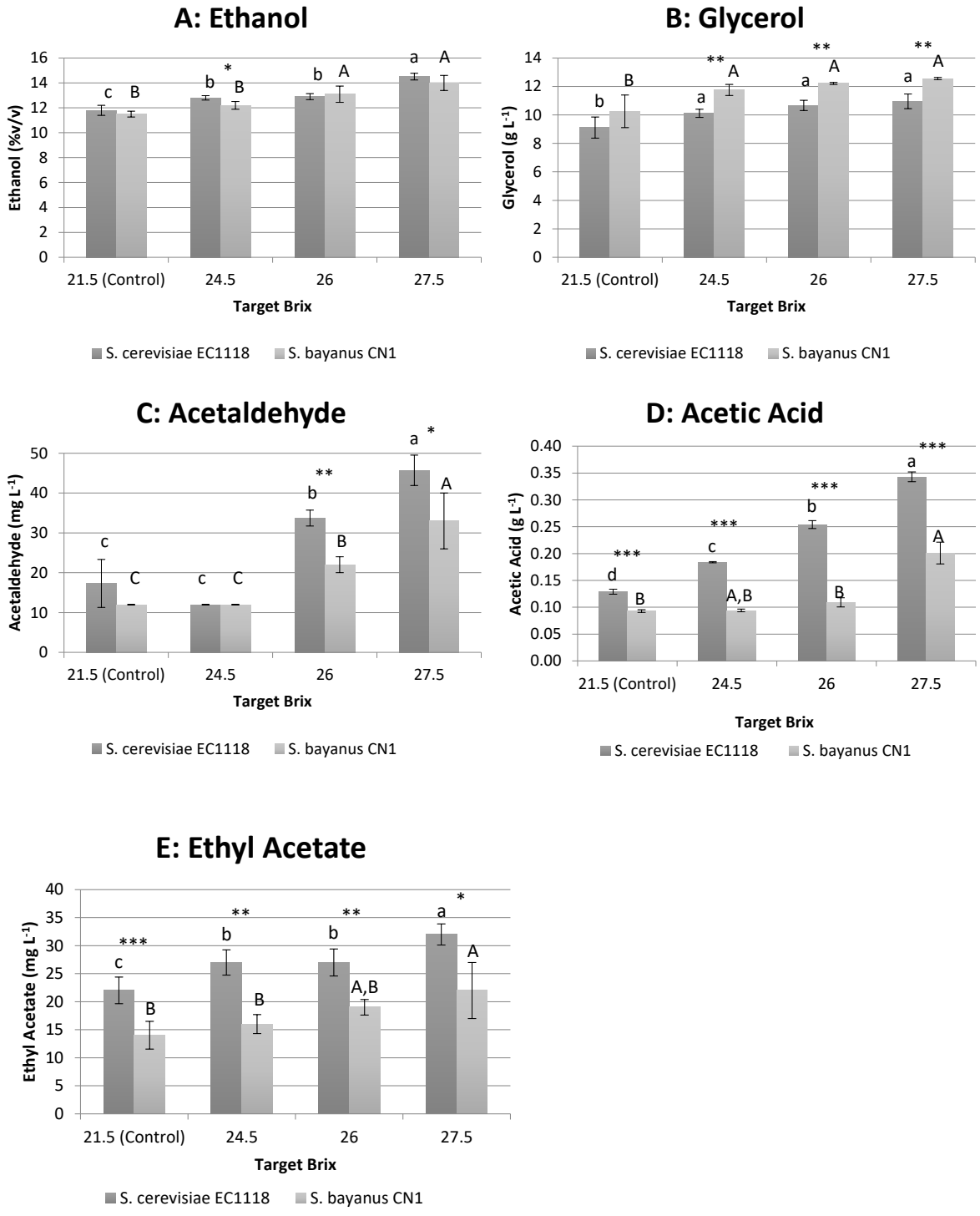

Figure 3.3: Yeast Metabolites, (A) ethanol, (B) glycerol, (C) acetaldehyde, (D) acetic acid and (E) ethyl acetate concentrations of control (21.5 °Brix), 24.5 °Brix, 26.0 °Brix and 27.5 °Brix target levels.

Fermentations were conducted in triplicate and each sample was tested in duplicate for all metabolites. Lowercase (EC1118) and uppercase (CN1) letters indicate statistical differences within the same yeast treatment determined by analysis of variance (ANOVA) with mean separation by Fisher's Least Significant Difference (LSD; $p < 0.05$). Asterisks (*$p<0.05$, **$p<0.01$, ***$p<0.0001$) indicate significant differences between yeast strains at the indicated dehydration target as determined by Student's *t*-Test.

3.4 Discussion

This study aimed to define the range of sugar concentrations that the indigenous *S. bayanus* CN1 yeast could ferment to less than 5 g/L in appassimento-style winemaking and to assess the impact this yeast has on reducing oxidative compounds in the final wines. Grapes in this study were dried in a commercial barn used in making Vin de Curé in Ontario, Canada as part of a commercial winemaking operation. Based on the time required to dry the grapes to 27.5 Brix (61 days), this method is considered a slow dry time (Mencarelli and Bellincontro, 2013). Traditionally, dry time is defined as follows: very fast: five to ten days, fast: two to three weeks, slow four to eight weeks, very slow: >eight weeks (Mencarelli and Bellincontro, 2013). Slow dry time for appassimento wine styles is noted as beneficial, as slow dry time in a controlled environment (versus sun drying) results in a reduction of the formation of potentially undesirable metabolites like acetaldehyde and acetic acid (Bellincontro et al., 2004).

For the range of grape dehydration targeted and the resulting must concentration tested for fermentation by CN1 versus EC1118, fermentations inoculated with CN1 only showed a slower sugar consumption rate compared to EC1118 for the 27.5°Brix treatment, with all other fermentations showing a comparable fermentation rate or slightly faster rate relative to EC1118. Although at 27.5°Brix CN1 required three additional days to complete the fermentation and left 2.4 g/L residual sugar in the wine,

the ethanol levels were not significantly different between the two yeasts (14.0 ±.0.6 % v/v ethanol for wines fermented with CN1 and 14.5 ± 0.2 % v/v ethanol for wines fermented with EC1118).

Previous work with CN1 (Kelly et al., 2018), demonstrated that when the starting must from partially dehydrated grapes was at 28°Brix, CN1 was not able to ferment to dryness, leaving 15.8 (±6.7) g/L residual sugar in the wine. The present study has established the upper concentration limit of must resulting from partially dehydrated grapes that CN1 can ferment to less than 5 g/L sugar at 27.5°Brix.

Traditional appassimento winemaking involves extended fermentation at low temperatures, suggesting the style allows for flavorants association with this technique (Accordini, 2013). The additional three days required for CN1 to reach less than 5 g/L residual sugar in the wine still allowed the fermentations to be completed within nine days, which is within acceptable ranges for this wine style as previously reported (Tosi et al., 2009).

Elevated concentrations of oxidation compounds have been reported to arise during the appassimento grape drying process (Bellincontro et al., 2004). Identifying yeast strains that generate low acetic acid, acetaldehyde and ethyl acetate during the fermentation of partially dehydrated grapes may be of commercial importance in making this wine style, as less oxidation compounds originating from the yeast would add into the wine. Fermentations with CN1 produced significantly lower levels of these oxidation compounds, in agreement with results for CN1 previously reported (Kelly et al, 2018). CN1 also produced high ethanol values and left low residual sugar in the wine

when using must up to 27.5°Brix. These traits of producing low oxidation compounds, high ethanol and leaving low residual sugar have been reported as advantageous for appassimento wine production (Accordini, 2013).

The findings in this current study agree with existing literature that reports the composition of wines produced with various strains of *S. bayanus* yeasts, where acetic acid levels are lower, and glycerol levels are higher when compared to wines made with *S. cerevisiae* (Eglinton et al., 2000). The response of *S. bayanus* CN1 in this present study in producing higher glycerol and lower acetic acid appears to differ from the well-characterized hyperosmotic stress response of *S. cerevisiae* wine yeast during high sugar fermentations (Yang et al., 2017; Pigeau and Inglis, 2005; Heit et al., 2018). The mechanism for these differing responses between *S. cerevisiae* and *S. bayanus* remains to be elucidated to understand the linkage of these two metabolites to hyperosmotic stress and the intracellular NAD+/NADH cofactor balance in the cells during fermentation leading to metabolite production.

Acetic acid is an important component and marker for influencing the final quality of wine, and at elevated levels, it is associated with spoilage, can reduce varietal character and can have a highly undesirable organoleptic effect (Jackson, 2008; Nurgel et al., 2004; Lambrechts and Pretorius, 2000). Consideration for the soundness of grapes included in this study and the slow dry time resulted in relatively low starting concentrations of acetic acid, but variations in vintage can result in varied grape quality. Therefore, utilization of appropriate yeast can assist with meeting requirements for legal allowable limits of oxidation compounds. In the present study, although fermentations

with CN1 consistently produced wines with lower acetic acid in comparison to EC1118, even the wines with EC1118 were relatively low in acetic acid concentration measuring only 0.34 g/L. In Canada, the legal limit of acetic acid present in wines made from dried grapes (Vin de Curé) is regulated based on the starting Brix measurement: at 27 to 28°Brix, the allowable limit is 1.5g/L, at 28 to 32°Brix, 1.8g/L and over 32°Brix 2.1g/L (VQA, 2019). Ethyl acetate, although often considered an oxidation fault, has also been reported as appassimento wine quality as a volatile constituent that may have an olfactory impact on wines made from dehydrated grapes (Moio and Piombino, 2013), providing an important contribution to the composition of appassimento wine that can potentially impact flavours and odorants at concentrations that do not contribute to the perception of spoilage.

In a study by Fedrizzi et al. (2011), dry Amarone wines vinified with EC1118 had a reported ethanol content of 14.3-14.9%, and acetic acid levels of 0.12-0.25 g/L. A 2018 study that investigated the impact of *Penicillium* infection on Amarone wines (Zapparoli et al., 2018) reported appassimento wines made with non-infected berries, vinified with EC1118, with an ethanol content of 17.1%, acetic acid as 0.7 g/L, glycerol as 11.8 g/L and ethyl acetate as 163 mg/L. In this current study, the ethanol ranged from 14.5% (EC1118) to 14.0% v/v (CN1) acetic acid from 0.34 (EC1118) to 0.20 (CN1) g/L, glycerol from 10.96 (EC1118) to 12.56 g/L (CN1), and ethyl acetate from 32 (EC1118) to 22 (CN1) mg/L for appassimento wines. Given the consistently high values of these oxidation compounds across current literature in appassimento style wines and in the starting musts fermented into wine, CN1 may prove to be a useful yeast for this style as a way to

mitigate potentially problematic wines that can be organoleptically faulted due to high levels of volatile acidity.

Utilization of CN1 yeast represents an opportunity to regionally tailor this wine style so to offer consumers a signature style that is suited to the climate and varietals available to industry personnel. Future work includes quantitative descriptive analysis on these wines, as CN1 wines can be sensorially distinguished from EC1118 wines, measurement of volatile organic compounds (VOCs) that may contribute to wine flavour and aroma, along with assessing consumer preference.

3.5 Conclusions

This study aimed to characterize *S. bayanus* CN1 and determine its upper fermentative limit for vinification of wine made from partially dehydrated grapes. Cabernet franc grapes were dried to 24.5°Brix, 26.0°Brix and 27.5°Brix targets and fermented with *S. bayanus* CN1 and *S. cerevisiae* EC1118. All wines within the starting sugar range fermented to dryness (<5 g/L residual sugar) with both yeast strains. During drying, high oxidation compounds may accumulate, combined with subsequent formation of oxidation compounds during fermentation that may negatively impact resultant appassimento style wines. CN1 produced wines with significantly lower levels of oxidation compounds (acetic acid, ethyl acetate and acetaldehyde), suggesting it may have commercial applicability. Applying CN1 yeast to the appassimento wine style can assist in fermentation management, to avoid bringing wines to market with above-threshold levels of volatile acidity, which could be rejected by consumers.

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Chapter 4 Sensorial and Volatile Analysis of Wines Made from Partially Dehydrated Grapes: An Ontario Case Study

Abstract:

Winemaking in cool climate viticultural areas can pose challenges due to difficulties achieving optimal ripeness from climatic conditions that tend to vary vintage-to-vintage. In order to stabilize quality, the use of partially dehydrated grapes has been indicated as a beneficial technique to produce high-quality wine in spite of climatic variation. Postharvest wine grape dehydration for the production of appassimento-style wines is a complex process that involves the concentration or formation of sugars, aromas and flavours. Some of these compounds contribute favourably to the profile, while others are undesirable. One of the quality challenges facing Appassimento winemaking is elevated levels of undesirable oxidation compounds, such as ethyl acetate, acetic acid and acetaldehyde. In this study we aim to characterize wines made from a local yeast isolate, *Saccharomyces bayanus*, which demonstrates limited osmotolerance and may have application to this wine style, as it is a known lower producer of such compounds. Wines made with the yeast of interest were compared to wines made with the accepted commercial standard, *Saccharomyces cerevisiae*, EC1118. Fermentations (n=24) were established at three target starting sugar concentrations and a control (control = 21.5, targets = 24.5, 26.0 and 27.5°Brix), and were chemically analyzed and subject to volatile organic compound (VOC) and volatile fatty acid (VFA) measurements via gas chromatography-mass spectrometry (GC-MS). Wines have also undergone quantitative descriptive analysis to identify and quantify attributes generated by a sensory panel

(n=11). Results indicate that the wines fermented with the yeast isolate have significantly lower concentrations (p<0.05) of oxidation compounds than the commercial yeast, along with comparable ethanol levels. We also observe significant differences in the concentrations of VOCs and VFAs in the wines, such as 2-phenylethanol and hexanoic acid. Sensorially, the wines differed in intensity for a number of attributes, including red fruit aroma, black fruit flavour and length of finish both within °Brix treatments and amongst yeast strains. The most important differentiating factor amongst these wines was the combination of yeast strain at the highest starting sugar concentration (27.5°Brix). These findings may assist winemakers by informing yeast strain choice for optimizing appassimento-style wine quality in cool climates.

4.1 Introduction

Wine aroma and flavour are important factors that influence quality. Wine is a complex matrix, with many above-threshold odorants that impact its profile, while non-impact compounds simultaneously contribute to the aroma, as well (Aznar et al., 2003). Further, the aromatic volatile compounds of both grapes and wine that are closely linked to a wine's sensory quality ultimately determine consumer response and acceptance (Scacco et al., 2010; Mencarelli and Bellincontro, 2018; Urcan et al., 2017). Volatile compounds in wine can arise from the grape berry, which can vary depending on cultivar, as well as from viticultural practices and winemaking practices. Compounds can also come from yeast and bacterial metabolism, as well as post-fermentative

treatments like oak wood extraction. Finally, compounds can form from chemical reactions during storage. The compounds that form along the vine to wine continuum provide diverse aroma and flavour profiles to the wines (Francis and Newton, 2005; Wang et al., 2016; Scacco et al., 2010).

Post-harvest practices like grape dehydration have a concentration effect on sugar and volatile compounds and impact final wine aroma and flavour (Costantini et al., 2006; Frangipane et al., 2012). Specifically, differences in the grape cultivars used; the post-harvest drying environment of the grapes; fermentation conditions; winemaking method and ageing time influence the chemical and sensory characteristics of wines made from dehydrated grapes (Giordano et al., 2009). Partial grape dehydration to produce special or diversified wines is used in winemaking regions globally (Urcan et al., 2017). In Ontario, Canada, a cool climate grape growing and winemaking region, this winemaking style is emerging as a mitigation technique to combat viticultural difficulties associated with a changing climate (Kelly et al., 2018). Drying grapes post-harvest in a protected environment allows for protection from predators and extreme weather events that can impact fruit and wine quality. Production of high-quality red wine in sub-optimal vintages where cold weather does not provide desirable conditions can be challenging. The benefits of producing wines in the appassimento style include an increase in body as well as riper flavours that garner consumer appeal. However, wines produced from partially dehydrated grapes may contain high initial concentrations of oxidation compounds like acetic acid, acetaldehyde and ethyl acetate that arise during the drying process (Bellincontro et al., 2016), as well as during high sugar fermentation.

Yeast choice can be impactful in this regard, as low oxidation compound-producing yeast can potentially mitigate this problem.

A locally isolated yeast has been utilized in mircovinification trials to ferment grapes that have been dried post-harvest (known as the appassimento process), that may positively influence wine quality by significantly reducing the concentration of oxidation compounds in the final wine. Two previous studies over two vintages (Kelly et al., 2018 and Chapter Three) have confirmed that *Saccharomyces bayanus* CN1 is a significantly lower producer of such compounds. It remains to be elucidated, however, what CN1's sensorial impact is on final wine quality. The impact of yeast in winemaking is crucial to the final aroma composition (Romano et al., 2003). Alcoholic fermentation produces secondary metabolites like acids, esters, aldehydes and other volatile compounds that contribute to the uniqueness of wine character, by modifying the organoleptic profile, which can vary depending on the choice of fermenting yeast (Fleet, 2003; Mencarelli and Bellincontro, 2018).

This study aims to characterize this yeast by sensorially evaluating wines made from partially dehydrated grapes, fermented with CN1, compared to a widely-used commercial yeast *S. cerevisiae* EC1118, as well as quantifying the volatile organic compounds (VOCs) and volatile fatty acids (VFAs) that can contribute to wine flavour and aroma

In this study, Cabernet franc wines made from partially dehydrated grapes were fermented at four starting sugar concentrations: control (21.5°Brix) and 24.5, 26.0 and 27.5°Brix fermented with two yeast strains: *S. bayanus* CN1 and commercial yeast *S.*

cerevisiae EC1118. Sensory and VOC/VFA data was collected, and data sets were statistically analyzed to find the fundamental relations between the two matrices.

This study aims to i) assess the impact of yeast strain and ii) starting sugar concentration on the aroma and flavour profile of wines made from partially dehydrated grapes. This study provides novel information on the indigenous yeast CN1 and its application to winemaking using partially dehydrated grapes, which may assist in developing this wine style in Ontario. Understanding the profile of this wine made with a local yeast and local grapes represents an opportunity to develop a regional signature: Ontario appassimento wine.

4.2 Materials and Methods

4.2.1 Winemaking

Detailed winemaking methods are fully described in Chapter Three. Wines were made with Cabernet franc grapes from the Niagara sub-appellation Niagara-on-the-Lake in 2013 after partial postharvest dehydration to their respective starting sugar concentration targets (24.5, 26.0 and 27.5°Brix), while control grapes (21.5°Brix) were harvested and processed immediately. Wines were fermented in triplicate with either the commercially available *S. cerevisiae* EC1118 or the autochthonous *S. bayanus* CN1 yeast until dry. CN1 was isolated from Riesling icewine grapes, sequenced and identified as *S. bayanus* via comparison of all available sequence databases of DNA using Basic Local Alignment Search Tool (BLAST) (Kelly et al., 2018). *S. cerevisiae* EC1118 was rehydrated according to manufacturer's directions and plated out on yeast extract peptone dextrose plates (YPD, 1% yeast extract, 2% peptone, 2% dextrose, 2% agar).

CN1 yeast was prepared from a frozen glycerol stock, and also plated out on YPD plates. Both yeasts were grown to appropriate colony size prior to preparing a starter culture in sterile-filtered grape juice. The starter cultures were built up in sterile-filtered Cabernet franc must, and then followed a step-wise acclimatization procedure as outlined in (Kontkanen et al., 2004). Must was inoculated at a rate of 5.0 x 10^6 cells/mL. After fermentation was complete (<5 g/L residual sugar), fermentation replicates were pressed separately with a small bladder press (Enotecnica Pillan, Vicenza, Italy) at one bar for two min into glass carboys. Before bottling, wines were suflited at 50 mg/L of sulfur dioxide (as potassium metabisulfite). After filtering, wines were bottled into 750mL glass bottles, closed with a natural cork, and stored in the wine cellar at the Cool Climate Oenology and Viticulture Institute (CCOVI) at 17.5°C and 74.5% humidity.

4.2.2 Descriptive Analysis

A prior triangle test (Kelly et al., 2018) demonstrated significant differences (p<0.001) in wines made from partially dehydrated grapes produced with CN1 and EC1118, thus indicating that a descriptive analysis could be conducted. In a preliminary bench tasting outlined in Chapter Three, wines fermented at 26.0 and 27.5°Brix starting sugar concentration were most representative of the appassimento style, while 24.5°Brix wines were perceptibly indistinguishable from table wine. Thus, the wines included in the descriptive analysis were: control, 26.0°Brix and 27.5°Brix treatments. The 18 control and wines made from partially dehydrated grapes (vinified in triplicate, with either EC1118 or CN1) were sensorially evaluated by a trained panel of 11 volunteers (three males and eight females) recruited from students and staff at the Cool Climate

Oenology and Viticulture Institute (CCOVI) at Brock University four months after bottling. Data collection was conducted after a total of ten training sessions. Panelists all had previous winetasting experience and were selected based on interest and availability. Research Ethics clearance was granted by the Research Ethics Office (File No. 14-021 INGLIS) at Brock University. Quantitative descriptive analysis methods followed the Manual on Descriptive Analysis Testing for Sensory Evaluation (Hootman, 1992).

Consensus terminology, language training, scoring wines on the scale and reference standards were developed over eight two-hour training sessions. During the first two training sessions, all wines were presented to the panelists (*Table A4.4*). Wine bottles were opened approximately 60 minutes before training sessions began and were assessed for non-treatment related faults like cork taint. A descriptor list for aroma, flavour and in-mouth sensations was generated in the first two training sessions, and the descriptor list was eventually reduced through panel discussion to avoid redundant or overlapping terms. The final descriptor list is as follows: cherry, green pepper, black fruit, herbal, spice, green vegetal, fresh/dried/cooked red fruit, leather/meat, earthy/toast, floral and candy/confection for aroma modalities and cooked/dried/fresh red fruit, confection, black fruit, spice, vegetal, herbal and earthy/toast flavour modalities, as well as astringency, alcohol, acidity, bitterness and length of finish for in-mouth sensations. After the descriptors were agreed upon by the panel, reference standards were presented for all aroma descriptors and the following training sessions optimized the standards to match the descriptors present in the wine. Reference

standards were made according to recipes outlined in *Table 4.1*, with the base wine consisting of the 24.5°Brix starting sugar concentration Cabernet franc appassimento wine.

Panel performance was evaluated throughout training. Panelists rated the intensity of the attributes on 15 cm scales (anchored with terms *low intensity* and *high intensity*) divided into quadrants on paper ballots. After eight sessions, the panel performance was considered adequate, as descriptors were being rated in a uniform manner. Before data collection, participants were subject to two additional training sessions on the Compusense™ system to become familiar with the sensory booths and electronic data collection method.

Following training, panelists evaluated 18 wines in duplicate over two sessions, broken down into three flights per session, where six wines were tasted per flight. The flights were scheduled over three two-hour intervals (for example, 11:00am, 1:00pm and 3:00pm). There were forced three-minute breaks between wines to prevent fatigue. Wine bottles were opened one hour before sessions began and were briefly assessed for non-treatment related faults. Panelists assessed the wines with digital 15-cm anchored line scales (anchored with terms *low intensity* and *high intensity*) that were divided into quadrants to mimic training sessions, based on feedback from the panelists. The formal tastings and data collection took place in the sensory lab at Cool Climate Oenology and Viticulture Institute. Wines were presented to the panelists in a complete randomized block design, and panelists were instructed to taste wines in the order presented. Each participant sat in their own booth equipped with a computer screen, mouse, wines,

unsalted crackers, water and a cup for expectorating. Participants were encouraged to consume crackers and water between wines to cleanse palates. Panelists were required to expectorate samples after tasting. One ounce (30mL) of each wine was poured at room temperature into *International Standards Organization* wine glasses and were covered with plastic lids (petri dishes). Each glass was with a three-digit blinding code. The lab was illuminated with red lights to correct for colour bias.

Table 4.1: Complete list of reference standards and describing terms used for descriptive analysis of wines made from partially dehydrated grapes.

Attribute	Includes Terms	Reference Standard Composition
Green Pepper		50mL wine + one drop of "green pepper" aroma*
Spice	Black pepper, baking spice, anise	50mL wine + four crushed all spice balls + one drop "anise" aroma* + two shakes of black pepper
Red Fruit (Cooked/dried/fresh)	Cherry, strawberry, raspberry, cranberry, jam	50mL prune juice + two teaspoons cooked strawberries + one sliced fresh strawberry + one teaspoon frozen raspberries
Black Fruit	Plum, blackberry, black currant	50mL Ribena† juice
Herbal	Mint, sage, rosemary, pine, eucalyptus	50mL wine + one drop eucalyptus oil + 10 dried bits rosemary + one drop "green herbaceous" aroma*
Canned Green Vegetable	Asparagus, green bean)	50 mL wine + one teaspoon canned asparagus + one teaspoon canned asparagus juice + one teaspoon canned green bean juice
Earthy/Toast	Dirty/dusty, leafy, straw, char/cigar	50mL wine + three dried leaves + one tablespoon dirt + one drop "toast" aroma*
Candy/Confection	Candied cherry, cotton candy	50mL wine + two Swedish berries‡ + ¾ cup cotton candy§
Floral		50mL wine + 5cm of inside of Turkish delight bar¶ + three Turkish delight candies#
Leather/Meat		50mL wine + one drop "leather"* + one drop "smoky bacon"*

All standards were prepared using EC1118 24.5°Brix Replicate 1 wine as base wine unless otherwise indicated.

*Wine Awakenings Kit: Niagara Falls, ON, Canada, http://www.wineawakenings.com, 1-877-595-5678

†Lucozade Ribena Suntory Ltd., 2 Longwalk Road, Stockley Park, Uxbridge, United Kingdom; sourced in Canada from Sobeys Inc.

‡ Maynards Sweets, 3 Robert Speck Parkways, Mississauga, ON, Canada; sourced from Sobeys Inc.

§Sweet Shoppe; 5805 av Royalmount, Mont-Royal, QC, Canada; sourced from Dollarama Inc.
¶ Nestle Canada Inc., 72 Sterling Road, Toronto, ON, Canada; sourced from Dollarama Inc.
# Bulk Barn Foods Limited, 320 Don Hillock Drive, Aurora, ON, Canada; sourced from Bulk Barn Foods Limited

4.2.3 Analysis and Quantification of Volatile Organic Compounds (VOC) and Volatile Fatty Acid Compounds (VFA)

Analysis of compounds (*Table 4.2*) was conducted using the method outlined in Botezatu et al. (2016). The compounds, representative of different classes of volatile compounds, were selected for analysis, as they are important to wine flavour. Full methods are full described in Appendix. Concentrations of standards were prepared based on Botezatu et al. (2016) but were adjusted to this project based on the outcome of model wine (EC1118 24.5°Brix) measurements. All samples were diluted 20-fold to ensure they fit within the standard curve. To conduct the solid-phase microextraction (SPME), a 2 cm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco Inc., Bellefonte, PA, USA), 23-gauge SPME fiber was used for sampling. An Agilent 7890A Gas-Chromatograph (GC) coupled with an Agilent MS 5975 Mass Spectrophotometer (MS) was equipped with a Deans Switch and two columns: a HP-5MS 5% phenyl methyl siloxane column (30 m, 0.25 mm i.d., 0.25 µm film thickness) coupled with a secondary DB-Wax capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness) (J&W Scientific). VFA analysis was conducted with similar methodology to VOC analysis, with some changes (described in appendix). Compounds were quantified by comparison of retention time and mass spectra (Chemstation/Wiley spectral databases, NIST 08) to pure standards. Six-point calibration curves were run for each compound in model wine solution to ensure linearity (r^2 >0.9, Figure A4.10, A-K). Standard curve concentrations were quantified based on the ratio of the peak area of the compound relative to the peak area of the deuterated internal standards to

determine the concentration of the analytes. Analysis was run in duplicate with coefficient of variation between replicates ranging from 2-18% (*Table A4.13*).

Table 4.2: Categories, CAS Numbers, odour descriptors and sensory threshold (μg/L) of all VOCs and VFAs measured in wines made from partially dehydrated grapes.

Category	Compound	CAS Number	Sensory Threshold	Purity (%)	Odour Descriptor
	Volatile Organic Compounds (VOCs)				
Internal Standard	d_{11} Ethyl hexanoate ISTD	2159-19-5	N/A	98.7	N/A
	Octanal-d_{16} ISTD	1219794-66-7	N/A	98	N/A
Acetate Ester	Isoamyl acetate	123-92-2	30[a]	97	Fruity, banana[b]
Ethyl Esters	Ethyl isobutyrate	97-62-1	15[a]	99	Sweet, rubber[c]
	Ethyl butyrate	105-55-4	20[d]	99	Apple[c]
	Ethyl isovalerate	108-64-5	3[a]	98	Fruity, sweet[e]
	Ethyl octanoate	106-32-1	580[f]	>99	Fruity, strawberry, sweet[e]
	Ethyl 2-methyl butyrate	7452-79-1	18[a]	99	Fruity, sweet, apple[e]
	Ethyl hexanoate	123-66-0	14[g]	99	Green apple[g]
Alcohol	Hexanol	111-23-3	8000[a]	99.5	Herbaceous, resin, flower[b]
	2-Phenylethanol	60-12-8	14,000[a]	99	Floral, rose, honey, spice[e,h]
Volatile Fatty Acids (VFAs)					
	Hexanoic Acid	142-62-1	420[a]	99.5	Sweat[e]
	Octanoic Acid	124-07-2	500[a]	99.5	Sweat, cheese[e]

[a]Ferreira et al., 2000 (determined in 11% ethanol/water solution with 7 g/L glycerol and 5 g/L tartaric acid at pH 3.4)
[b]Bellincontro et al., 2016
[c]Rice et al., 2018
[d]Guth et al. (1997) (determined in 10% ethanol/water solution)
[e]Francis and Newton, 2005

[f]Etiévant, 1991
[g]López de Lerma et al, 2012
[h]Cordente et al., 2018

4.2.3.1 Identification and Quantification

The analytical data software (Chemstation, MSD E.02.00.493 by Agilent Technologies) was used to extract the quantifying ions, and the ratio of the standard over the internal standard was plotted against each analyte concentration to fit a quadratic equation where the intercept was set to zero. Recovery was calculated by measuring triplicate spiked samples after every 20 wines were measured.

4.2.4 Statistical Analysis

Descriptive analysis data was collected using Compusense™ software (Guelph, ON, Canada). Analysis of Variance (ANOVA), including one-way ANOVA [factors: VOC concentration], two-way ANOVA [Factors: Tasting replicate, winemaking replicate and tasting replicate*winemaking replicate interaction] (p<0.05) and three-way ANOVA [f=Tasting replicate, Judge, Wine and Tasting Replicate*Judge, Tasting Replicate*Wine and Judge*Wine interactions] was performed using XLSTAT software (Addinsoft, Paris, France), at 95% confidence interval (p<0.05). Attributes that differed were separated by least significant difference (LSD) post-hoc tests. Principal component analysis (PCA) (Observations/variables were chemical compounds, supplementary variables were winemaking treatments, no rotation, PCA type: Pearson (n), type of biplot: correlation biplot/ coefficient=automatic) and partial least squares regression analysis (PLS) (Y/Quantitative variables were chemical compounds, X/Quantitative variables were sensory attributes, algorithm: fast, stop conditions: automatic, cross-validation: Jackknife (LOO), variables: centre and

reduce, confidence interval: 95%) were performed with XLSTAT software (Addinsoft, Paris, France).

4.3 Results

4.3.1 Basic must parameters

The starting sugar concentration for each treatment is outlined in Table 4.3.

Table 4.3 Wine grapes were dried to a target sugar concentration, and delivered to the winery for processing when ready, while control grapes were processed without drying. In some cases, the must was diluted with water to homogenize the winemaking replicates, as needed. Generally, pH increased as grapes dried, while titratable acidity (TA, g/L tartaric acid) decreased. Grape sugars increased as a function of drying, as indicated by the significant increase at each starting sugar concentration.

4.3.2 Basic wine parameters

Trends for TA in wine were observed as previously described in the must, with control treatments having higher TA than wines made from partially dehydrated grapes, and EC1118 wines having higher TAs than CN1, with the exception of the 26.0°Brix treatment (Table 4.3).

Wines were fermented to dryness (<5 g/L residual sugar), with only CN1 27.5°Brix wine being significantly higher than all other treatments (2.35±1.23 g/L remaining). Ethanol concentration in the final wines was not significantly different between yeast strains, except for 24.5°Brix. As reported in the previous data chapter, other important metabolite trends were as follows: glycerol concentration was significantly higher in CN1 wines, while oppositely, acetic acid and ethyl acetate were significantly lower in comparison to EC1118, and less significantly less acetaldehyde at the 26 and 27.5 °Brix treatments (see chapter three).

Table 4.3:Metabolites and basic physio-chemical characteristics in must and wine samples (mean ± standard deviation).

Fermentations were conducted in triplicate and each sample was tested in duplicate for all metabolites other than soluble solids. Lowercase (EC1118) and uppercase (CN1) letters indicate statistical differences within the same yeast treatment determined by analysis of variance (ANOVA) with mean separation by Fisher's Least Significant Difference (LSD; $p< 0.05$). Asterisks (*=$p<0.05$, **=$p<0.01$, ***p=<0.001) indicate differences between yeast strains within a target Brix level as det.

MUST					
Basic Physio-Chemical Characteristic	**Yeast**	**Control (21.5°Brix) Target**	**24.5°Brix Target**	**26.0°Brix Target**	**27.5°Brix Target**
Soluble Solids (°Brix)	*S. cerevisiae* EC1118	21.5±0.5	24.3±0.1	26.0±0.2	27.4±0.1
	S. bayanus CN1	21.7±0.5	24.4±0.1	25.9±0.1	27.5±0.0
Reducing Sugar (g/L)	*S. cerevisiae* EC1118	236±7d*	267±8c	293±4b	313±3a
	S. bayanus CN1	227±5D*	269±7C	290±4B	315±6A
pH	*S. cerevisiae* EC1118	3.41±0.07d	3.76±0.02b	3.65±0.02c	3.84±0.02a
	S. bayanus CN1	3.43±0.01C	3.71±0.03B	3.69±0.03B	3.85±0.01A
Titratable Acidity (g/L tartaric acid)	*S. cerevisiae* EC1118	5.1±0.3a	4.8±0.1b	4.4±0.1c	4.7±0.0b
	S. bayanus CN1	4.9±0.3A	4.7±0.1A,B	4.5±0.1B	4.6±0.1B
WINE					
Basic Physio-Chemical Characteristic /Metabolite	**Yeast**	**Control (21.5°Brix) Target**	**24.5°Brix Target**	**26.0°Brix Target**	**27.5°Brix Target**
Reducing Sugar (g/L)	*S. cerevisiae* EC1118	0.07±0.00a	0.11±0.07a	0.07±0.00a	0.09±0.03a*
	S. bayanus CN1	0.07±0.00b	0.26±0.15b	0.07±0.00b	2.35±1.23a*
pH	*S. cerevisiae* EC1118	3.82±0.05b	3.87±0.01b**	3.84±0.02b**	4.02±0.01a**
	S. bayanus CN1	3.78±0.07b	3.77±0.03b**	3.72±0.04b**	3.9±0.03a**

Titratable Acidity (g/L tartaric acid)	*S. cerevisiae* EC1118	8.5±0.3a***	6.5±0.1b***	6.7±0.1b***	6.4±0.1b**
	S. bayanus CN1	6.7±0.1c***	7.7±0.1b***	7.8±0.1a***	6.8±0.1c**
Ethanol (%v/v)	*S. cerevisiae* EC1118	11.8±0.4c	12.8±0.2b*	12.9+0.2b	14.5±0.3a
	S. bayanus CN1	11.5±0.2b	12.2±0.3b*	13.1±0.7a	14.0±0.6a

4.3.3 Descriptive Analysis (Analysis of Variance)

A preliminary two-way ANOVA was conducted with tasting replicate and winemaking replicate as factors (*Table A4.5, Table A4.6, Table A4.7, Table A4.8, Table A4.9, Table A4.10*). The purpose of this was to determine whether data that was collected for 18 wines, (EC1118 control, 26.0°Brix and 27.5°Brix as well as CN1 control, 26.0°Brix and 27.5°Brix and their respective triplicate winemaking replicates), could be collapsed to represent six treatments. Few significant values [factor: tasting replicate] came from this output. Thus, data could be collapsed to represent the six wine treatments without including winemaking replicates. Further analysis (PLS and PCA biplots *Figure 4.3*, *Figure A4.8*) take this model into consideration.

A three-way ANOVA [F=Tasting replicate, Judge, Wine and interactions] (*Table A4.11A*) was then conducted with the collapsed data. There were no significant differences in tasting replicate. All attributes were significant for judge factor (p<0.0001, alpha 0.05), likely indicating that the judges were using the scales differently. 17 of the 22 attributes were significantly different amongst the wines. Mean intensity scores are shown as spider plots (*Figure 4.1*, *Figure 4.2*). As shown, the EC1118 wines generally have higher ratings for in-mouth sensations and flavour attributes as the °Brix increase, while aroma attributes are not as clearly separated

by starting sugar concentration. CN1 wines are generally characterized by having higher flavour scores, and high alcohol and length of finish at high starting sugar concentrations (27.5°Brix). Aroma attributes were not as clearly separated as flavour and in-mouth sensations scores.

Attributes that did not differ by wine were spice aroma, black fruit aroma, herbal aroma, red fruit flavour (cooked/dried/fresh) and bitterness. There were some significant interactions between tasting replicate*judge (canned green vegetable aroma, vegetal flavour, earthy/toast flavour and astringency). There was a significant interaction for tasting replicate*wine for alcohol and judge*wine interactions were significant for some attributes (*Table A4.11*).

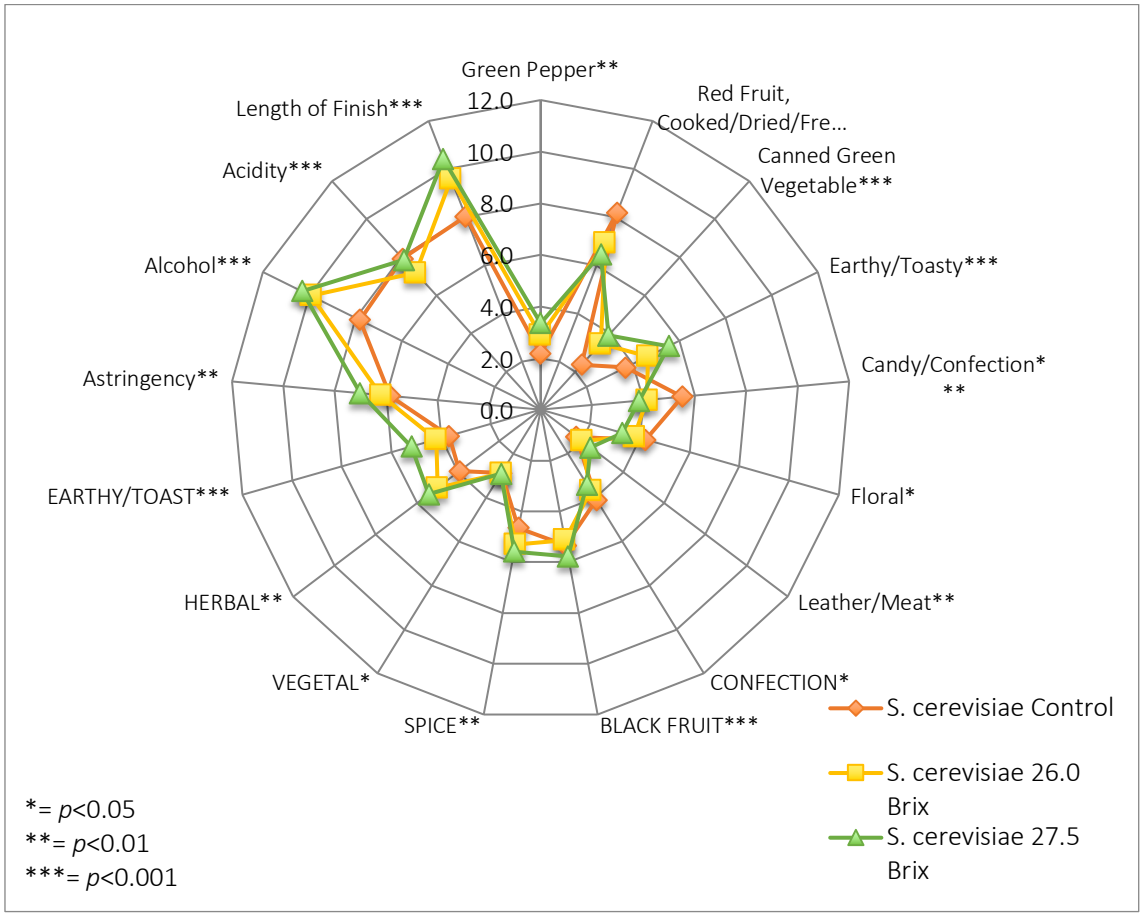

Figure 4.1: Spider plot of mean intensity ratings of significant attributes for S. cerevisiae EC1118 wines at all starting sugar concentrations levels (control (21.5 °Brix), 26.0 °Brix and 27.5 °Brix).

Aroma attributes are indicated in lowercase letters, flavour attributes in capital letters.

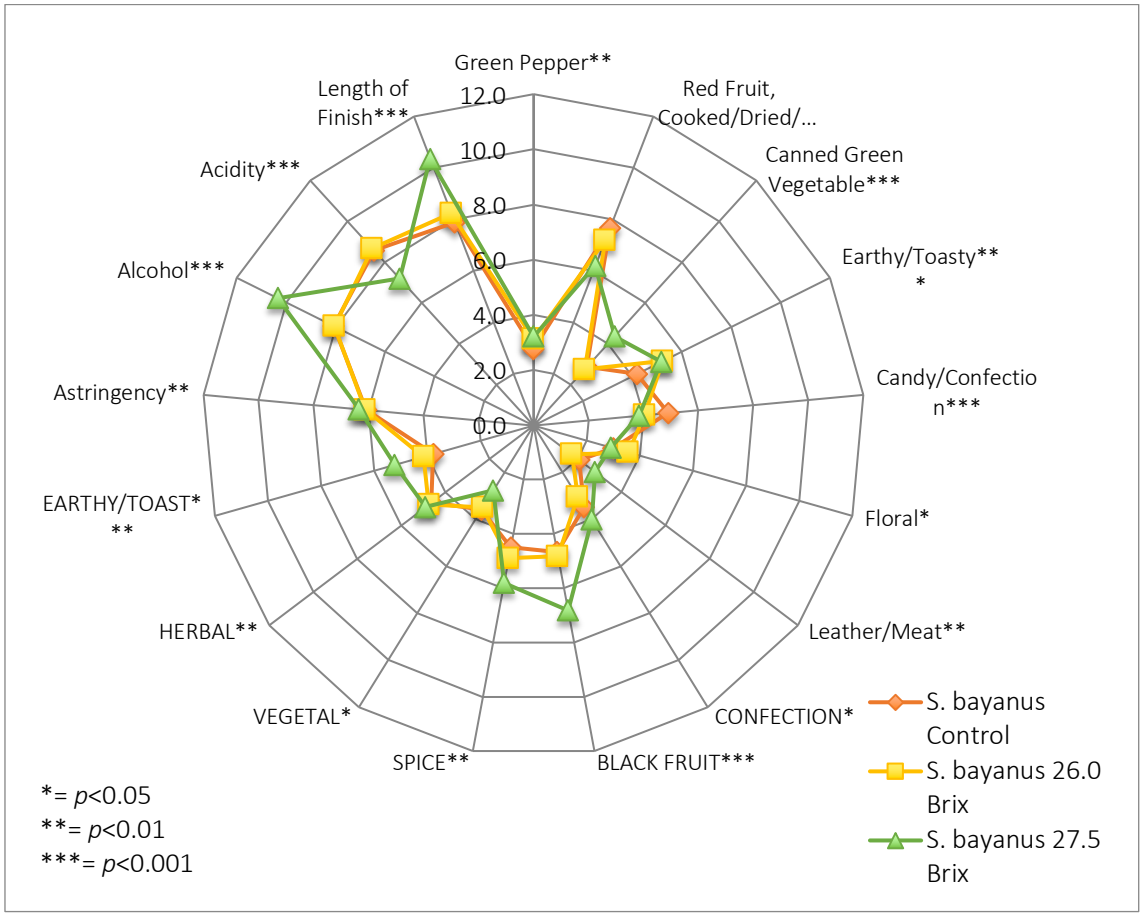

Figure 4.2:Spider plot of mean intensity ratings of significant attributes for S. bayanus CN1 wines at all starting sugar concentrations levels (control (21.5 °Brix), 26.0 °Brix and 27.5 °Brix).

Aroma attributes are indicated in lowercase letters, flavour attributes in capital letters.

4.3.4 Principal Component Analysis

A principal component analysis (*Figure 4.3*) was performed with all sensory attributes and all wines, including winemaking replicates. The PCA explains 61.34% of the variation on two factors which were retained; on F1, 43.54% and F2 17.18%. Most of the attributes are positively loaded on F1, while black fruit aroma, confection flavour, vegetal flavour, acidity and bitterness are loaded on F2. The small angles between canned green vegetable aroma, alcohol, length of finish, spice flavour, leather/meat aroma represent positive correlation amongst those attributes. Similarly, herbal aroma, herbal flavour, spice aroma, earthy/toast aroma, green pepper aroma and astringency are strongly correlated. On left side of the plot, floral aroma, red fruit flavour (cooked/dried/fresh), candy/confection aroma and red fruit aroma (dried/cooked/fresh) are also positively correlated, and negatively correlated with the aforementioned groupings of attributes. Vegetal flavour, acidity and bitterness are grouped together, representing a positive correlation amongst those attributes, and are positioned oppositely (180°) to confection flavour, indicating a negative correlation.

EC1118 control replicates one, two and three falls mostly in the upper left quadrant, correlated with floral aroma, red fruit flavour (cooked/dried/fresh), candy/confection aroma and red fruit aroma (cooked/dried/fresh). CN1 control replicates one, two and three falls within the lower left quadrant, and overlap with both EC1118 control replicate one and CN1 26.0°Brix replicate two. Generally, all CN1 26.0°Brix replicates and all CN1 control replicates are grouped together, while all EC1118 26.0°Brix replicates fall in the middle of the plot. All 27.5°Brix treatments

fall on the right side of the plot, within the top and bottom right quadrants. Biological replicates were reasonably grouped together on the plot (i.e. winemaking replicates for a given yeast and sugar concentration) except for EC1118 control replicates one, two and three and CN1 26.0°Brix replicates one, two and three.

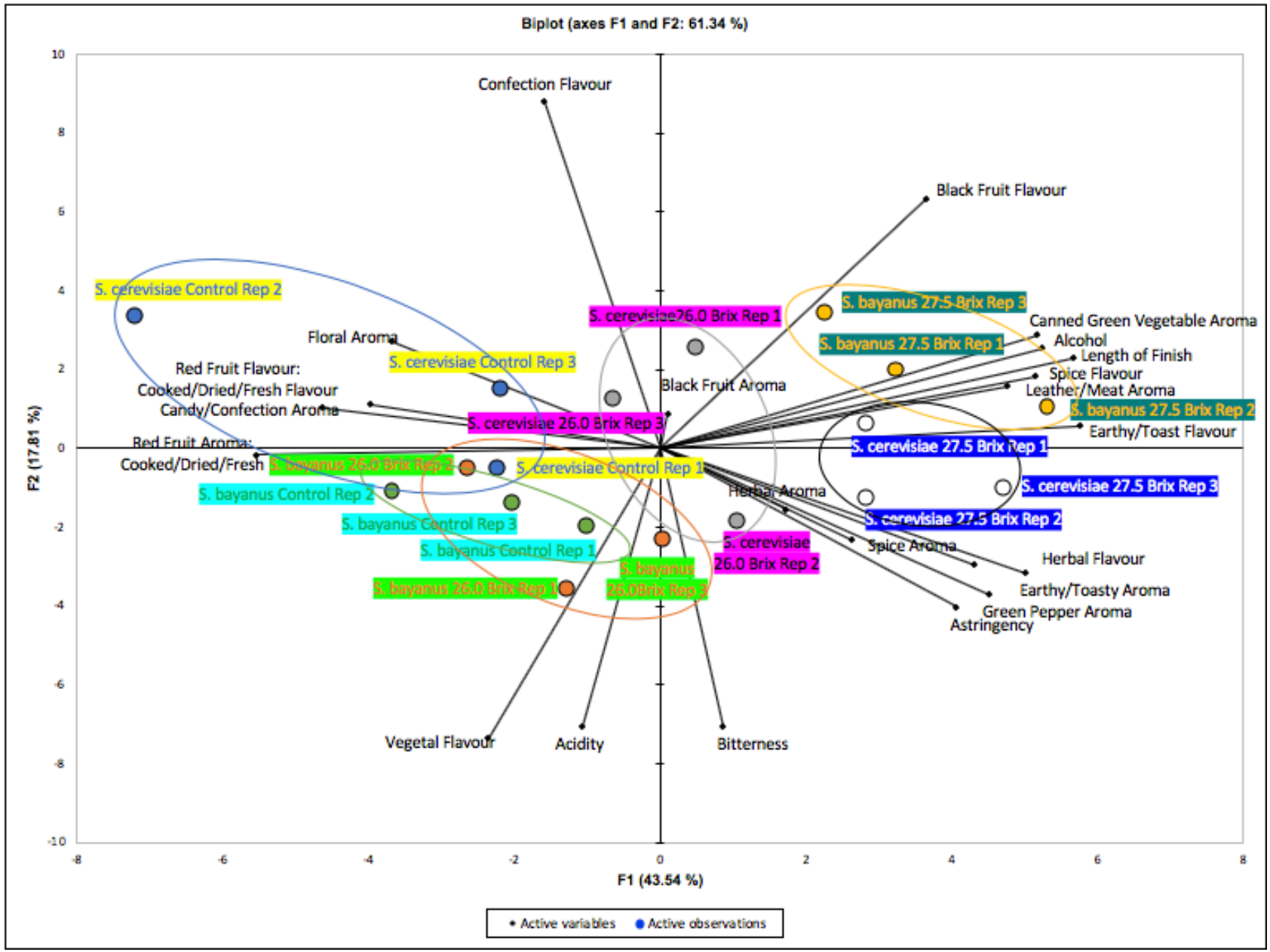

Figure 4.3: Sensory map of wines made from partially dehydrated grapes including winemaking replicates fermented with S. cerevisiae EC1118 and S. bayanus CN1 at different starting sugar concentrations (control (21.5 °Brix), 26.0 °Brix and 27.5 °Brix) on Factors 1 and 2.

Ellipses around data points group winemaking replicates from the same yeast and starting sugar concentration.

4.3.5 VOCs:

4.3.5.1 Ethyl esters:

Several ethyl esters were measured in this study (*Figure 4.4*). Ethyl hexanoate concentrations were significantly higher in all EC1118 sugar treatments, increasing as sugar concentration increases. There was a range of 2.1 to 2.6-fold increase in ethyl hexanoate in CN1 wines at each starting sugar concentration.

Generally, ethyl octanoate was found in higher concentrations in EC1118 treatments, with the highest concentrations measured in 26.0°Brix wine.

The concentration of ethyl isobutyrate was significantly higher in all *S. bayanus* CN1 wines than in *S. cerevisiae* EC1118 wines. The concentration did not increase in a linear trend as sugar increased; the highest concentration was measured in control and 26.0°Brix wine, and significantly lower concentrations (within yeast strain) at 24.5 and 27.5°Brix. Between yeast strains, there was a 3.4 to 5.6-fold increase in ethyl isobutyrate.

Ethyl butyrate was significantly higher in *S. cerevisiae* EC1118 wines and was found in the highest concentration in the control wine.

Ethyl isovalerate was generally found in higher concentrations in the wines fermented with *S. bayanus* CN1, with the exception of the 27.5°Brix treatment, with *S. cerevisiae* EC1118 27.5°Brix measuring approximately 2.4-fold higher.

Ethyl 2-methyl butyrate was significantly higher in *S. bayanus* CN1 wines across all treatments. The highest concentration of this compound was found in *S. bayanus* CN1 control and 26.0°Brix wines, a trend similar to ethyl isobutyrate. The highest concentration of ethyl 2-methylbutyrate was measured in *S. bayanus* CN1 control

treatment, and lowest in *EC 1118* 27.5°Brix and 24.5°Brix treatments.

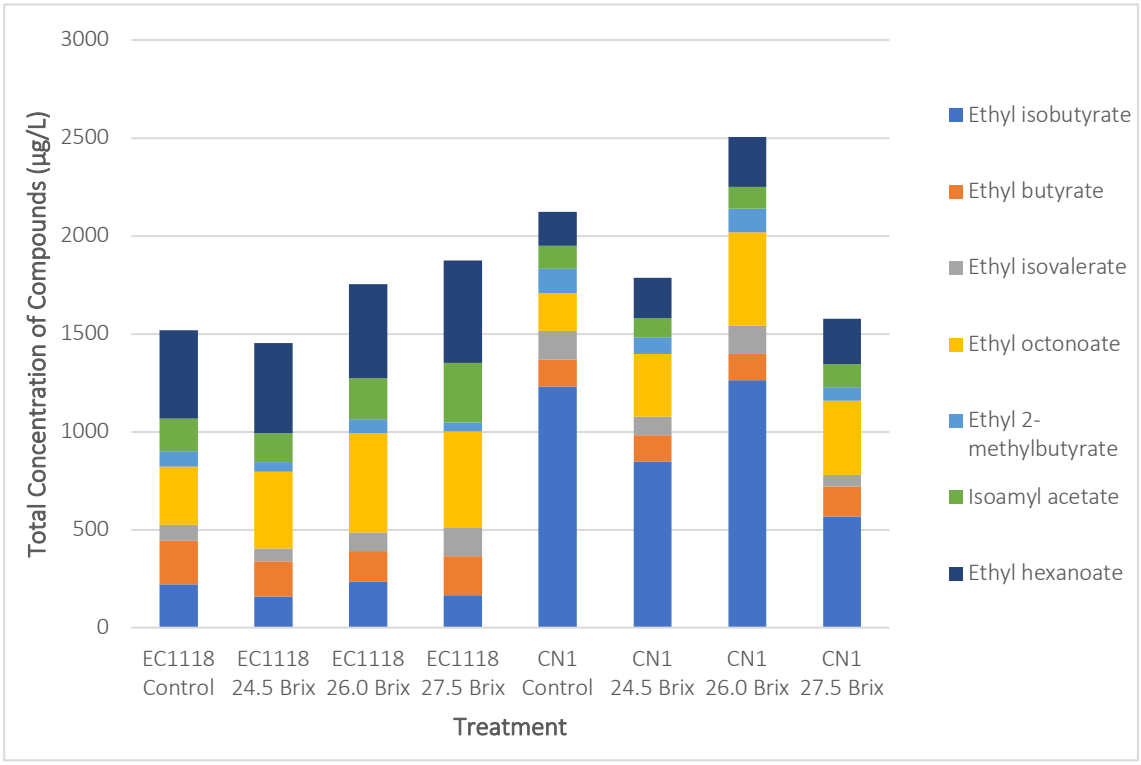

Figure 4.4: Selected volatile organic compound concentrations for control and wines made from partially dehydrated grapes: S. cerevisiae EC1118 and S. bayanus CN1.

Values are averages of 8 data points: duplicate measurements of 2 winemaking replicates, 2 bottles per replicate.

4.3.5.2 Alcohols

Concentration of 2-phenylethanol in all wines fermented with CN1 were higher than in the EC1118 wines (*Figure 4.5*). In the control treatment, there was a 3.2-fold difference in 2-phenlyethanol concentration of *S. bayanus* CN1 wine when compared to *S. cerevisiae* EC1118. This trend occurred at all starting sugar levels (*Table A4.14*). Hexanol concentrations were not statistically different between yeasts in control treatments but the remaining treatments were statistically different, with the higher concentrations measured in wines fermented with *S. bayanus* CN1.

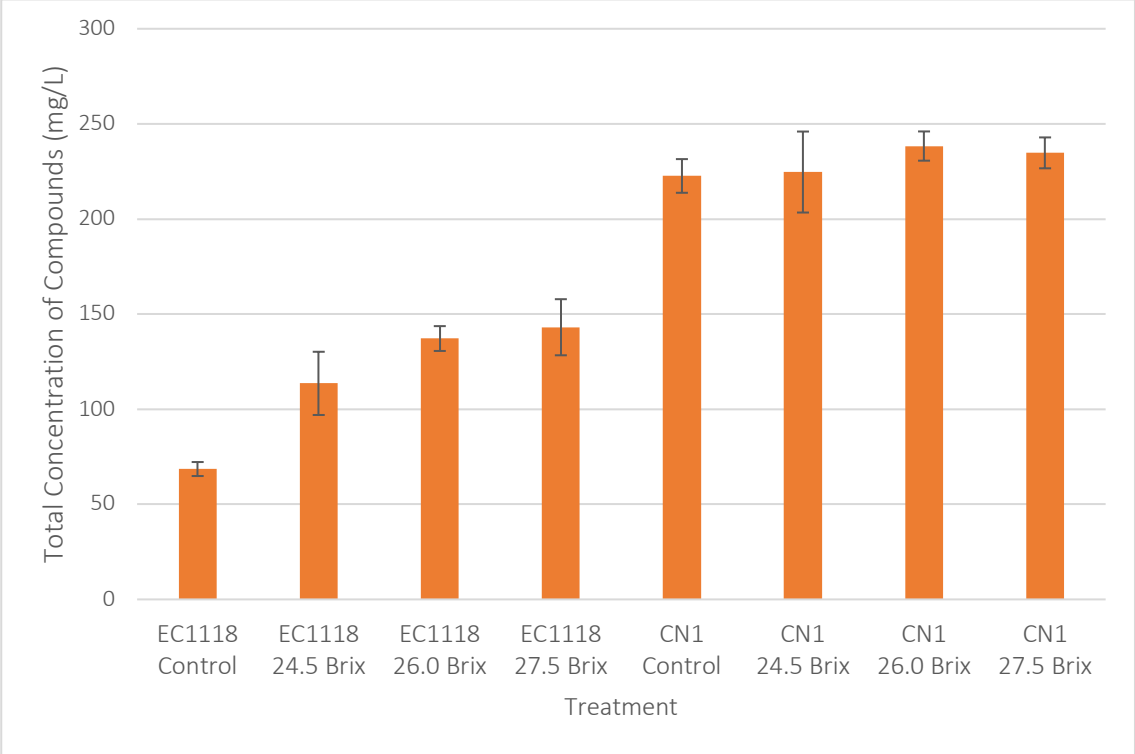

Figure 4.5: 2-Phenylethanol concentrations for control and treatment wines made from partially dehydrated grapes: S. cerevisiae EC1118 and S. bayanus CN1.

Values are averages of 8 data points (duplicate measurements of 2 winemaking replicates, 2 bottles per replicate) ± standard deviation.

4.3.5.3 Acids

Isoamyl acetate was significantly higher in wines fermented with *S. cerevisiae* EC1118, with the biggest difference found in the highest Brix treatments. At 26.0°Brix, there is approximately 2-times more isoamyl acetate in *S. cerevisiae* EC1118 wine, and at 27.5°Brix there is a 2.6-fold increase in *S. cerevisiae* EC1118 wines.

4.3.5.4 VFAs

For both VFAs measured (hexanoic acid and octanoic acid), wines fermented with *S. cerevisiae* EC1118 had higher concentrations in all treatments. Hexanoic acid concentrations are as follows: control; EC1118: 3183.63 ± 112.87^{a}, CN1: 1160.22 ± 66.97^{b}, 24.5°Brix; EC1118: 2501.71 ± 134.62^{a}, CN1: 1272.53 ± 72.08^{b}, 26.0°Brix; EC1118: 2394.35 ± 88.41^{a}, CN1: 1148.21 ± 220.42^{b}, 27.5°Brix; EC1118: 1955.13 ± 161.45^{a}, CN1: 990.17 ± 46.93^{b}.

Octanoic acid concentrations are as follows: control; EC1118: 3230.86 ± 192.37^{a}, CN1: 1616.67 ± 108.64^{b}, 24.5°Brix; EC1118: 2560.33 ± 55.84^{a}, CN1: 1995.42 ± 105.59^{b}, 26.0°Brix; EC1118: 2466.13 ± 111.19^{a}, CN1: 1889.04 ± 126.5^{b}, 27.5°Brix; EC1118: 1848.44 ± 221.04^{a}, CN1: 1430.00 ± 71.78^{b}.

4.3.6 Partial Least Squares (PLS) Regression Analysis

The PLS (Figure 4.6) of the 22 sensory attributes and 11 VOC and VFA compounds is described below.

The cumulated R^2Y and R^2X index that corresponds to the correlations between the explanatory (x) and dependent (y) variables explains 80.0% (x) and 90.2% (y) of variability. The PLS plot can be interpreted in the same way that a PCA plot can be

interpreted: the angles formed by the vector-variables in the plot give an indication of the correlations between the original variables (Tenenhaus and Vinzi, 2005).

PLS indicates that control wines from CN1 are correlated to candy/confection aroma, associated with the presence of ethyl isobutyrate. Similar to the PCA plot generated for the sensory attributes and the treatments, *S. bayanus* CN1 control is associated with attributes like candy/confection aroma and red fruit aroma (cooked/dried/fresh). PLS predicts the explanatory variables (VOC and VFA data) from response variables (sensory analysis), predicting that CN1's attributes are due to the relative abundance of ethyl isobutyrate, ethyl butyrate and ethyl 2-methylbutyrate, based on their groupings on the plot. Conversely, *S. cerevisiae* EC1118 27.5°Brix is associated with herbal flavour, earthy/toasty aroma, astringency, green pepper aroma and herbal aroma, correlated with ethyl octanoate and isoamyl acetate and inversely related to the CN1 attributes previously described. *S. bayanus* CN1 27.5°Brix is correlated with black fruit flavour, leather/meat aroma and black fruit aroma, and associated with hexanol and 2-phenylethanol.

Generally, the control wines from each yeast strain and the 26.0°Brix wines from each yeast strain were positioned closely to each other on the plot. These wines were therefore described with similar sensory attributes and are associated with similar volatiles. The high Brix wines (27.5°Brix) are separated by yeast strain on the plot, meaning they are described differently and are association with different volatiles. Sensory attributes are separated on the plot. Fresher aromas and flavours are negatively

loaded on t1, while the sensory descriptors positively loaded on t1 stray from fruit-like descriptors. Rather, they are more vegetal, spicy and earth.

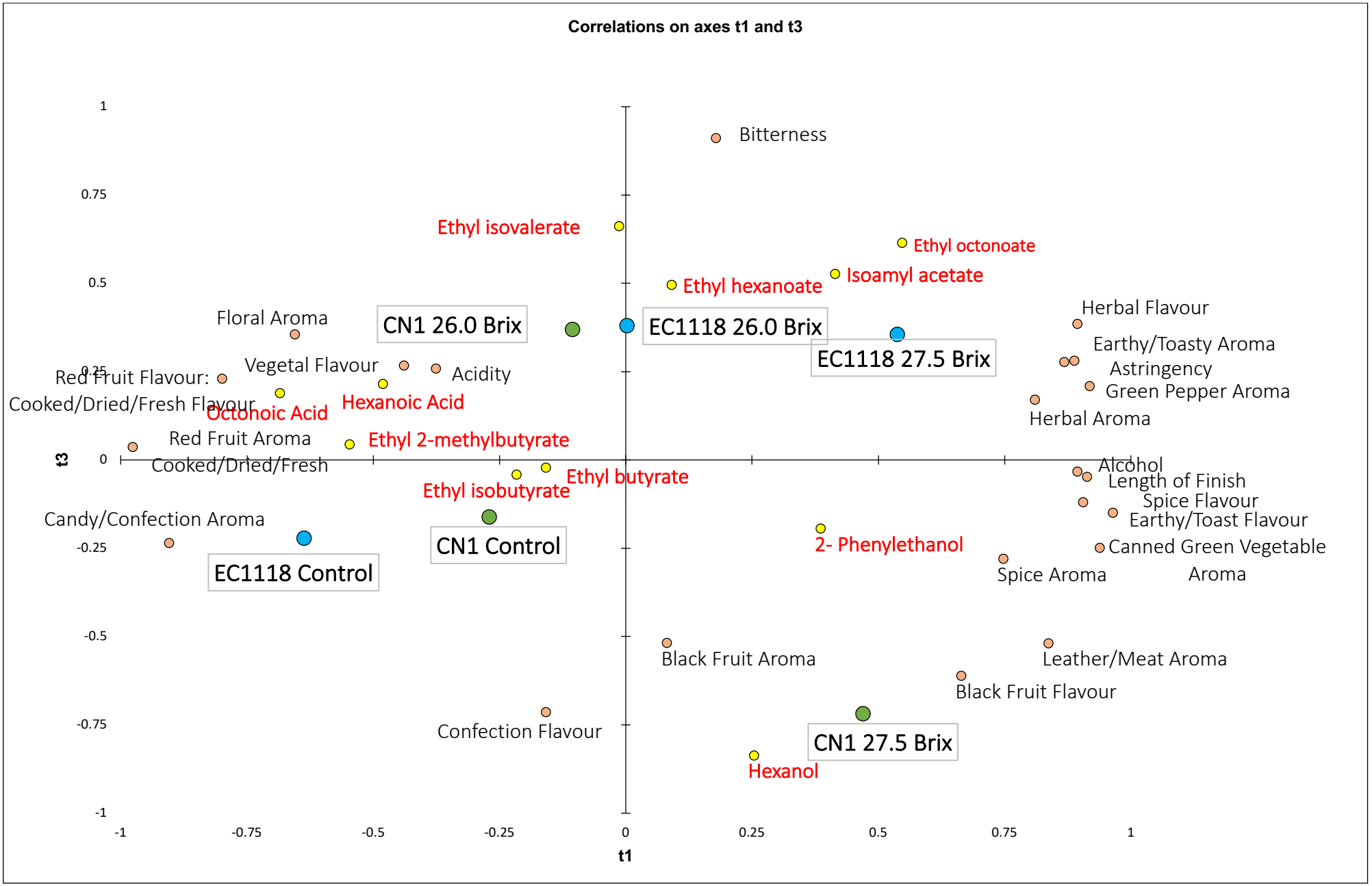

Figure 4.6: Impact of yeast strain and starting sugar concentration on the sensory and chemical profiles of Cabernet franc control and wines made from partially dehydrated grapes as determined by PLS.

Each treatment point is the mean of attribute intensity rating of three winemaking replicates.

4.4 Discussion

The aim of this study was to assess the differences in aroma and flavour in wines made from partially dehydrated grapes with varying initial sugar concentration fermented with two yeast strains. VOC and VFA concentrations amongst treatments were compared to identify the major contributors to the sensory profile of the wine. PLS was used to establish relationships between the sensory and instrumental data. This study provides novel insight on the sensory profile of wines made with partially dehydrated grapes fermented with CN1 yeast. The association of chemical compounds to sensory descriptors may be attributed to possible relations between volatiles and their associated odours, to the presence of other compounds which were not analyzed, or to some associations and interactions among the analyzed compounds (Vilanova et al., 2010).

Previous studies on wines fermented with partially dehydrated grapes have considered the sensory impact of ageing (Pagliarini et al., 2004; González-Álvarez et al., 2013), the presence of the pathogenic agent *Penicillium* (Zapparoli et al., 2018), the presence of *Botrytis cinerea* (Tosi et al., 2013; Tosi et al., 2012), the drying method (Marquez et al., 2013; Panceri et al., 2017) and the drying temperature and humidity (Bellincontro et al., 2017). Relevant studies on the volatile composition of wines made from partially dehydrated grapes have focused on the impact of starting sugar concentration (Moreno et al., 2008, Dall'Agnol & Rizzon, 2002), grape variety and mass loss (Bellincontro et al., 2016), drying methods (Guarrera et al., 2005), berry maturity and dehydration rate (Urcan et al., 2017) and oxidative ageing (Fedrizzi et al., 2011).

4.4.1 Effect of Yeast Strain on Sensory and Chemical Data

In order to determine the effect of yeast strain on sensory and chemical data, prudent results will be discussed.

Sensory Descriptors

Generally, for this study, sensory terms that were generated by the descriptive analysis panel should represent both Cabernet franc typicity and wines made from partially dehydrated grapes. In a study conducted by Cadot et al. (2012), terms generated for Cabernet franc wines included stewed red fruits, empyreumatic (smoky, toast), spicy, vegetal and animal (leather, stable), all of which were also identified in this current study. Wines made from partially dehydrated grapes can be described aromatically with terms like jam, cherries in alcohol and fresh fruit, as well as herbal, tobacco, spice and vanilla notes (Accordini, 2013). There is overlap in the terms generated for Cabernet franc table wine, and wines made from partially dehydrated grapes, an example being spice and stewed fruits (jam). Interestingly, in this study, attributes like red fruit (cooked/dried/fresh) aroma and flavour and floral and candy/confection aroma were associated with the control wines (representative of Cabernet franc table wines) for both yeast strains, which were negatively correlated to herbal aroma and flavour, spice aroma and flavour earthy/toast aroma and flavour. The sensorial impact of yeast strain was observed only at the highest starting sugar concentration (27.5°Brix), a result consistent with the findings of López de Lerma et al. (2012), where autochthonous yeast differed from selected yeasts sensorially in wines made with partially dehydrated

grapes. In the case of this study, though, the yeast strain difference arose only at a particular starting sugar concentration.

Importantly, sensory attributes that are associated with oxidation compounds such as vinegar (acetic acid), nail polish remover (ethyl acetate) and bruised apple (acetaldehyde), (Jackson, 2008) were not included on this list of descriptors, likely because the values of these compounds were measured at below threshold levels (acetic acid in table wine 0.7-1.1g/L, ethyl acetate 0.07-0.17 g/L (Cliff and Pickering 2006), acetaldehyde 0.5 mg/L in 10% ethanol/water solution (Guth et al., 1997)) in this study. Even though there were significantly higher concentrations of oxidation compounds in wines made with EC1118, they were not detected sensorially.

VOCs

Of the VOCs measured, 2-phenylethanol and ethyl isobutyrate had the most pronounced differences amongst yeast strains. Higher alcohols are important compounds in wine, and 2-phenylethanol is the most important phenol-derived higher alcohol. This class of compounds commonly accounts for about 50% of the aromatic constituents in wine, excluding ethanol (Jackson, 2008). Importantly, grape dehydration impacts amino acid catabolism (due to water stress), which can increase the concentration of higher alcohols (Bellincontro et al., 2004). The concentration of 2-phenylethanol in wines fermented with CN1 yeast is significantly higher for all treatments than EC1118. This compound can be described as “floral”-like, or “spice”-like (Cordente et al., 2018). Perhaps the association with descriptors such as spice aroma and flavour (*Figure 4.6*) are due to 2-phenylethanol content, or another unquantified

metabolite like 2-phenethyl acetate are contributing to the association with these descriptors. Further, concentration alone is insufficient to explain the properties of a product (Voilley and Lubbers, 1998), as an important link between VOCs and the sensorial perception of aromas and flavour is the concept of the sensory odour detection threshold (ODT). ODT values are defined as the lowest concentration of odorant that could be recognised by at least 50% of the individuals as different from that of the blank (Ferreira et al., 2000). Compounds that are odour active have an odour activity value (OAV; calculated by dividing the concentration of a compound present in a matrix by the ODT for that compound in that specific matrix) greater than one (McKay et al., 2018). However, the effect of volatile compounds on the wine sensory profile is complex, as the effects may be synergistic, additive or suppressive. Interestingly, in this study, the concentration of 2-phenylethanol is above threshold for all wines fermented with CN1 yeast, and only in the 27.5 °Brix wine fermented by EC1118.

Evidence from literature suggests that desirable rose-like aromas are often difficult to achieve with existing *S. cerevisiae* strains, and that alternate yeasts, or modified yeast are required to overproduce 2-phenylethanol (Cordente et al., 2018). Fusel alcohols are generally formed through metabolic pathways involving the formation of amino acids. The most common pathway of formation, which account for more than 80% of the production (Nesbit et al. 2014), is the anabolic pathway which occurs during the biosynthesis of amino acids from hexose sugars. By this mechanism, sugar degradation leads to the formation of α-keto acids which are decarboxylated to aldehydes and reduced to fusel alcohol. The other pathway of formation is of lesser importance and happens by the Ehrlich pathway, which is

the formation of new amino acids by catabolism of grape amino acids. In this mechanism, grape amino acids are deaminated to form α-keto acids. These α-keto acids are transformed into fusel alcohol following the steps above (Chen, 1978). It has been established that fermentation with *S. bayanus* strains result in a greater concentration of some higher molecular weight alcohols, particularly 2- phenylethanol (Eglinton et al., 2000). It is also important to note that higher alcohols tend to increase in higher Brix must (Cordente et al., 2012). This is consistent with the findings of this current study.

Few esters are present in grapes, most are formed during fermentation and are found in the finished wine. Esters are condensation products of the carboxyl group of an organic acid and the hydroxyl group of an alcohol or a phenol. They are important fermentation-derived aroma compounds, produced by yeast after cell division has ceased (Tsakiris et al., 2014). The choice of yeast has a great influence on the production of esters (Rapp and Mandery, 1986). Generally, it has been accepted that esters contribute to the fruity aroma of young wines (Antalick et al., 2014). In this study high concentrations of ethyl isobutyrate were measured in CN1 wines. This compound has been identified as the most abundant ethyl ester of branched esters measured in red wines (Antalick et al., 2014), and also contributes to a higher overall concentration of ethyl esters in wine fermented with *S. bayanus* yeast (Gil et al., 1996). This compound is formed by the esterification of ethanol with the acids formed by yeast from corresponding amino acids or amino acid derivatives, such as ketoacids. In fact, when considering the total composition of the wines in the study, ethyl esters are present in the highest concentration (by percentage) in CN1 wines control, 24.5 and 26.0°Brix, but in the

highest Brix wines, it is EC1118 that contains more ethyl esters. Ethyl esters impart a 'fruitiness' component to the wine, but also can mask 'vegetative' odours (Bindon et al., 2013). This may have occurred in this study, as red fruit flavour (cooked/dried/fresh) aroma and flavour were negatively correlated to canned green vegetable aroma.

4.4.2 Effect of starting Sugar Concentration on Sensory and Chemical Data

The second part of this discussion will review the prudent results with respect to the effect of starting sugar concentration, rather than yeast strain, on the sensory and chemical data of the wines. Wines fermented with different yeast strains at each starting sugar concentration (with the exception of 24.5°Brix wines) did not differ in their ethanol content, an important result for CN1 yeast. This is consistent with the findings of a preliminary study of CN1 where 28.0°Brix must was fermented with CN1 and EC1118, and ethanol differences were not significant (Kelly et al., 2018). Ethanol is an important component to the wine matrix, as it can elicit sweetness and bitterness; in an aqueous solution it can elicit sourness and saltiness (Thibideau and Pickering, 2017; Vidal et al., 2004). Further, ethanol can modify the perception of wine aroma compounds (Le Berre et al., 2007), and higher ethanol matrices (>16%v/v) seem to require the addition of sweetness to mask bitterness (Panovska et al., 2008). Ethanol is therefore able to modify the sensory profile of the wine significantly. With increasing ethanol concentration, different responses are elicited, namely tactile sensations like burning or tingling (Nolden and Hayes, 2015). Further, with regards to the abundance of descriptors associated to the wines, most of the sensory attributes are correlated with the 27.5°Brix wines, which contain the highest ethanol values of all wines evaluated.

Meillon et al. (2010) reported that wines with a higher alcohol content were perceived as more complex than lower alcohol wines, and panelists rated them as "persistent" and "with many aromas". In our study, the alcohol content in the 27.5°Brix wines could contribute to the perceived complexity and persistence (length of finish), and thus differentiate them from the other treatments.

VOCs

It has been established that in the aroma formation of wines made from partially dehydrated grapes, a significant contribution to the wine aroma comes from the dehydration process (Bellincontro et al., 2016). Wines made from dehydrated grapes have contain high concentrations of acetic acid, hexanol, esters, (Bellincontro et al., 2004), isoamyl alcohols, 2-phenylethanol (Moreno et al., 2008), ethyl acetate, phenylethyl alcohol (Ruiz et al., 2010), ethyl hexanoate, ethyl octanoate and acetaldehyde (Budic-Leto et al., 2010), all of which are in agreement with this study, and most of which differ based on starting sugar concentration. Isoamyl acetate is important to *Passito* (dried) wines and present in high values in white wines made from dehydrated grapes (Giordano et al., 2009). This compound was measured in above-threshold concentrations for all wines, though higher in EC1118 wines. Contrary to this current study, *S. bayanus* wines generally contain high concentration of isoamyl acetate (Eglinton et al., 2000). Interestingly, even the control EC1118 wines had higher isoamyl acetate values in this study.

Hexanol generally increased as starting sugar concentration increased, a result that is consistent with the findings of Bellincontro et al. (2016) and Urcan et al. (2017).

When both factors are considered, it becomes clear that the impact of yeast strain is most clearly demonstrated when starting sugar concentration is highest. This is an important finding to this study, as it provides a quantitative value to industry personnel for production of this wine style.

4.4.3 Other Considerations

Alkyl-methoxypyrazines (MPs) are a class of compounds that have been detected in wine grapes that may have a positive impact on wine flavour at low levels, but at higher concentrations, can be regarded as unfavourable due to the “green” or “unripe” characteristics they impart. MPs are characteristic of wines like Sauvignon blanc and Cabernet sauvignon and are elevated in under ripe fruit and in cooler climate viticultural areas (Pickering et al., 2010). These compounds can be grape-derived or may be from the presence of Coccinellidae beetles (*Harmonia axyridis*) when grape crushing occurs, resulting in wines that are faulted due to ladybug taint. The aroma-active methoxypyrazines of interest include isobutyl methoxypyrazine (IBMP), isopropyl methoxypyrazine (IPMP), secbutyl methoxypyrazine (SBMP) and 2,5-dimethyl-3-methoxypyrazine (DMMP) (Botezateau and Pickering, 2012). Of these compounds, IBMP and IPMP contribute the most sensorially to wines (Pickering et al., 2008). Associated detrimental sensory descriptors include vegetative, green and herbaceous (Pickering et al., 2010). There has been anecdotal concern about utilizing a grape variety that is already associated with MPs for producing appassimento wine. It has been suggested that MPs may be susceptible to further concentration from drying, and thus grapes picked at suboptimal ripeness that may be “green” in flavour will only be negatively

impacted further. Given that sensory detection thresholds for IPMP and IBMP in wine are in the 0.3-10 ng/L range (Pickering et al., 2007), these concerns should be considered seriously so that wine quality is not impacted.

Methoxypyrazine content does not increase as starting sugar concentration increases. Generally, for IPMP concentration, the content is higher in wines fermented with EC1118, but only at 26.0°Brix are there significant differences in the wines made with different yeast strains (Figure 4.7). These concentrations are present at above-sensory threshold levels, so the question remains whether or not there is a sensorial impact. In the current project, descriptors like green pepper and green vegetal aroma and vegetal flavour came from the descriptive analysis. The intensity ratings for all of these descriptors were low, and generally, increased as starting sugar concentration increased (*Figure 4.1*, *Figure 4.2*). Although these compounds are present at above-threshold levels, it appears that there may be no detrimental sensorial implication. Further research on this topic is of great interest to this wine style and its quality. Methoxypyrazine concentration on the wines included in this study (*Figure A4.9*, A-C) were measured after this thesis was approved and will be included in subsequent publications of this data.

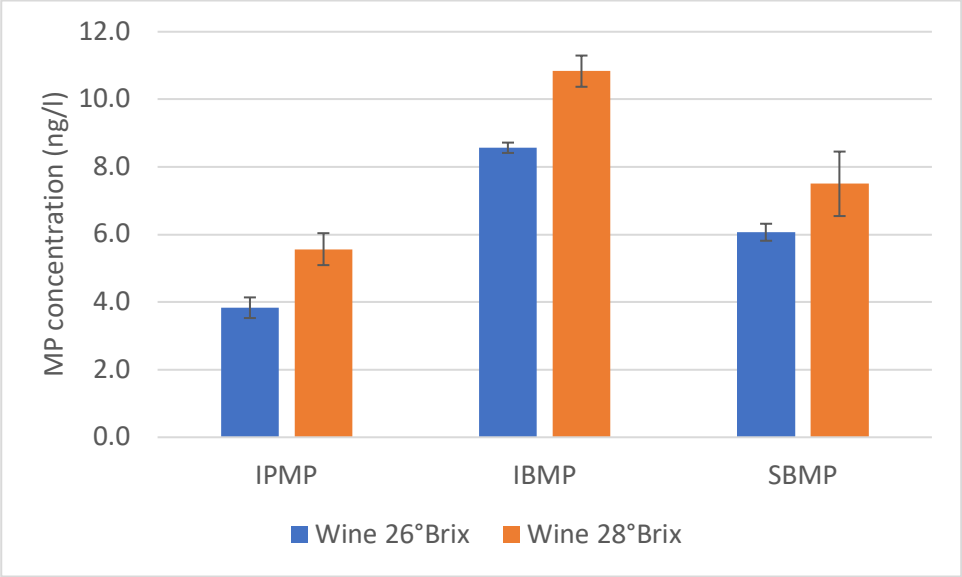

Figure 4.7: Concentration of methoxypyrazines in 2013 appassimento wines from CCOVI Appassimento Project dried in Cave Spring Winery Barn.

4.5 Conclusions

Both sensory profiles and chemical composition of the wines made from partially dehydrated grapes were impacted by starting sugar concentration and yeast strain selection. The most significant impact, however, is from yeast strain selection. Important to this study is the comparable ethanol values of wines fermented with the same starting sugar concentration between yeast strains. Oxidation compounds that accumulate during grape drying, that also arise from high sugar fermentation are reduced in CN1 wines. Oxidation-related compounds were not characteristic of any wines due to their low starting concentration. However, wines made from partially dehydrated grapes containing high starting concentration of volatile acidity due to rot or disease that could impact quality could benefit from a yeast that reduces these compounds in the final wine. In addition to grapes with affected by rot, high starting sugar concentration fermentations can be problematic for the management of volatile

acidity associated with a corrective hyperosmotic stress response in yeast cells. The production of acetic acid has been correlated to the overproduction of glycerol. The shift in redox balance ($NADH/NAD^+$) caused by the accumulation of glycerol is compensated by the production of acetic acid to reduce NAD^+ back to NADH. The acetic acid is produced by yeasts via the oxidation of acetaldehyde (Accordini, 2013). Thus, wines produced under these conditions (e.g. wines made from partially dehydrated grapes), may contain high concentrations of acetic acid. Since oxidation compounds present at higher than threshold concentrations may negatively impact quality, the use of CN1 may positively impact final wine quality.

Regarding the impact of starting sugar concentration, we observed that increased complexity (longer length of finish and more describing attributes) was associated with wines fermented at the highest starting sugar concentration. The influence of grape drying is evident in our sensorial data, as control wines were differentiated the most from the highest Brix wines. Further, higher Brix wines contain elevated concentrations of compounds like ethyl hexanoate, ethyl isovalerate, ethyl octanoate, 2-phenylethanol and hexanol, all varying with yeast strain. Sensorially, it is the interaction between yeast strain and starting sugar concentration that makes the biggest impact. The so-called "sweet spot" for this study is 27.5°Brix, where resultant wines are differentiated.

To our knowledge, this is the first time that appassimento wine made in Ontario at varying starting sugar concentrations fermented with indigenous yeast, have been sensorially and chemically profiled. This is the first time that CN1 yeast used to ferment partially dehydrated grapes to produce Ontario appassimento wine has been

characterized sensorially. Future direction of this study will include consumer preferences for these wines, to inform the impact of these differences. Next, considerations for the development of appassimento wines (such as the inclusion of *Botrytis cinerea* infected grapes) will also be evaluated. This information can be used to assist with the optimization of winemaking practices to enhance wine quality when fermenting partially dehydrated grapes.

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4.7 Appendix

Table A4.4: Training schedule for panel and exposure to wines.

In training sessions one and two, all panelists were exposed to all 18 wines in order to develop lexicon of attributes. Reference standards were used in sessions three to eight, and the base wine was the same every time new standards were made. Upper case letters indicate the blinding codes for sessions one to seven. In session eight, the codes were three-digits, as they were during data collection. Some wines (example: CN1 26.0 Rep 2 during session four) were presented to the panel twice as a check of panel performance.

		Sept. 30	**Oct. 7**	**Oct. 14**	**Oct. 21**	**Oct. 28**	**Nov. 4**	**Nov. 11**	**Nov. 25**
Control	**Rep**	**Session 1**	**Session 2**	**Session 3**	**Session 4**	**Session 5**	**Session 6**	**Session 7**	**Session 8**
EC1118- *S. cerevisiae*	1	✓(G)			✓ (C)				
	2		✓(D)	✓ (A)				✓ (C)	
	3		✓ (A)			✓ (B)			
CN1- *S. bayanus*	1	✓ (D)					✓ (D)		
	2		✓ (F)	✓ (E)					✓(119)
	3	✓(A)				✓ (E)			
26.0°Brix		**Session 1**	**Session 2**	**Session 3**	**Session 4**	**Session 5**	**Session 6**	**Session 7**	**Session 8**
EC1118- *S. cerevisiae*	1	✓(C)		✓ (F)	✓ (F)			✓ (A)	
	2		✓ (G)						
	3	✓(I)				✓ (A)			
CN1- *S. bayanus*	1	✓(B)					✓ (B)		
	2		✓ (B)	✓ (C)	✓ ✓ (B)(D)				
	3		✓ (E)			✓ (F)			✓(860)
27.5°Brix		**Session 1**	**Session 2**	**Session 3**	**Session 4**	**Session 5**	**Session 6**	**Session 7**	**Session 8**
EC1118- *S. cerevisiae*	1	✓(E)			✓ (E)				✓(931)
	2	✓(H)		✓ (B)			✓✓(A/ C)		
	3		✓ (I)			✓ (C)			
CN1- *S. bayanus*	1	✓(F)				✓ (D)			
	2		✓ (H)	✓ (D)				✓✓ (B)+(?)	
	3		✓ (C)		✓ (A)				✓(419)
24.5°Brix				**Session 3**	**Session 4**	**Session 5**	**Session 6**	**Session 7**	**Session 8**
BASE WINE (for reference standards)				EC1118 Rep 1	EC1118 Rep 1	EC1118 Rep 1	EC1118 Rep 1	EC1118 Rep 1	EC1118 Rep 1

*Table A4.5: S. cerevisiae EC1118 control, 2-way ANOVA [Factors: Tasting replicate, winemaking replicate and tasting replicate*winemaking replicate interaction] (p<0.05).*

Attribute		Tasting Replicate	Winemaking Replicate	Tasting Replicate* Winemaking Replicate
Green Pepper Aroma	F-Value	1.011	1.316	0.433
	p-Value	0.319	0.276	0.651
Spice Aroma	F-Value	0.456	0.114	0.034
	p-Value	0.502	0.893	0.966
Red Fruit Aroma (Cooked/ Dried/Fresh)	F-Value	0.028	0.197	0.717
	p-Value	0.867	0.822	0.492
Black Fruit Aroma	F-Value	0.036	0.011	0.228
	p-Value	0.850	0.989	0.797
Herbal Aroma	F-Value	0.003	1.306	0.476
	p-Value	0.959	0.279	0.624
Canned Green Vegetable Aroma	F-Value	0.062	2.030	0.212
	p-Value	0.804	0.140	0.809
Earthy/ Toasty Aroma	F-Value	0.024	1.934	0.100
	p-Value	0.877	0.153	0.905
Candy/ Confection Aroma	F-Value	0.002	0.533	0.004
	p-Value	0.963	0.590	0.996
Floral Aroma	F-Value	0.044	1.608	0.063
	p-Value	0.835	0.209	0.939
Leather/ Meat Aroma	F-Value	2.003	0.220	0.116
	p-Value	0.162	0.803	0.891
Red Fruit Flavour	F-Value	1.385	0.395	0.209

(Cooked/ Dried/Fresh)	*p*-Value	0.244	0.676	0.812
Confection Flavour	F-Value	0.092	0.677	0.068
	p-Value	0.762	0.512	0.935
Black Fruit Flavour	F-Value	0.030	0.451	0.118
	p-Value	0.862	0.639	0.889
Spice Flavour	F-Value	0.205	0.157	0.294
	p-Value	0.652	0.855	0.747
Vegetal Flavour	F-Value	0.029	0.132	0.219
	p-Value	0.866	0.877	0.804
Herbal Flavour	F-Value	0.121	1.147	1.929
	p-Value	0.730	0.325	0.154
Earthy/ Toast Flavour	F-Value	0.169	0.169	0.169
	p-Value	0.682	0.496	0.987
Astringency	F-Value	0.025	1.060	0.090
	p-Value	0.876	0.353	0.914
Alcohol	F-Value	0.004	1.042	0.685
	p-Value	0.948	0.359	0.508
Acidity	F-Value	0.201	0.102	0.051
	p-Value	0.655	0.903	0.950
Bitterness	F-Value	0.362	1.388	0.677
	p-Value	0.549	0.258	0.512
Length of Finish	F-Value	0.420	2.577	0.057
	p-Value	0.519	0.084	0.945

*Table A4.6: S. bayanus CN1 control, 2-way ANOVA [Factors: Tasting replicate, winemaking replicate and tasting replicate*winemaking replicate interaction] (p<0.05).*

Attribute		Tasting Replicate	Winemaking Replicate	Tasting Replicate* Winemaking Replicate
Green Pepper Aroma	F-Value	1.790	0.471	0.017
	p-Value	0.186	0.627	0.983
Spice Aroma	F-Value	**4.987**	1.613	0.946
	p-Value	0.029	0.208	0.394
Red Fruit Aroma (Cooked/ Dried/Fresh)	F-Value	0.671	0.331	0.812
	p-Value	0.416	0.719	0.449
Black Fruit Aroma	F-Value	2.875	1.322	0.152
	p-Value	0.095	0.274	0.859
Herbal Aroma	F-Value	0.174	0.854	0.451
	p-Value	0.678	0.431	0.639
Canned Green Vegetable Aroma	F-Value	0.621	0.971	0.070
	p-Value	0.434	0.385	0.933
Earthy/ Toasty Aroma	F-Value	1.379	0.043	2.496
	p-Value	0.245	0.958	0.091
Candy/ Confection Aroma	F-Value	0.002	1.306	1.853
	p-Value	0.963	0.279	0.166
Floral Aroma	F-Value	0.055	0.188	0.747
	p-Value	0.815	0.829	0.478
Leather/ Meat Aroma	F-Value	1.562	0.517	1.785
	p-Value	0.216	0.599	0.177
Red Fruit Flavour	F-Value	0.222	1.098	0.177

(Cooked/ Dried/Fresh)	*p*-Value	0.639	0.340	0.838
Confection Flavour	F-Value	0.026	0.114	0.180
	p-Value	0.873	0.892	0.835
Black Fruit Flavour	F-Value	**4.298**	4.298	4.298
	p-Value	0.042	0.963	0.199
Spice Flavour	F-Value	**7.899**	0.500	0.489
	p-Value	0.007	0.609	0.616
Vegetal Flavour	F-Value	1.907	0.434	0.106
	p-Value	0.172	0.650	0.899
Herbal Flavour	F-Value	0.358	1.002	0.295
	p-Value	0.552	0.373	0.746
Earthy/ Toast Flavour	F-Value	3.521	0.315	1.911
	p-Value	0.065	0.731	0.157
Astringency	F-Value	2.180	0.055	0.011
	p-Value	0.145	0.947	0.989
Alcohol	F-Value	**14.760**	0.109	0.249
	p-Value	0.000	0.897	0.781
Acidity	F-Value	**7.337**	0.164	0.758
	p-Value	0.009	0.849	0.473
Bitterness	F-Value	0.022	0.423	0.003
	p-Value	0.883	0.657	0.997
Length of Finish	F-Value	**38.358**	0.883	0.814
	p-Value	< 0.0001	0.124	0.207

*Table A4.7: S. cerevisiae EC1118 26. °Brix, 2-way ANOVA [Factors: Tasting replicate, winemaking replicate and tasting replicate*winemaking replicate interaction] (p<0.05).*

Attribute		Tasting Replicate	Winemaking Replicate	Tasting Replicate* Winemaking Replicate
Green Pepper Aroma	F-Value	0.298	0.578	2.831
	p-Value	0.587	0.564	0.067
Spice Aroma	F-Value	0.781	0.802	0.225
	p-Value	0.380	0.453	0.799
Red Fruit Aroma (Cooked/ Dried/Fresh)	F-Value	0.001	0.044	2.177
	p-Value	0.982	0.957	0.122
Black Fruit Aroma	F-Value	0.241	0.069	1.896
	p-Value	0.625	0.934	0.159
Herbal Aroma	F-Value	0.423	0.019	1.594
	p-Value	0.518	0.981	0.212
Canned Green Vegetable Aroma	F-Value	0.086	0.468	0.720
	p-Value	0.771	0.628	0.491
Earthy/ Toasty Aroma	F-Value	1.267	0.375	0.779
	p-Value	0.265	0.689	0.463
Candy/ Confection Aroma	F-Value	0.077	0.702	0.543
	p-Value	0.783	0.499	0.584
Floral Aroma	F-Value	0.492	0.975	0.152
	p-Value	0.477	0.026	1.943
Leather/ Meat Aroma	F-Value	0.435	0.008	0.231
	p-Value	0.512	0.993	0.795
Red Fruit Flavour	F-Value	0.002	0.784	0.386

(Cooked/ Dried/Fresh)	*p*-Value	0.965	0.461	0.682
Confection Flavour	F-Value	0.270	0.436	0.312
	p-Value	0.605	0.648	0.733
Black Fruit Flavour	F-Value	0.092	0.279	0.291
	p-Value	0.763	0.757	0.748
Spice Flavour	F-Value	0.734	0.151	0.805
	p-Value	0.395	0.860	0.452
Vegetal Flavour	F-Value	1.531	0.206	0.084
	p-Value	0.221	0.814	0.919
Herbal Flavour	F-Value	0.314	0.090	0.139
	p-Value	0.577	0.914	0.870
Earthy/ Toast Flavour	F-Value	0.073	0.623	0.020
	p-Value	0.788	0.540	0.980
Astringency	F-Value	1.239	0.801	0.035
	p-Value	0.270	0.454	0.966
Alcohol	F-Value	1.769	0.319	0.227
	p-Value	0.189	0.728	0.797
Acidity	F-Value	0.027	0.592	0.073
	p-Value	0.869	0.556	0.930
Bitterness	F-Value	0.178	1.152	0.020
	p-Value	0.675	0.323	0.981
Length of Finish	F-Value	**8.694**	0.095	0.852
	p-Value	0.005	0.910	0.432

*Table A4.8: S. bayanus CN1 26.0°Brix, 2-way ANOVA [Factors: Tasting replicate, winemaking replicate and tasting replicate*winemaking replicate interaction] (p<0.05).*

Attribute		Tasting Replicate	Winemaking Replicate	Tasting Replicate* Winemaking Replicate
Green Pepper Aroma	F-Value	0.010	0.305	0.302
	p-Value	0.921	0.738	0.741
Spice Aroma	F-Value	0.022	0.752	0.830
	p-Value	0.882	0.476	0.441
Red Fruit Aroma (Cooked/ Dried/Fresh)	F-Value	1.176	0.420	0.595
	p-Value	0.283	0.659	0.555
Black Fruit Aroma	F-Value	0.316	1.548	0.132
	p-Value	0.576	0.221	0.877
Herbal Aroma	F-Value	0.917	0.769	0.126
	p-Value	0.342	0.468	0.881
Canned Green Vegetable Aroma	F-Value	0.560	0.140	0.356
	p-Value	0.457	0.870	0.702
Earthy/ Toasty Aroma	F-Value	1.006	1.150	1.523
	p-Value	0.320	0.324	0.226
Candy/ Confection Aroma	F-Value	1.124	0.638	0.062
	p-Value	0.293	0.532	0.940
Floral Aroma	F-Value	1.053	2.366	0.341
	p-Value	0.309	0.103	0.713
Leather/ Meat Aroma	F-Value	2.326	0.144	0.018
	p-Value	0.132	0.866	0.982
Red Fruit Flavour	F-Value	1.538	0.348	0.020

(Cooked/ Dried/Fresh)	*p*-Value	0.220	0.707	0.980
Confection Flavour	F-Value	0.239	0.105	0.341
	p-Value	0.627	0.901	0.712
Black Fruit Flavour	F-Value	0.299	0.521	0.072
	p-Value	0.586	0.597	0.931
Spice Flavour	F-Value	2.538	0.179	0.071
	p-Value	0.116	0.837	0.931
Vegetal Flavour	F-Value	1.273	1.022	0.081
	p-Value	0.264	0.366	0.922
Herbal Flavour	F-Value	0.000	0.167	0.447
	p-Value	0.990	0.847	0.641
Earthy/ Toast Flavour	F-Value	0.329	0.004	0.072
	p-Value	0.568	0.996	0.931
Astringency	F-Value	0.274	0.402	0.351
	p-Value	0.603	0.671	0.705
Alcohol	F-Value	1.433	0.596	0.000
	p-Value	0.236	0.554	1.000
Acidity	F-Value	3.035	0.226	0.027
	p-Value	0.087	0.799	0.974
Bitterness	F-Value	0.002	0.156	0.587
	p-Value	0.967	0.856	0.559
Length of Finish	F-Value	2.341	0.173	0.364
	p-Value	0.131	0.841	0.697

*Table A4.9: S. cerevisiae EC1118 27.5 °Brix 2-way ANOVA [Factors: Tasting replicate, winemaking replicate and tasting replicate*winemaking replicate interaction] (p<0.05).*

Attribute		Tasting Replicate	Winemaking Replicate	Tasting Replicate* Winemaking Replicate
Green Pepper Aroma	F-Value	1.723	0.019	1.252
	p-Value	0.194	0.981	0.293
Spice Aroma	F-Value	1.042	0.503	0.487
	p-Value	0.312	0.607	0.617
Red Fruit Aroma (Cooked/ Dried/Fresh)	F-Value	0.605	0.241	0.434
	p-Value	0.440	0.787	0.650
Black Fruit Aroma	F-Value	3.658	0.653	0.757
	p-Value	0.061	0.524	0.473
Herbal Aroma	F-Value	0.357	1.228	0.222
	p-Value	0.553	0.300	0.802
Canned Green Vegetable Aroma	F-Value	3.077	0.237	0.398
	p-Value	0.085	0.790	0.674
Earthy/ Toasty Aroma	F-Value	1.129	0.961	0.583
	p-Value	0.292	0.388	0.561
Candy/ Confection Aroma	F-Value	2.723	0.555	0.607
	p-Value	0.104	0.577	0.548
Floral Aroma	F-Value	3.002	1.543	0.021
	p-Value	0.088	0.222	0.979
Leather/ Meat Aroma	F-Value	2.556	0.517	0.191
	p-Value	0.115	0.599	0.827
Red Fruit Flavour	F-Value	0.276	0.028	0.152

(Cooked/ Dried/Fresh)	*p*-Value	0.601	0.972	0.859
Confection Flavour	F-Value	0.006	0.073	0.041
	p-Value	0.938	0.930	0.960
Black Fruit Flavour	F-Value	0.020	0.122	0.700
	p-Value	0.887	0.885	0.501
Spice Flavour	F-Value	0.106	0.384	0.206
	p-Value	0.746	0.683	0.815
Vegetal Flavour	F-Value	0.060	0.299	0.230
	p-Value	0.808	0.743	0.795
Herbal Flavour	F-Value	1.912	0.374	0.922
	p-Value	0.172	0.689	0.403
Earthy/ Toast Flavour	F-Value	0.001	0.709	0.875
	p-Value	0.978	0.496	0.422
Astringency	F-Value	0.012	0.293	0.008
	p-Value	0.913	0.747	0.992
Alcohol	F-Value	3.119	0.007	0.231
	p-Value	0.082	0.993	0.794
Acidity	F-Value	1.289	0.199	0.104
	p-Value	0.261	0.820	0.901
Bitterness	F-Value	0.366	0.048	0.354
	p-Value	0.548	0.953	0.704
Length of Finish	F-Value	3.841	0.743	0.082
	p-Value	0.055	0.480	0.921

*Table A4.10: S. bayanus CN1 27.5 °Brix, 2-way ANOVA [Factors: Tasting replicate, winemaking replicate and tasting replicate*winemaking replicate interaction] (p<0.05).*

Attribute		Tasting Replicate	Winemaking Replicate	Tasting Replicate* Winemaking Replicate
Green Pepper Aroma	F-Value	0.603	0.413	0.811
	p-Value	0.441	0.664	0.449
Spice Aroma	F-Value	1.000	0.819	0.377
	p-Value	0.321	0.446	0.688
Red Fruit Aroma (Cooked/ Dried/Fresh)	F-Value	0.220	1.621	0.199
	p-Value	0.640	0.206	0.820
Black Fruit Aroma	F-Value	1.844	0.355	0.264
	p-Value	0.180	0.703	0.769
Herbal Aroma	F-Value	0.347	0.060	0.316
	p-Value	0.558	0.942	0.730
Canned Green Vegetable Aroma	F-Value	0.364	0.842	0.390
	p-Value	0.549	0.436	0.679
Earthy/ Toasty Aroma	F-Value	0.057	0.248	0.021
	p-Value	0.812	0.781	0.979
Candy/ Confection Aroma	F-Value	2.008	0.366	0.308
	p-Value	0.162	0.695	0.736
Floral Aroma	F-Value	3.188	0.159	0.281
	p-Value	0.079	0.853	0.756
Leather/ Meat Aroma	F-Value	0.140	0.789	0.124
	p-Value	0.709	0.459	0.884
Red Fruit Flavour	F-Value	1.355	0.771	0.847

(Cooked/ Dried/Fresh)	*p*-Value	0.249	0.467	0.434
Confection Flavour	F-Value	1.337	0.586	0.308
	p-Value	0.252	0.560	0.736
Black Fruit Flavour	F-Value	2.827	0.480	0.125
	p-Value	0.098	0.621	0.883
Spice Flavour	F-Value	0.143	0.207	0.526
	p-Value	0.707	0.814	0.593
Vegetal Flavour	F-Value	0.019	0.503	0.004
	p-Value	0.891	0.607	0.996
Herbal Flavour	F-Value	0.126	0.264	0.072
	p-Value	0.724	0.769	0.931
Earthy/ Toast Flavour	F-Value	0.141	0.162	0.133
	p-Value	0.709	0.851	0.875
Astringency	F-Value	0.066	0.300	0.027
	p-Value	0.798	0.742	0.973
Alcohol	F-Value	0.847	0.638	0.150
	p-Value	0.361	0.532	0.861
Acidity	F-Value	0.000	0.003	0.323
	p-Value	0.989	0.997	0.726
Bitterness	F-Value	0.000	0.278	0.224
	p-Value	0.991	0.759	0.800
Length of Finish	F-Value	0.659	0.603	0.205
	p-Value	0.420	0.551	0.815

Table A4.11: Output of 3-way ANOVA [F=Tasting replicate, Judge, Wine] and interactions amongst factors (p<0.05).

Attribute		Tasting Replicate	Judge	Wine	Tasting Replicate* Judge	Tasting Replicate *Wine	Judge*Wine
Green Pepper Aroma	F-Value	0.472	**35.054**	**3.051**	0.773	1.694	1.037
	p-Value	0.492	< 0.0001	0.010	0.654	0.136	0.413
Spice Aroma	F-Value	0.680	**17.370**	1.104	0.959	0.341	**1.404**
	p-Value	0.410	< 0.0001	0.358	0.480	0.888	0.045
Red Fruit Aroma (Cooked/ Dried/Fresh)	F-Value	0.646	**23.142**	**5.406**	1.506	0.431	1.248
	p-Value	0.422	< 0.0001	< 0.0001	0.136	0.827	0.135
Black Fruit Aroma	F-Value	0.192	**19.727**	1.235	0.925	0.613	**1.621**
	p-Value	0.662	< 0.0001	0.292	0.510	0.690	0.008
Herbal Aroma	F-Value	0.088	**22.717**	0.447	0.935	0.604	1.105
	p-Value	0.767	< 0.0001	0.815	0.501	0.697	0.301
Canned Green Vegetable Aroma	F-Value	0.046	**23.555**	**5.833**	**2.187**	1.459	**1.776**
	p-Value	0.830	< 0.0001	< 0.0001	0.018	0.203	0.002
Earthy/ Toasty Aroma	F-Value	0.229	**26.261**	**5.421**	1.742	0.483	**1.444**
	p-Value	0.633	< 0.0001	< 0.0001	0.071	0.789	0.034
Candy/ Confection Aroma	F-Value	3.260	**27.907**	**3.875**	0.645	0.687	1.049
	p-Value	0.072	< 0.0001	0.002	0.775	0.633	0.391
Floral Aroma	F-Value	0.188	**12.683**	**2.319**	0.856	0.183	1.391
	p-Value	0.665	< 0.0001	0.043	0.575	0.969	0.050
Leather/ Meat Aroma	F-Value	0.011	**35.383**	**2.807**	0.547	0.231	**1.506**
	p-Value	0.918	< 0.0001	0.017	0.856	0.949	0.021
Red Fruit Flavour	F-Value	1.673	**26.036**	0.947	1.225	0.923	1.014

(Cooked/ Dried/Fresh)	*p*-Value	0.197	< 0.0001	0.451	0.274	0.466	0.453
Confection Flavour	F-Value	0.517	**72.997**	**2.781**	1.142	0.444	**2.427**
	p-Value	0.473	< 0.0001	0.018	0.330	0.817	< 0.0001
Black Fruit Flavour	F-Value	1.103	**27.287**	**6.674**	0.453	0.964	**1.679**
	p-Value	0.294	< 0.0001	< 0.0001	0.919	0.440	0.005
Spice Flavour	F-Value	1.117	**30.783**	**2.885**	1.290	0.441	0.807
	p-Value	0.291	< 0.0001	0.015	0.235	0.820	0.821
Vegetal Flavour	F-Value	1.866	**74.810**	**2.523**	**2.929**	0.519	**1.470**
	p-Value	0.173	< 0.0001	0.029	0.002	0.762	0.027
Herbal Flavour	F-Value	0.012	**55.596**	**2.947**	1.274	1.185	1.232
	p-Value	0.914	< 0.0001	0.013	0.244	0.316	0.148
Earthy/ Toast Flavour	F-Value	0.105	**0.105**	**0.105**	**0.105**	0.105	0.105
	p-Value	0.746	< 0.0001	< 0.0001	0.015	0.360	0.165
Astringency	F-Value	0.001	**68.224**	**3.084**	**2.793**	1.383	**1.906**
	p-Value	0.972	< 0.0001	0.010	0.003	0.230	0.001
Alcohol	F-Value	0.366	**52.300**	**24.724**	1.724	**2.281**	**1.564**
	p-Value	0.546	< 0.0001	< 0.0001	0.075	0.047	0.013
Acidity	F-Value	1.812	**98.455**	**7.770**	1.721	1.306	1.266
	p-Value	0.179	< 0.0001	< 0.0001	0.075	0.261	0.120
Bitterness	F-Value	1.843	**41.421**	1.286	0.492	1.001	**1.436**
	p-Value	0.176	< 0.0001	0.270	0.895	0.417	0.036
Length of Finish	F-Value	2.783	**8.360**	**17.675**	1.417	0.648	1.263
	p-Value	0.096	< 0.0001	< 0.0001	0.171	0.663	0.122

Table A4.12: Mean intensity ratings for each sensory describing attribute.

Lowercase letters indicate statistical differences within the same attribute indicate differences between winemaking treatments (yeast strain and starting sugar concentration) determined by analysis of variance (ANOVA) with mean separation by Fisher's Least Significant Difference (LSD; $p < 0.05$).

Aroma										
Treatment	Green Pepper	Spice	Red Fruit (Cooked/ Dried/Fresh)	Black Fruit	Herbal	Canned Green Vegetable	Earthy/ Toast	Candy/ Confection	Floral	Leather/ Meat
EC1118 Control	2.2 b	4.9 a	8.2 a	5.8 a	4.6 a	2.4 c	3.7 d	5.5 a	4.2 a	1.7 b
CN1 Control	2.8 a,b	5.0 a	7.7 a,b	5.2 a	5.0 a	2.9 b,c	4.2 cd	4.9 a,b	3.0 b	2.1 b
EC1118 26.0°Brix	2.9 a	4.7 a	6.9 b,c,d	5.0 a	4.8 a	3.5 a,b	4.7 b,c	4.1 b,c	3.8 a,b	2.0 b
CN1 26.0°Brix	3.1 a	5.2 a	7.2 b,c	5.7 a	5.1 a	2.8 b,c	5.2 ab	4.0 b,c	3.6 a,b	1.7 b
EC1118 27.5°Brix	3.4 a	5.6 a	6.5 c,d	5.5 a	5.3 a	3.9 a	5.5 a	3.8 c	3.3 b	2.4 a,b
CN127.5°Brix	3.2 a	5.6 a	6.2 d	5.9 a	5.1 a	4.4 a	5.2 ab	3.8 c	2.9 b	2.8 a

Flavour							
	Red Fruit (Cooked/ Dried/Fresh)	Confection	Black Fruit	Spice	Vegetal	Herbal	Earthy/ Toast
EC1118 Control	7.7 a	4.1 a	5.3 b,c	4.6 c	2.9 b,c	3.9 b	3.7 b
CN1 Control	7.5 a	3.5 a,b	4.7 c	4.5 c	3.6 a	4.6 a,b	3.8 b
EC1118 26.0°Brix	7.6 a	3.7 a,b	5.1 b,c	5.3 a,b,c	2.9 b,c	5.0 a	4.2 b
CN1	7.8 a	3.0 b	4.8 c	4.9 b,c	3.5 a,b	4.7 a,b	4.1 b

26.0°Brix								
EC1118 27.5°Brix	7.1 a	3.4 a,b	5.8 b	5.6 a,b	2.9 b,c	5.4 a	5.1 a	
CN127.5°Brix	7.2 a	4.0 a	6.8 a	5.8 a	2.8 c	4.9 a	5.2 a	
In-Mouth Sensations								
	Astringency	Alcohol	Acidity	Bitterness	Length of Finish			
EC1118 Control	5.8 b	7.8 b	7.9 b	4.1 a	8.0 c			
CN1 Control	6.1 b	8.1 b	8.6 a	4.6 a	7.9 c			
EC1118 26.0°Brix	6.2 b	9.9 a	7.2 c	4.7 a	9.6 b			
CN1 26.0°Brix	6.1 b	8.1 b	8.7 a	4.7 a	8.2 c			
EC1118 27.5°Brix	7.0 a	10.3 a	7.9 b	4.8 a	10.4 a			
CN127.5°Brix	6.4 b	10.4 a	7.2 c	4.0 a	10.3 a,b			

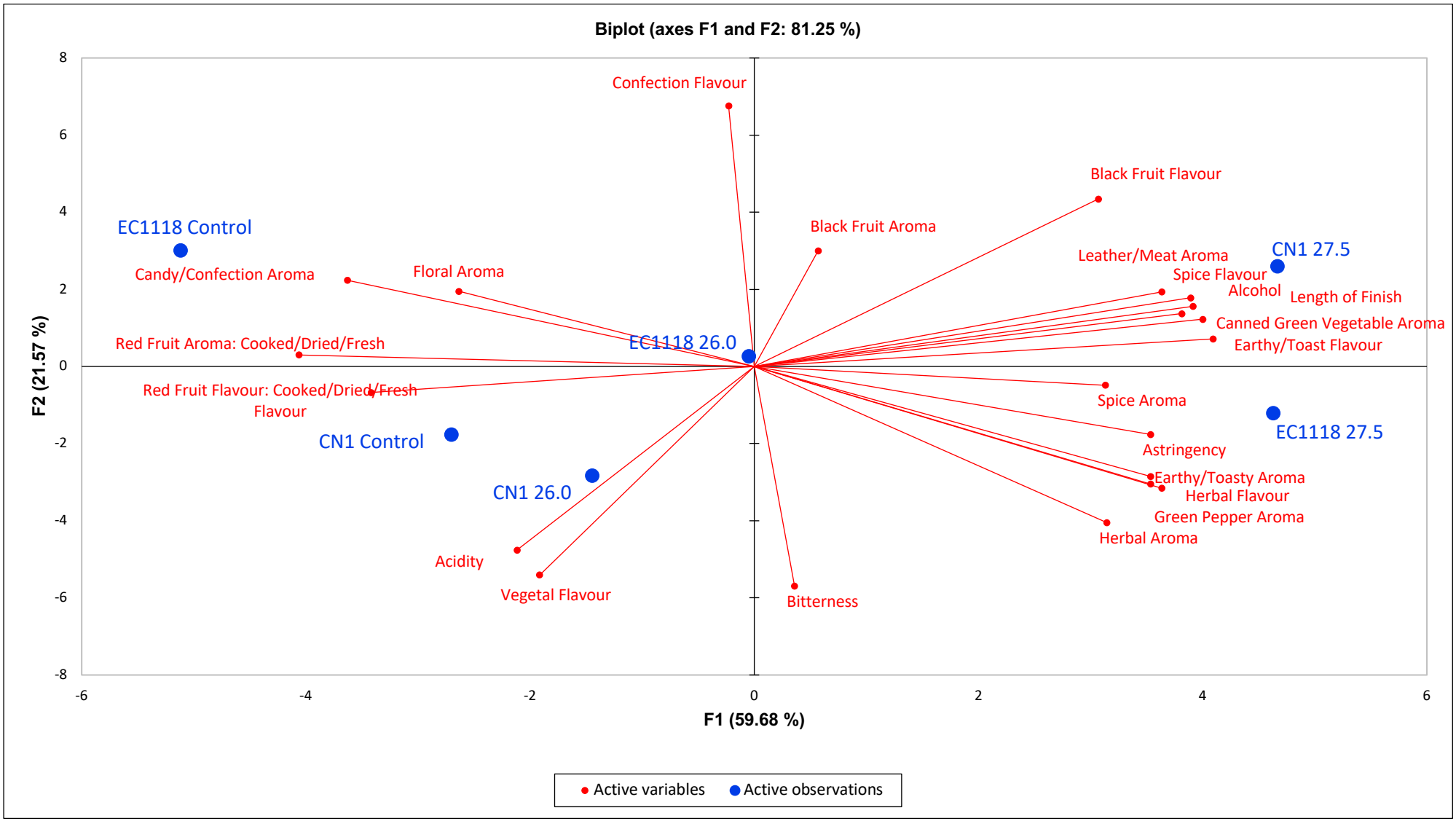


Figure A4.8: Sensory map of wines made with partially dehydrated grapes (with winemaking replicate data collapsed) fermented with S. cerevisiae EC1118 and S. bayanus CN1 at different starting sugar concentrations (control (21.5 °Brix), 26.0 °Brix and 27.5 °Brix) via PCA on Factors 1 and 2.

Ellipses around data points group winemaking replicates from the same yeast and starting sugar concentration.

Table A4.13: Volatile aroma compounds, retention times, target and confirming ions, standard curves, % recovery, calibration ranges and % coefficient of variation (CV).

Compound	Retention Time (min)	Target Ion (*m/z*)	Standard Curve (R^2)	% Recovery	Calibration Range Lowest to Highest (µg/L)	%CV
2- Phenylethanol*	47.1	91	0.9974	116	720.3-11024.5	3-11, Average: 7.4
Ethyl isobutyrate*	12.7	71	0.9907	109	3.2-38.2	3-16, Average: 9.5
Ethyl butyrate	14.9	88	0.9919	104	5.2-62.2	3-13, Average: 6.6
Ethyl isovalerate	17.6	88	0.9917	96	1.1-12.7	5-11, Average: 8.1
Ethyl octanoate	40.5	88	0.9956	111	6.4-75.7	2-11, Average: 6.3
Ethyl 2-methylbutryate	17.3	57	0.9942	98	0.5-5.9	3-7, Average: 4.6
Isoamyl acetate*^	19.0	87	0.9944	97	1.6-37.6	3-18, Average: 5.0
Hexanol	21.1	56	0.9967	102	36.4-433.6	2-9, Average: 5.1
Ethyl hexanoate^	25.6	99	0.9992	103	6.0-142.1	1-16, Average: 5
Hexanoic Acid	14.9	60	0.9933	116	28.6-226.2	4-19, Average: 7.0
Octanoic Acid	17.9	60	0.9944	127	28.6-226.2	2-12, Average: 6.1

*Curve has been adjusted from original standard curve based on sample concentrations

^Additional standard added to capture low-end concentrations based on sample concentrations

%cv= Standard deviation / mean of 8 data points (2x2 winemaking replicates, 2 bottles in measured in duplicate)

Analysis of VOCs

Concentrations of samples were based on the Botezatu et al., (2016), as well as preliminary analysis of model wine. Milli-Q water (Biocel MilliQ, EMD Millipore, Cillerica, MA, USA) was used for sample preparation, and was filtered through 0.22 μM filter (Millipore). All stock solutions (Standard A) were prepared using Chromasolv® HPLC standard ethanol (Sigma-Aldrich, Oakville, ON, Canada). From Standard A, a composite standard solution was made (Standard C), which was then used to prepare a working standard, made fresh for every day wines were analyzed.

Preparation of VOC Standards

Standards were prepared in a 20mL round-bottomed amber glass vial (MicroLiter Analytical Supplies Incorportaed, Millville, New Jersey, United States of America), first by adding 3g of reagent grade NaCl (BioShop, Burlington, ON, Canada) and a magnetic stir bar, then 8.06mL of Milli-Q water, followed by a wine matrix that was de-volatilized by using a rotary-evaporator at 40°C for 30 minutes then topped up to 15% ethanol, along with 10% ethanol. Composite aroma standards (Standard C) was added according to the calibration range. 40 μL of ethyl hexanoate-d_{11} was added and the vial was immediately capped with a magnetic screw/thread headspace cap (PTFE / silicone; MicroLiter).

Preparation of VOC Samples

Standards were prepared in a 20mL round-bottomed amber glass vial (MicroLiter Analytical Supplies Incorporated, Millville, New Jersey, United States of America), first by adding 3g of reagent grade NaCl and a magnetic stir bar, then 8.06mL of Milli-Q water, and 0.45mL of wine for a 20-fold dilution. Finally, 40 μL of the deuterated internal standard ethyl hexanoate-d_{11}

standard C was added and the vial was closed with a magnetic screw/thread headspace cap (PTFE / silicone; MicroLiter). Samples were then incubated at 40 C for 1 min at 600 rpm before being exposed to the fiber for 30 min at 40°C with stirring at 600 rpm.

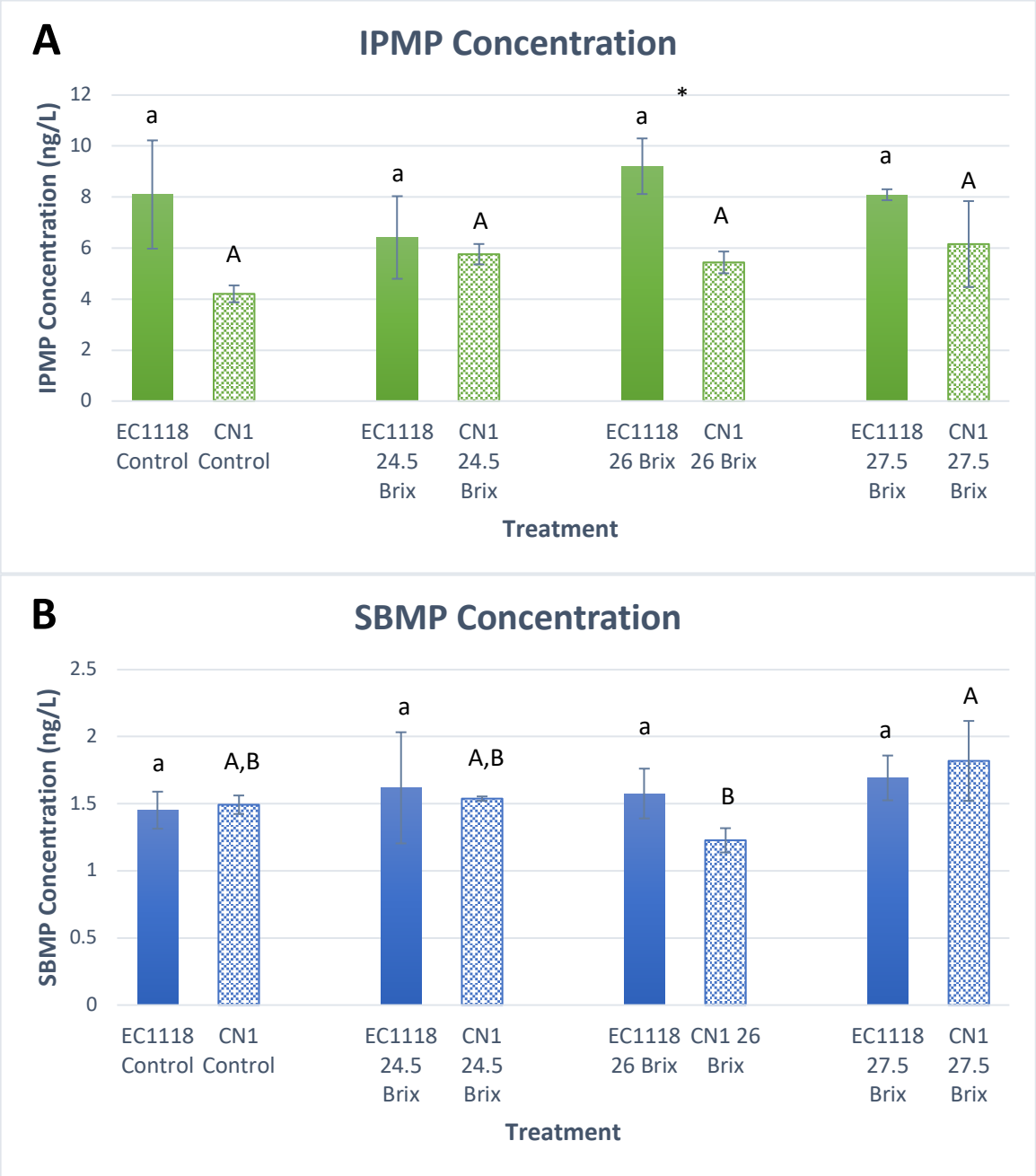
Preparation VFA Standards and Samples

Fatty acid sample and standard preparation generally followed the same protocol described above for VOCs. Standard A and C composite standards were made fresh on the day of analysis and the Milli-Q water and matrix were acidified to pH 3.6 with 1M HCl (Anachemia Canada Inc., Montreal, QC, Canada). Wine samples were diluted 20-fold with the acidified matrix. Vials were sealed with magnetic screw/thread headspace cap PTFE/silicone closures and octanal-d_{16} was used as the internal standard.

Headspace Solid- Phase Micro-Extraction Gas Chromatography-Mass Spectrometry (HS-SPME-GC-MS)

The HS-SPME-GC-MS method from Botezatu et al. (2016) was used to analyse VOCs. A 2 cm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco Inc., Bellefonte, PA, USA), 23-gauge SPME fiber was used for sampling. Samples were incubated at 40 °C with a conditioned stir bar before exposing the fiber for 30 min at 40°C at 600 rpm. The samples were analysed using an Agilent (Mississauga, ON, Canada) 7890A gas chromatograph coupled to a 5975C mass selective detector (MSD) equipped with a Gerstal MPS2 XL autosampler (Linthicum Heights, MD, USA). The GC was equipped with a Deans Switch and two columns: a HP-5MS 5% phenyl methyl siloxane column (30 m, 0.25 mm i.d., 0.25 µm film thickness) coupled with a secondary DB-Wax capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness) (Agilent Technologies Inc., Santa Clara, CA, USA). The liner was a SPME inlet liner (0.7 mm i.d.; Supelco).

Helium was used as the carrier gas with a flow rate of 0.5 mL/min in the first column, and 1.5 mL/min in the second column. Oven temperature programming began at 35°C for 3 min, and then increased 3°C/min up to 105°C where it was held for 10 min. Temperature was then increased by 2°C/min up to 140°C, before holding for 10 min. Temperature went through one more ramp up of 4°C/min up to 250°C, before holding for a final 10 min. The run time for this method was 101 min. The MSD interface was held at 250°C. The inlet temperature was 250°C and the SPME fiber was desorbed in splitless mode. The solvent delay was 5 min. The fiber was prebaked for 10 min and post baked for 20 min. Samples were warmed at 40°C and stirred at 600 rpm for 1 min before being exposed to the fiber for 30 min at 40°C with stirring at 600 rpm, followed by desorption in the inlet for 10 min. The samples were measured using synchronous scan and selected ion monitoring (SIM mode). The scan parameters ran from 35 m/z to 400 m/z, and both scan and SIM acquisitions were performed with an EMV Gain Factor of 7. All wine analyses were carried out in duplicate.


A
IPMP Concentration
IPMP Concentration (ng/L)
12
10
8
6
4
2
0
a
A
a
A
a
*
A
a
A
EC1118 Control
CN1 Control
EC1118 24.5 Brix
CN1 24.5 Brix
EC1118 26 Brix
CN1 26 Brix
EC1118 27.5 Brix
CN1 27.5 Brix
Treatment
B
SBMP Concentration
SBMP Concentration (ng/L)
2.5
2
1.5
1
0.5
0
a
A,B
a
A,B
a
B
a
A
EC1118 Control
CN1 Control
EC1118 24.5 Brix
CN1 24.5 Brix
EC1118 26 Brix
CN1 26 Brix
EC1118 27.5 Brix
CN1 27.5 Brix
Treatment

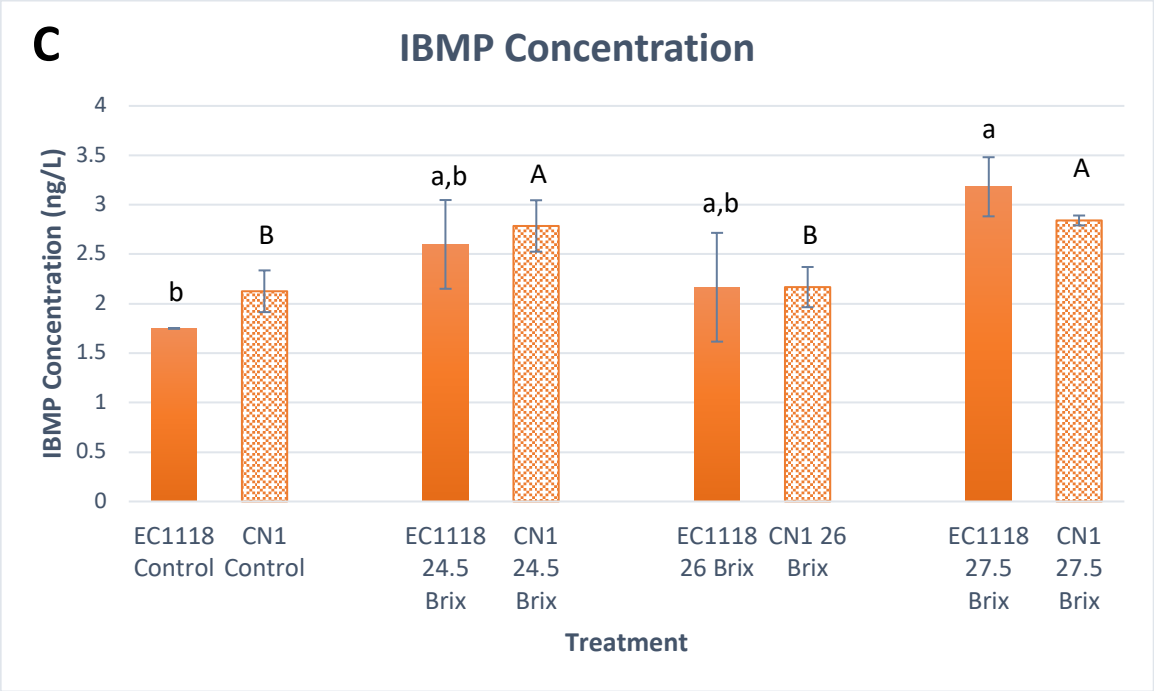


Figure A4.9, A-C: Methoxypyrazines (IPMP (A), SBMP (B) and IBMP (C)) in 2013 wines made from partially dehydrated grapes dried in Cave Spring Winery Barn.

Lowercase (EC1118) and uppercase (CN1) letters indicate statistical differences within the same yeast treatment determined by analysis of variance (ANOVA) with mean separation by Fisher's Least Significant Difference (LSD; $p < 0.05$). Asterisks ($*p<0.05$) indicate significant differences between yeast strains at the indicated dehydration target as determined by Student's *t*-Test.

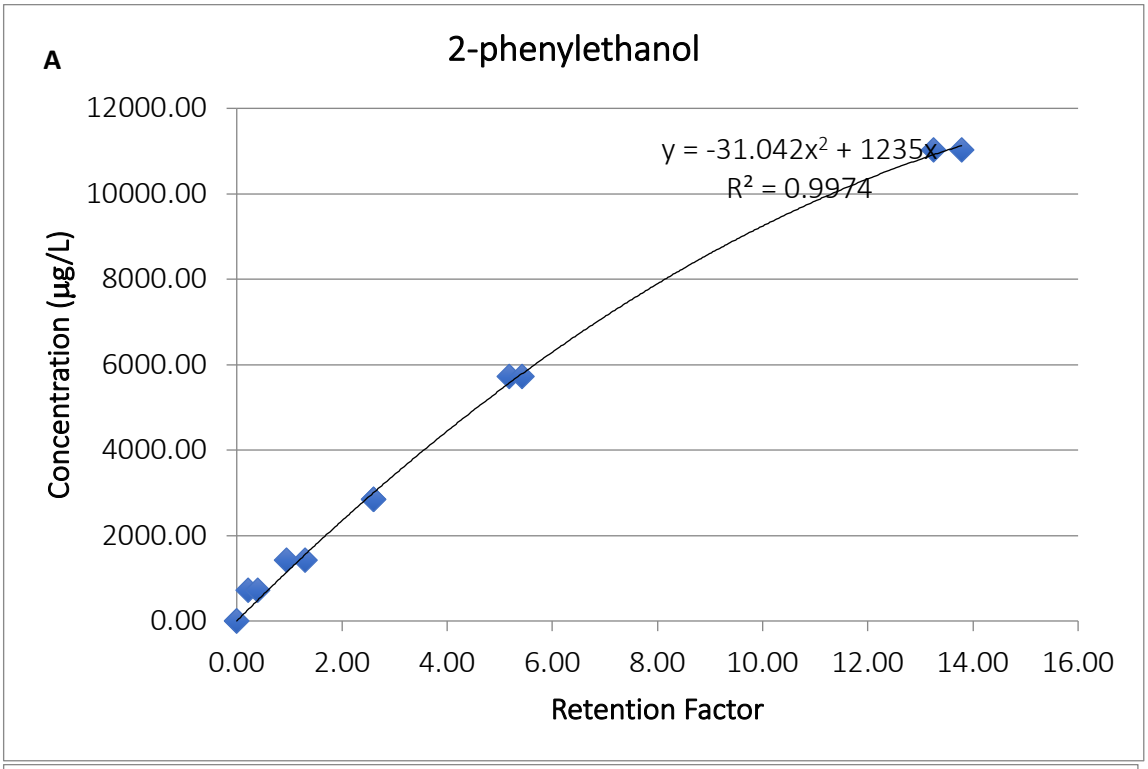
Table A4.14: VOC and VFA composition of wines.

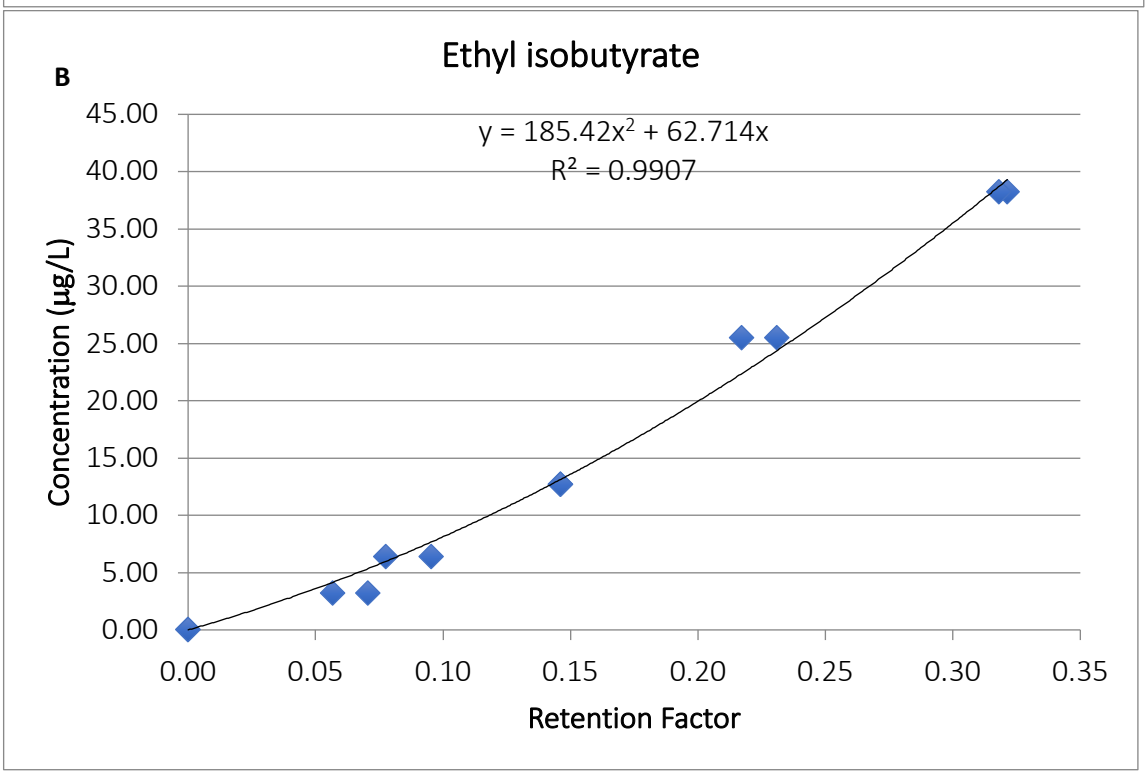
Results are the mathematical average of 2 representative wine bottles and 2 sample replicates of each treatment (8 data points per result) ± standard deviation. Analysis of variance (ANOVA) with mean separation by Fisher's Least Significant Difference (LSD; $p < 0.05$). Lowercase letters within the same starting sugar concentration yeast indicate differences using Fisher's $LSD_{0.05}$.

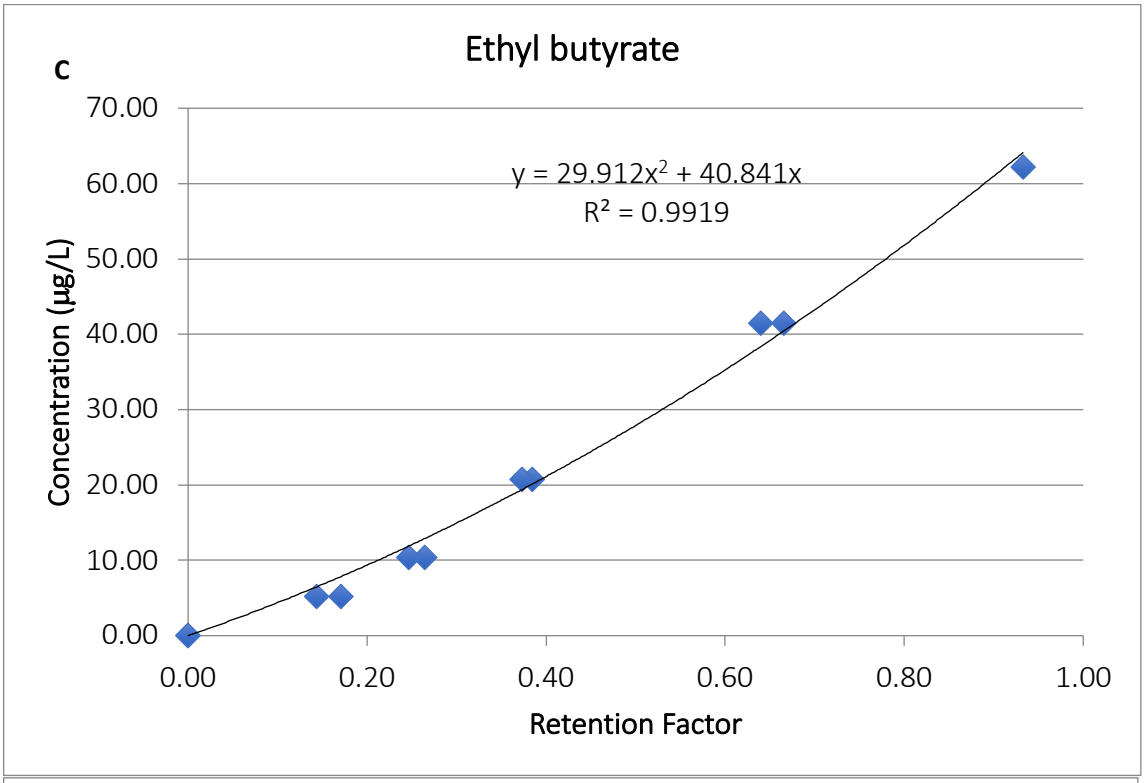
Treatment	Volatile Organic Compounds and Concentrations (µg/L)								
	2-Phenyl ethanol	Ethyl isobutyrate	Ethyl butyrate	Ethyl isovalerate	Ethyl octanoate	Ethyl 2-methyl butyrate	Isoamyl acetate	Hexanol	Ethyl hexanoate
S. cerevisiae Control	68543.6± 3679.6^{b}	220.3± 50.1^{b}	222.1± 31.6^{a}	82.3± 13.2^{b}	298.1± 32.2^{a}	78.04± 13.32^{b}	165.5± 38.9^{a}	3246.7± 240.3^{a}	451.4± 18.7^{a}
S. bayanus Control	222628.2 ± 8852.6^{a}	1232.1± 440.4^{a}	135.3± 13.6^{b}	146.2± 31.5^{a}	193.4± 32.2^{b}	125.31± 28.94^{a}	116.4± 21.6^{b}	3565.1± 399.9^{a}	175.3± 8.5^{b}
S. cerevisiae 24.5°Brix	113575.6 ±16616.1^{b}	160.0± 13.7^{b}	177.9± 7.8^{a}	67.4± 4.5^{b}	392.1± 23.5^{a}	48.09± 1.13^{b}	147.4± 1.1^{a}	2072.0± 6.6^{b}	460.6± 6.6^{a}
S. bayanus 24.5 °Brix	224681.0 ± 21288.7^{a}	849.7± 196.4^{a}	132.3± 6.0^{b}	95.5± 9.2^{a}	320.6± 46.6^{b}	84.26± 8.33^{a}	97.1± 13.2^{b}	2493.4± 9.7^{a}	208.1± 9.7^{b}
S. cerevisiae 26.0 °Brix	137148.5 ± 6525.2^{b}	232.9± 30.0^{b}	156.7± 13.7^{a}	95.9± 5.8^{a}	505.4± 47.2^{a}	72.22± 2.72^{b}	210.1± 18.7^{a}	2125.8± 13.9^{b}	479.6± 13.9^{a}
S. bayanus 26.0 °Brix	238341.3 ± 7685.4^{a}	1262.6± 204.9^{a}	134.4± 3.9^{a}	145.1± 7.4^{a}	475.3± 4.3^{b}	122.33± 5.59a	110.8± 24.4^{b}	2504.9± 40.6^{a}	253.3± 40.6^{b}
S. cerevisiae 27.5 °Brix	143087.7 ± 14744.2^{b}	164.8± 10.9^{b}	198.5± 12.9^{a}	144.1± 9.8^{a}	493.3± 12.4^{a}	48.42± 1.32^{b}	303.8± 4.8^{a}	3296.5± 8.4^{b}	520.3± 8.4^{a}
S. bayanus	234763.4	568.0±	153.0±	59.7±	378.8±	67.84±	116.11±	4061.7±	233.3±

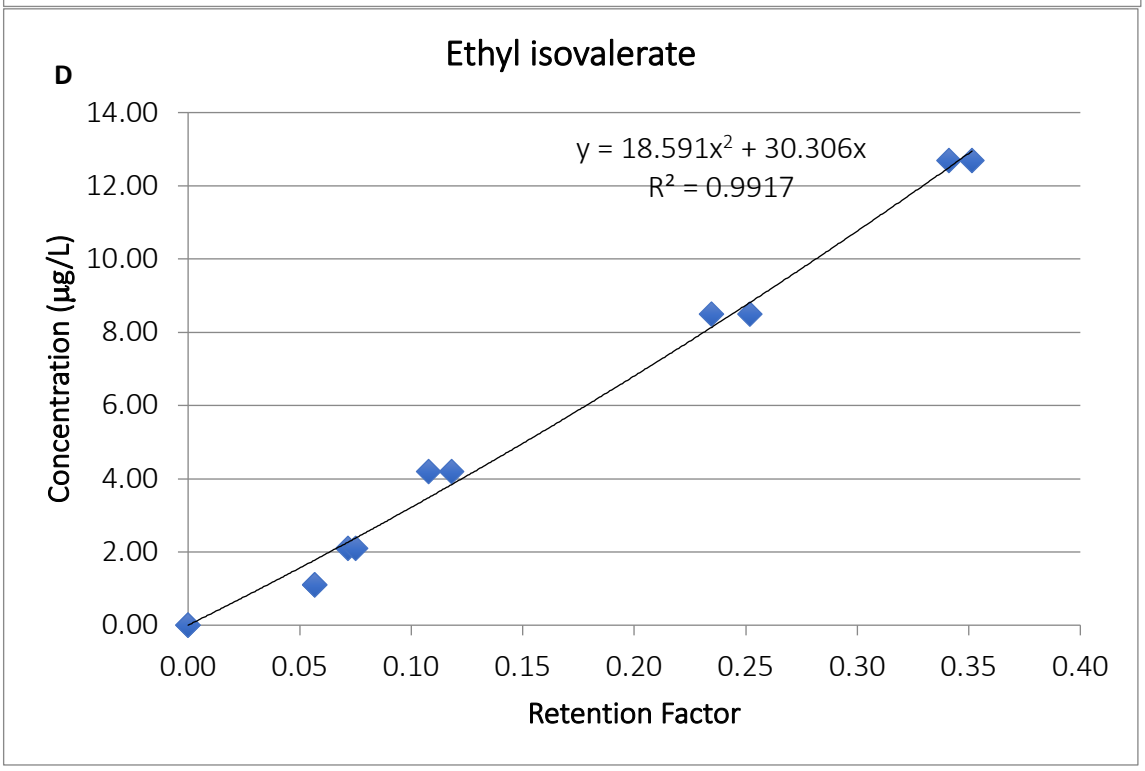
27.5 °Brix	± 8119.9[a]	82.1[a]	12.9[b]	2.8[b]	45.7[b]	7.38[a]	4.1[b]	14.7[a]	14.7[b]

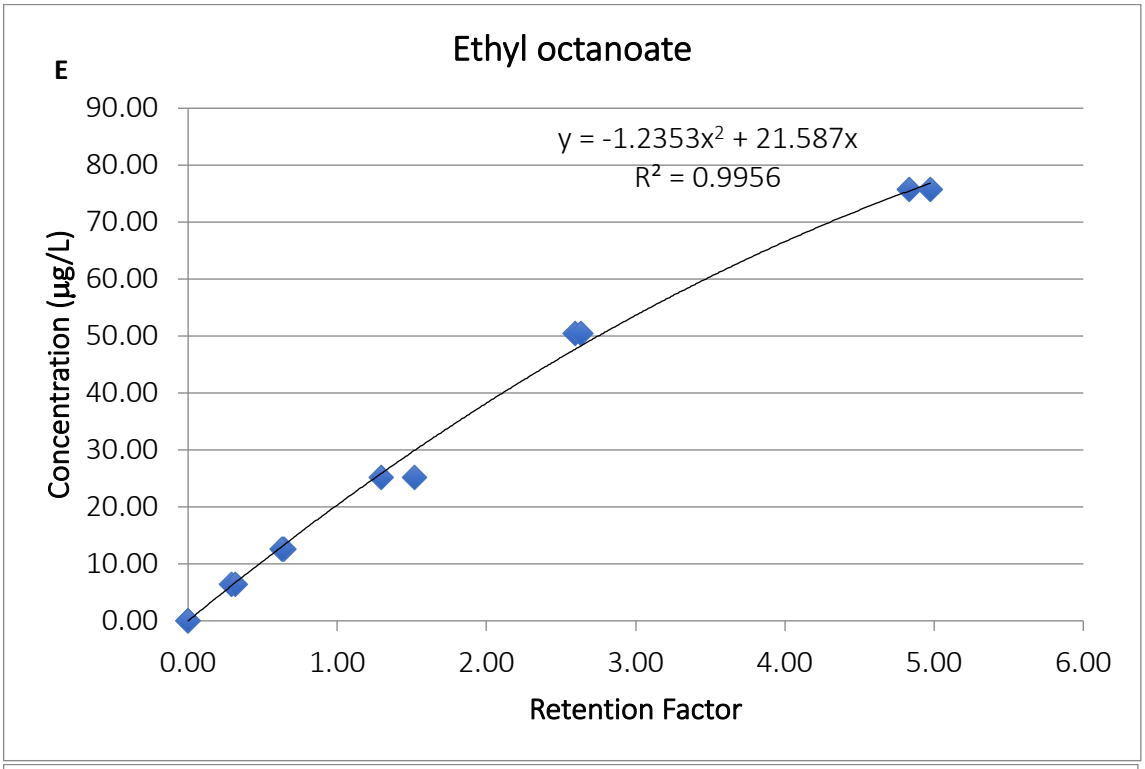
Treatment	Volatile Fatty Acid Concentrations (μg/L)	
	Hexanoic Acid	Octanoic Acid
S. cerevisiae Control	3183.6±112.9[a]	3230.9±192.4[a]
S. bayanus Control	1160.2±66.9[b]	1616.7±108.6[b]
S. cerevisiae 24.5°Brix	2501.7±134.6[a]	2560.3±55.8[a]
S. bayanus 24.5 °Brix	1272.5±72.1[b]	1995.4±105.6[b]
S. cerevisiae 26.0 °Brix	2394.4±88.4[a]	2466.1±111.2[a]
S. bayanus 26.0 °Brix	1148.2±220.4[b]	1889.1±126.5[b]
S. cerevisiae 27.5 °Brix	1955.1±161.5[a]	1848.4±221.1[a]
S. bayanus 27.5 °Brix	990.2±46.9[b]	1430.0±71.8[b]

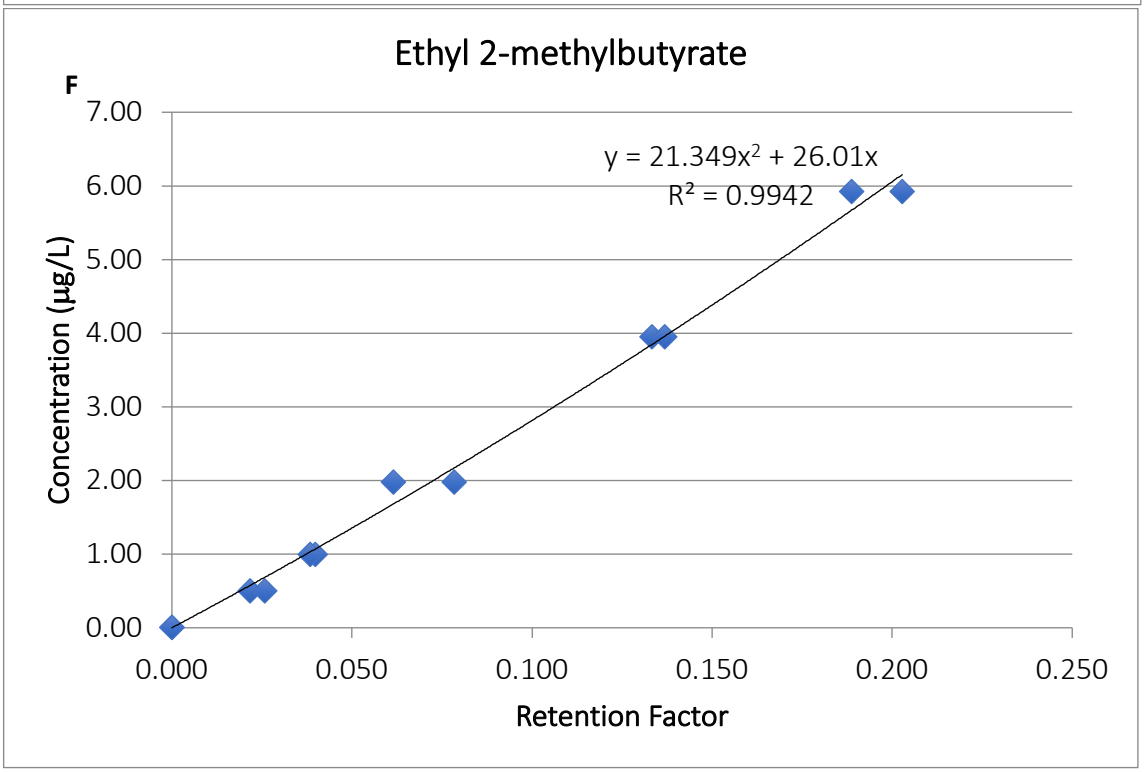

A
2-phenylethanol
y = -31.042x² + 1235x
R² = 0.9974
Concentration (µg/L)
12000.00
10000.00
8000.00
6000.00
4000.00
2000.00
0.00
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
Retention Factor

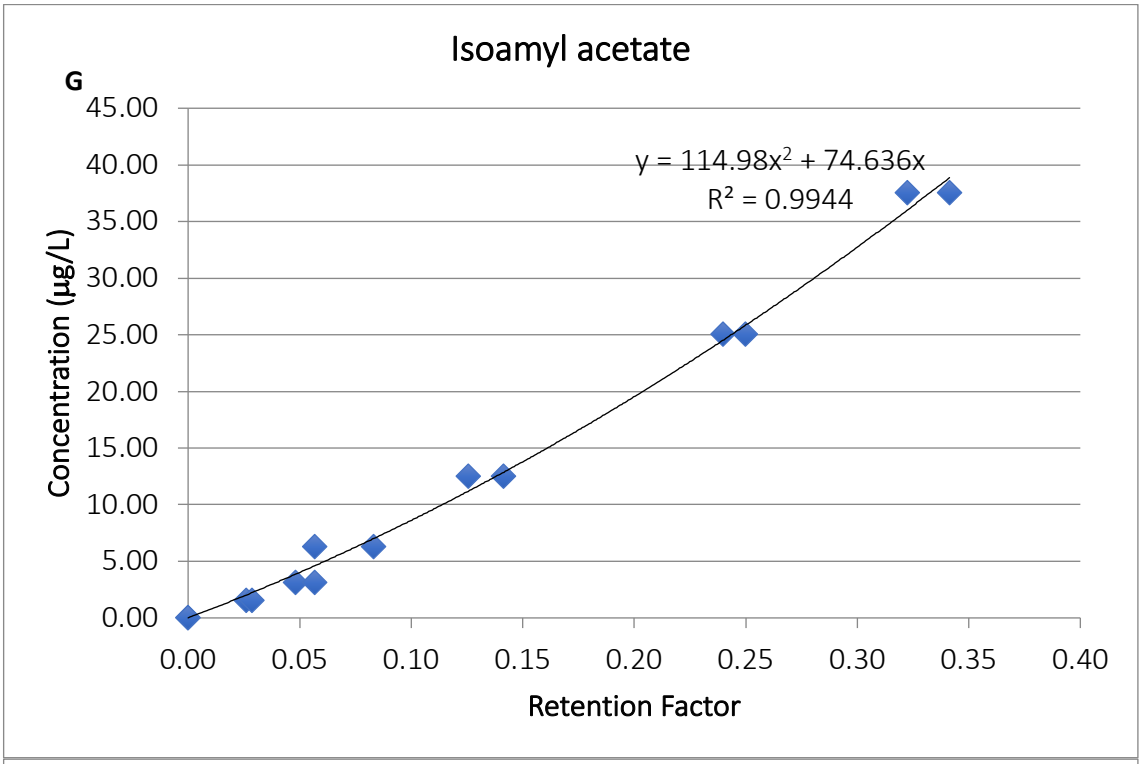

B
Ethyl isobutyrate
y = 185.42x² + 62.714x
R² = 0.9907
Concentration (µg/L)
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
Retention Factor

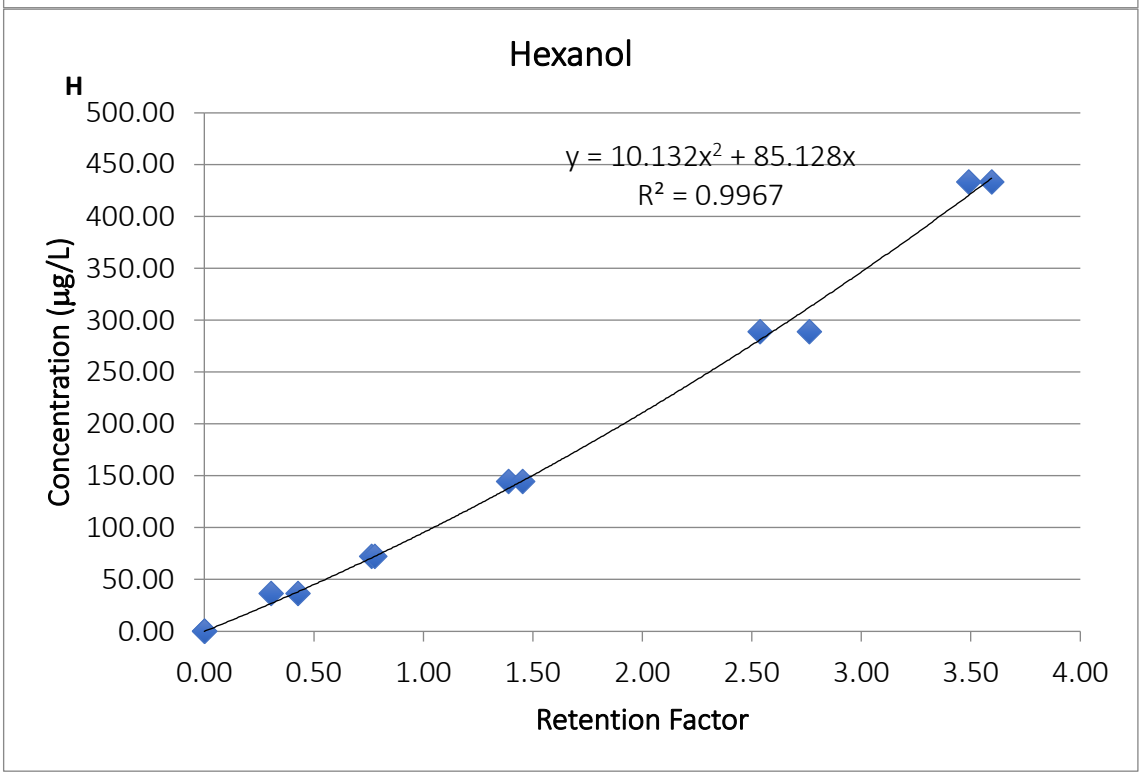

C
Ethyl butyrate
y = 29.912x² + 40.841x
R² = 0.9919
Concentration (µg/L)
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0.00
0.20
0.40
0.60
0.80
1.00
Retention Factor

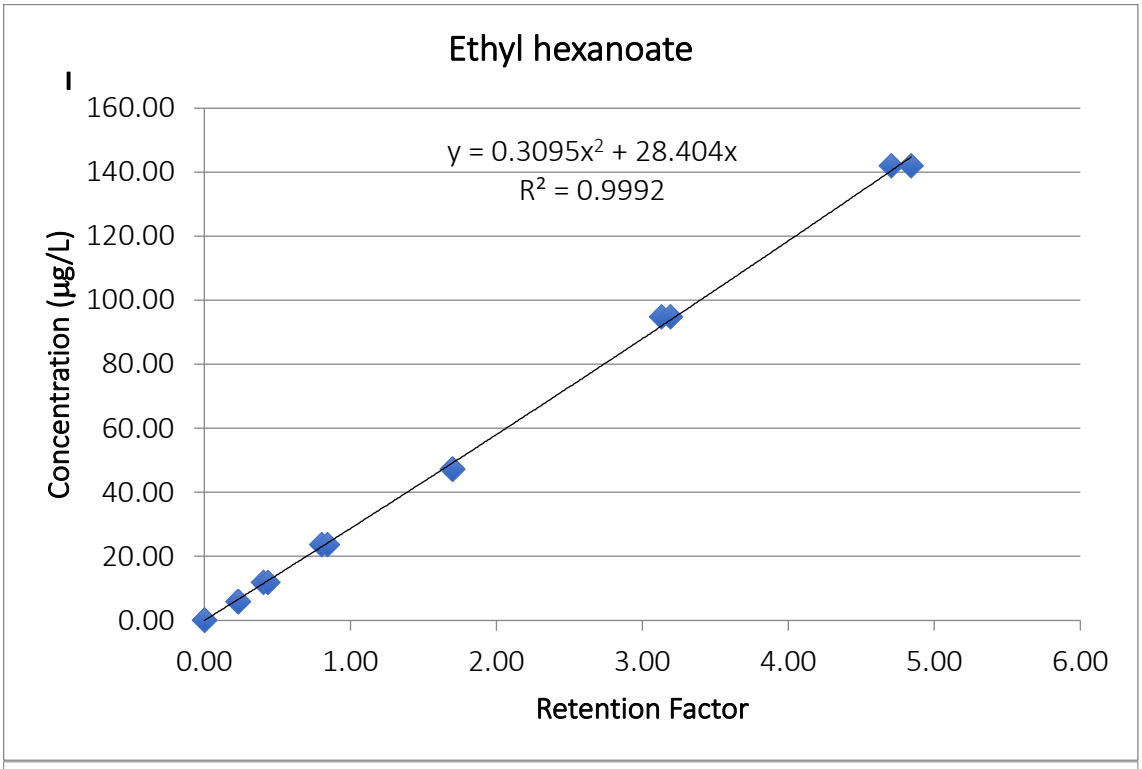

D
Ethyl isovalerate
y = 18.591x² + 30.306x
R² = 0.9917
Concentration (µg/L)
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
Retention Factor

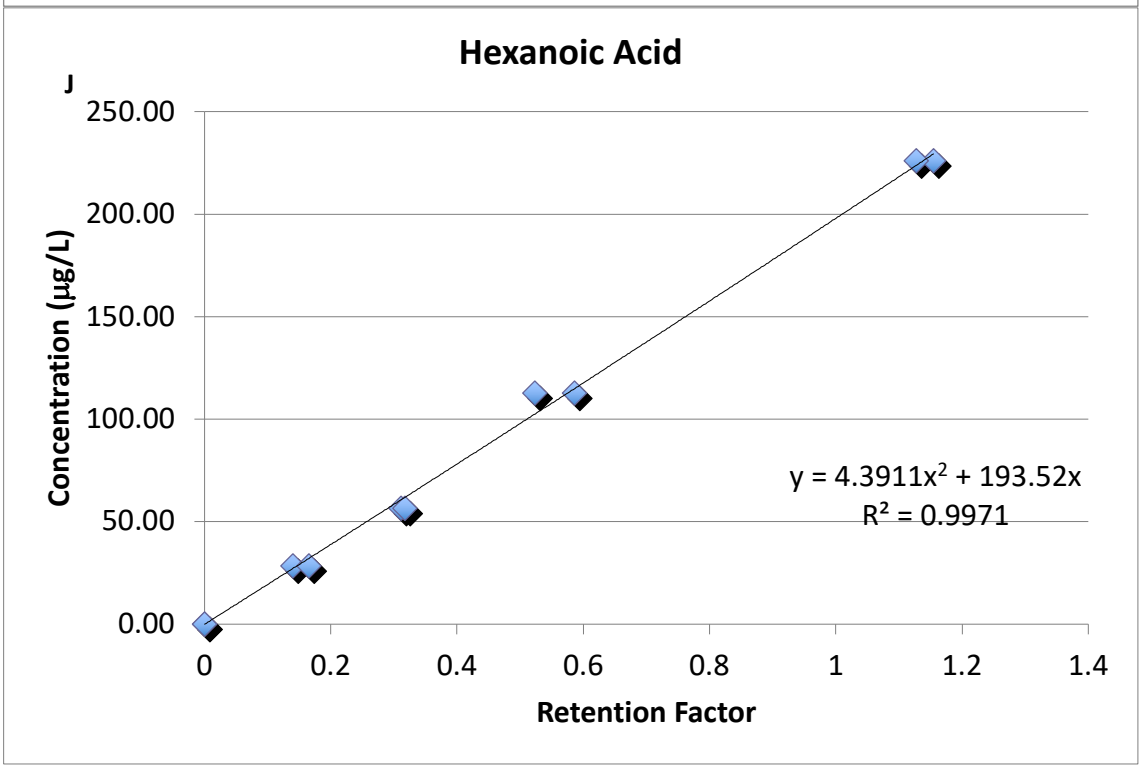

E
Ethyl octanoate
y = -1.2353x² + 21.587x
R² = 0.9956
Concentration (µg/L)
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
Retention Factor


F
Ethyl 2-methylbutyrate
y = 21.349x² + 26.01x
R² = 0.9942
Concentration (µg/L)
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0.000
0.050
0.100
0.150
0.200
0.250
Retention Factor


G
Isoamyl acetate
y = 114.98x² + 74.636x
R² = 0.9944
Concentration (µg/L)
Retention Factor
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40


H
Hexanol
y = 10.132x² + 85.128x
R² = 0.9967
Concentration (µg/L)
Retention Factor
0.00
50.00
100.00
150.00
200.00
250.00
300.00
350.00
400.00
450.00
500.00
0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00


Ethyl hexanoate
I
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Concentration (µg/L)
y = 0.3095x² + 28.404x
R² = 0.9992
0.00
1.00
2.00
3.00
4.00
5.00
6.00
Retention Factor


Hexanoic Acid
J
250.00
200.00
150.00
100.00
50.00
0.00
Concentration (µg/L)
y = 4.3911x² + 193.52x
R² = 0.9971
0
0.2
0.4
0.6
0.8
1
1.2
1.4
Retention Factor

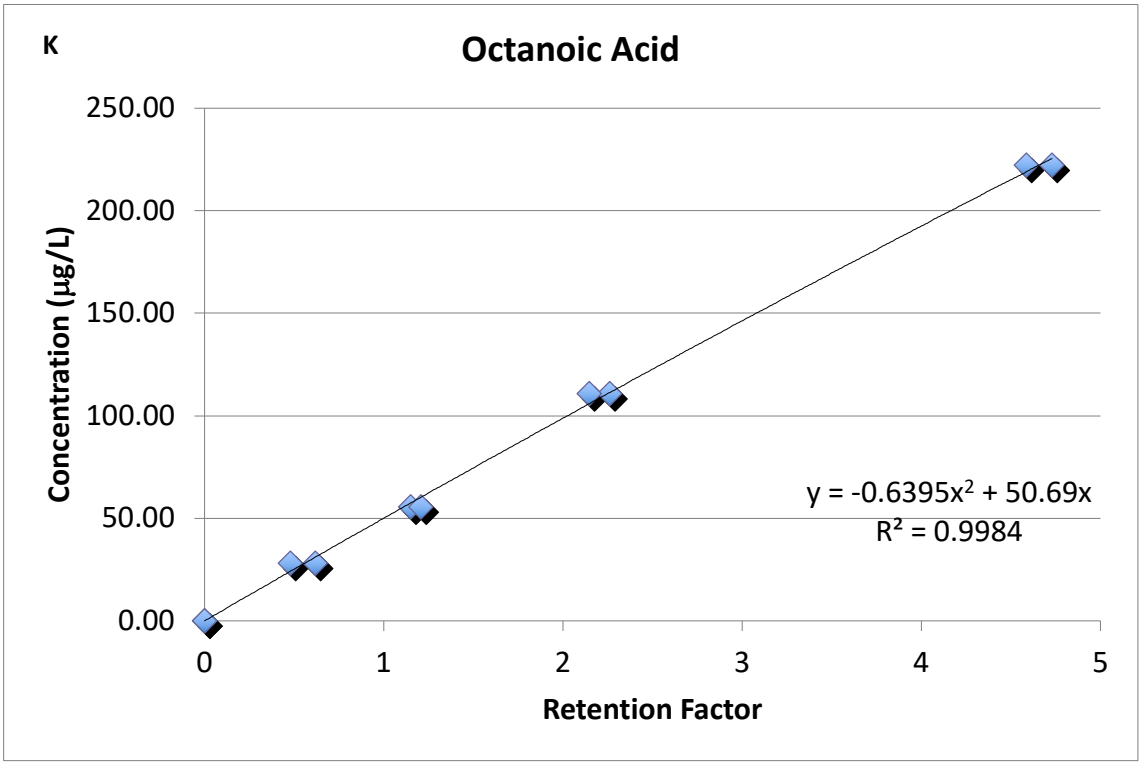


Figure A4.10, A-K: Standard curves for VOCs (2-phenylethanol, ethyl isobutyrate, ethyl butyrate, ethyl isovalerate, ethyl octanoate, ethyl 2-methylbutyrate, isoamyl acetate, hexanol and ethyl hexanoate) and VFAs (hexanoic acid and octanoic acid).

Chapter 5 Impact of *Botrytis cinerea*-Infected Grapes on Quality Parameters of Wine Made from Partially Dehydrated Grapes

Abstract: *Botrytis cinerea* is a fungal infection that takes on two forms in wine grapes: the undesirable grey mould, and the desirable noble rot infection that occurs under specific weather conditions. The presence of noble rot may contribute to the formation of favourable aromas and flavours in specialized sweet dessert wines. Modification of important aroma categories induced by *B. cinerea* have been reported in dry wine (Amarone) made from partially dehydrated grapes. There is a gap in the literature regarding the influence of this fungus on dry wines produced from partially dehydrated grapes (appassimento) in Ontario, Canada. Appassimento-style wines are produced after grapes are dried to concentrate sugars and volatile constituents. Cabernet franc grapes were dried to 28.0°Brix and fermented with either healthy grapes (0% infection, representing our control) or a combination of healthy and botrytized grapes (10% *B. cinerea* infection by weight). Analysis was carried out on physio-chemical characteristics and metabolites (enzymatic), volatile organic compounds (SPME GC-MS), and sensory evaluation (descriptive analysis) and consumer preference (nine-point hedonic scale) also conducted. Results indicate that the inclusion of *B. cinerea* at 10% infection rate had some impact on the wines. Expected markers such as gluconic acid and glycerol, acetic acid were significantly higher in the *B. cinerea*- affected treatments, as well as primary amino nitrogen. Differences in some ethyl esters, isoamyl acetate and hexanol were observed between the control and botrytized wines. These differences were not detectible sensorially when these wines were analyzed via descriptive analysis, as only one attribute (dried red fruit) differentiated the treatments. A consumer preference test (n=153) that compared the liking

scores of the appassimento-style wines from this current study (control and 10% *B. cinerea* infection) to wine made from partially dehydrated grapes fermented with an autochthonous yeast from a previous study revealed that all wines were preferred the same. The wines were assessed for preference with the use of a nine-point hedonic scale. These findings can be used to inform industry decisions regarding discarding infected fruit, as well as informing considerations for the development of wines made with partially dehydrated grapes in cool climate grape growing and winemaking regions.

5.1 Introduction

A changing climate represents an ongoing economic and agricultural challenge to winemakers and grape growers worldwide (Ashenfelter and Storchmann, 2016), as variation in wine quality may be unpredictable vintage-to-vintage. Production technologies, harvest timing and enological adaptations are examples of techniques that may assist in mitigating such quality challenges (Webb et al., 2007). One technique that may be pertinent to climatic mitigation is the production of dry wines from partially dehydrated grapes (appassimento). Wine made using the appassimento method represents a complex product with sensory characteristics that arise from biochemical changes in the grapes that affect compounds such as volatile constituents and polyphenols (Consonni et al., 2011). This style is gaining traction in Ontario, Canada as a way to diversify wine portfolios, and improve quality when climatic conditions are not ideal. During grape drying, controlled environmental conditions are important for obtaining high-quality dehydrated berries and modifications of the drying chamber, such as temperature, relative humidity, and airflow, can impact wine quality (Bellincontro et al., 2004; Chkaiban et al., 2007). Other opportunities for stylistic considerations within this wine style include grape

cultivar (Accordini, 2013), yeast selection and starting sugar concentration (Kelly et al., 2018), maceration time (Paronetto and Dellaglio, 2011), cooperage selection (Del Alamo Sanza et al., 2004) and ageing (Fedrizzi et al, 2011a). Another consideration for the development of wines made from partially dehydrated grapes is the impact of botrytization of the grapes; that is, the presence of grapes infected with *Botrytis cinerea*.

B. cinerea is the anamorphic state of the ascosporogenous species *Botryotinia fuckeliana*, a facultative parasitic fungus that is part of the most geographically wide-spread group of plant pathogens (Magyar, 2011). It causes economic losses in many crops worldwide; it is a pathogen of grapes, lettuces, tomatoes, carrots, tobacco and strawberries, among a total of more than 235 identified plant species (Aleu and Collado, 2001). It is the agent responsible for the disease known as "grey mould", as it produces a grey powdery mould on the crops infected. When infecting wine grapes, grey mould drastically reduces yield at harvest, negatively alters wine composition such as sugar, organic acids, aroma compounds which may be responsible for organoleptic defects in the wine (Ky et al., 2012). This has been characterized by the presence of off-aromas and flavours described as "damp earth", "vegetal/herbal like" and "mushroom" (La Guerche et al., 2006).

Known as the 'Jekyll and Hyde' fungus, *B. cinerea* manifests in two forms; the devastating grey mould (grey rot), or the desirable noble rot that has been historically used to produce late-harvest specialized dessert wines like Tokaji and Sauternes since the 16th century (Jackson, 2008; Negri et al., 2017). Favorable weather conditions in the vineyard (alternation of wet and dry periods and relatively low air temperature) lead to the formation of noble rot, while heavy

rain and high humidity favor the formation of grey rot (Barbanti et al., 2008; Accordini, 2013). For the production of sweet dessert wines, some vineyard factors that enhance the development of noble rot include weather conditions, selection of appropriate varieties, and harvest timing (Magyar, 2011). These wines intentionally made with the inclusion of *B. cinerea* in the berries are known as botrytized wines and are renowned for particular aromas like citrus, dried fruits and honey (Fedrizzi, 2011b).

Italian Amarone is the most well-known dry red wine made from partially dehydrated grapes, and the addition of *B. cinerea* infected grapes in fermentation of Amarone occurs traditionally. To date, a significant proportion of Amarone wine is still obtained from the traditional withering process (uncontrolled drying chamber), where unfavourable mould infection is difficult to control. When wines made with traditional drying method are compared to wines made with grapes in controlled chambers, the organoleptic impact of rot can be higher (Tosi et al., 2012). The modern approach to this style of wine is to therefore control the conditions in the drying chamber to limit the development of grey mould. Contrary to the sweet dessert wines mentioned above, Amarone is an example of a wine made with unintentional inclusion of *B. cinerea,* rather, it forms based on endogenous and exogenous factors key to this wine production. During drying, grapes are susceptible to fungal infection, likely originating from latent fruit infections acquired in the spring during flowering (Jackson, 2008; Fedrizzi et al., 2011a). The resulting influence on quality is variable and uncertain, as spontaneous fungal growth is dependent on the occurrence of favourable seasonal conditions and withering conditions (Tosi et al., 2013; Magyar and Soós, 2016). To better control noble rot development, artificial induction of *B. cinerea* strains in harvested grapes has been tested with promising

results (Azzolini et al., 2013). A “standardized” rate of infection is therefore difficult to determine, given the numerous factors that contribute to the development of this desirable factor in the winemaking process.

The sensory aspects of wine are an integral part of the consumer experience (Bruwer et al., 2011). Wine is also a complicated matrix with several mouthfeel and taste interactions that contribute to preference (Sena-Esteves et al., 2018). Further, some of the attributes in red wine such as bitterness and astringency are well known for eliciting negative consumer reactions when present at high intensity (Lesschaeve and Noble, 2005). Combining the sensory characterization of wines with hedonic tests carried out under controlled conditions can inform consumer preference or liking (Francis and Williamson, 2015). In addition to individual preferences, consumer segmentation is considered essential to understand preferences for different types of wine (Francis and Newton, 2005). In wine, the most frequent studied segments consist of geographic, demographic (gender, age, income) and behavioural (level of expertise, consumption habits or culture) (Sena-Esteves et al., 2018; Thach and Olsen, 2006). This study will segment consumers based on some of these characteristics.

In Ontario, Canada, winemaking with partially dehydrated grapes is emerging as a production technique as a way to mitigate challenges associated with climate change and to improve wine quality (Kelly et al., 2018). Drying grapes can result in post-harvest flavour development, as sugars, phenolics and aroma compounds are either concentrated or produced (Moreno et al., 2008). Some potentially problematic oxidation compounds that may arise during the drying process and during high sugar fermentation may cause organoleptic defects in final wine (Kelly

et al., 2018). The recently identified yeast, *Saccharomyces bayanus* CN1, a low producer of oxidation compounds such as acetic acid, ethyl acetate and acetaldehyde appears to be a good fit for this wine style. A consideration for the development of this wine style in Ontario is the inclusion of grapes infected with *B. cinerea* during fermentation. It is hypothesized that the controlled inclusion of grapes infected with noble rot may positively contribute to Ontario appassimento style wine quality by impacting its chemical composition and its subsequent sensory profile, with potential favourable impact on wine complexity. Cabernet franc grapes were dried to 28.0°Brix in a controlled drying chamber, and a comparative analysis was done between wines obtained from healthy grapes, our control (0% *B. cinerea* infection) and a mix of healthy and botrytized grapes (10% *B. cinerea* infection by weight). This percentage was chosen based on naturally occurring rates under these drying conditions. Chemical and volatile profiles of the wines were determined, and their sensory profiles were compared. A consumer preference test was conducted to evaluate the consumers' response to these wines, along with wine made from CN1 yeast.

The aim of this study was to understand the impact of 10% *B. cinerea* infection on high sugar wine made from partially dehydrated grapes by assessing i) chemical differences, including volatile composition, ii) sensory profile, and iii) consumer preference on such wines. This study will inform the development of appassimento-style wines in cool climate winemaking regions that are seeking ways to innovate in the face of a changing climate. Further, this may inform processing decisions regarding sorting infected berries, as hand-sorting can be a timely and expensive process due to personnel requirements. This will also be the first time that Ontario appassimento style wines made with the autochthonous *S. bayanus* CN1 yeast have been

evaluated from the perspective of consumer preference, as well as the first time consumers assessing this wine will be segmented based on liking and additional characteristics.

5.2 Materials & Methods

5.2.1 Grape Drying: *B. cinerea* Trial

Cabernet franc grapes (225 kg) were harvested from the Mazza Vineyards in the Niagara sub-appellation of Niagara-on-the-Lake at 21.5°Brix (normal maturity) and dried in the Cave Spring Winery Barn (described in data chapter two) to a target starting sugar concentration of 28.0°Brix. Grapes were sampled weekly (15 randomly selected clusters), and 105 randomly selected berries from the 15 clusters were weighed. The clusters and berries were then crushed by hand in a plastic bag and strained through a metal strainer to collect must for immediate determination of soluble solids, pH and titratable acidity. Once the fruit reached the drying target (53 days after harvest), the parcel was delivered to the CCOVI pilot winery for processing.

5.2.1.1 Grape Drying: CN1 Yeast Trial

Cabernet franc grapes (226 kg) were harvested from the same vineyard as grapes from study one at 21.5°Brix and dried in the same drying barn. Once the fruit reached the drying target (27.5°Brix), the parcel was delivered to the CCOVI pilot winery for processing.

5.2.2 Identification of *B. cinerea* Infection

During drying, the formation of the fungus *B. cinerea* was observed. It was necessary to confirm the presence of *B. cinerea* in order to i) ensure the berry infection was correctly identified, and to ii) aid the processing team with visually identify infected grapes during culling.

While grapes were drying, samples of infected berries were divided into three categories: black, with opaque dark blue to black skins, their normal colour when healthy; red with puckered light-purple to red skins, typically a sign of *B. cinerea* infection causing weakening skin (Fournier et al., 2013); and sporulating, with puckered red skins showing signs of grey mycelial growth

and spore on the surface (Fournier et al., 2013). Three separate methods for identification were utilized and are described below.

5.2.2.1 Must analysis

Must was extracted from a subset of berries 15 from each category for analysis of soluble solids (°Brix), glycerol and gluconic acid.

5.2.2.2 Cultured berries

Another subset of 50 berries from each of the visual identification categories was processed and cultured on PDA (potato dextrose agar) plates. Berries were surface sterilized by submerging them in 95% ethanol and brief exposure to flame. Each berry was then macerated in 1mL sterile peptone water (1g/L peptone + 0.1% Tween-20) and 100 µL was plated directly on the PDA plate in triplicate. After five days at room temperature, plates underwent visual examination (Huber, 2016).

5.2.2.3 Incubation in moist chamber

Intact berries represented the third subset for identification. Ten berries from each category were surface sterilized (agitated in 10% commercial bleach for 20 minutes, then rinsed three times with sterile distilled water), then placed in a moist chamber for eight days at room temperature. Berries were then crushed and must was analyzed for soluble solids (°Brix), glycerol and gluconic acid.

5.2.3 Grape Processing: *B. cinerea* Trial

Once the grapes were ready for processing, the dried grapes were hard sorted by four laboratory technicians to remove infected grapes from the clusters. Grapes were visually inspected, and all berries that were “red” or “sporulating” were removed with narrow-tipped grape snips. These grapes were placed in a separate container, while the clean grapes were divided equally into fermentation vessels.

A total of six fermentations were carried out in 20L steel fermentation pots; three control (0% *B. cinerea* infection) and three *B. cinerea* infected (10% *B. cinerea* infection inclusion by weight). These treatments will be denoted "control" and "*Bot*10%" throughout this paper. Each replicate was processed separately through the crusher/destemmer (model Gamma 50, Mori-TEM; Florence, Italy). Each fermentation pot contained a total of 19kg of grapes. There were no modifications made to the control treatments. By using the weight of the clean grapes (kg x0.65 to account for desiccation effects), the weight of the *B. cinerea*-infected berries required to compose 10% of the final weight was calculated. Thus, the clean pots contained 19kg of grapes (x0.65 = 11.4L), while the 10% treatment pots contained an additional 2.1 kg, which equals a total of 21.1kg (x0.65 = 12.7L). The portions of infected grapes were weighed, added to the fermentation lots and then mixed by hand, and 100 mL of sample from each replicate was taken for chemical analysis.

5.2.3.1 Grape Processing: CN1 Yeast Trial

Full methods are described in chapter three. To summarize, dried grapes were divided randomly and equally into three replicates based on weight (approximately 20 kg grapes/replicate), and each replicate was processed separately through the crusher/destemmer into 20L steel fermentation vessels with tight-filling lids.

5.2.4 Fermentation: *B. cinerea* Trial

Dried grapes were brought to the CCOVI winery for processing, and fermentation took place the following day after temperature stabilization overnight at room temperature (18°C). Fermentations were conducted in triplicate. Sulfite (from potassium metabisulfite) was added to each fermentation vessel at a rate of 25 mg/L, and the grapes were blanketed with CO_2 and sealed with steel lids. Grapes were brought to 22°C before inoculation. Commercial yeast,

Saccharomyces cerevisiae EC1118 (Lallemand, Montreal, QC, Canada) was rehydrated according to manufacturer's instructions, added to each vessel at 0.35g/L and mixed via punch down to initiate fermentation. Diammonium phosphate (DAP) was added at a rate of 500mg/L at the beginning of fermentation, and again on the third day of fermentation to avoid yeast nutrient stress. All fermentations were kept at 22°C, punched down twice daily and monitored once daily by recording soluble solids (hydrometer, °Brix), specific gravity (hydrometer, specific gravity) and temperature (thermometer, °C). Winemaking replicates received 20 plunges per vessel, and as the fermentation progressed, this number was gradually reduced to four plunges per vessel by the end of fermentation, using a separate punch down tool for each yeast trial to prevent cross-contamination. Once fermentations were complete (<5g/L reducing sugars, as determined by WineScanTM; Hillerød, Denmark), they were pressed separately with a small bladder press Enotecnica Pillan; Vicenza, Italy) at one bar for two minutes into 11L glass carboys. Treatments were sulfited at 50 mg/L of sulfur dioxide (as potassium metabisulfite) and settled at room temperature. Wines were then racked and moved to a -2°C chamber for cold stabilization until bottling. Wines were subsequently pad filtered through filter pads and bottled as separate treatments into 750mL glass bottles with a manual bottler (Criveller Group; Niagara Falls, Ontario, Canada), and automated corker (model ETSILON-R, Bertolaso; San Vito, Italy) and natural cork and stored in the CCOVI wine cellar (17.5°C and 74.5% humidity).

5.2.4.1 Fermentation: CN1 Yeast Trial

Full methods are described in chapter three. To summarize, a yeast culture (*S. bayanus* CN1) was built up in sterile-filtered 10°Brix must, and a step-wise acclimatization method (Kontkanen et al., 2004) was utilized to reduce yeast stress. The 27.5°Brix must was inoculated at a rate of

$5.0x10^6$ cells/mL. Fermentations were conducted in triplicate. Fermentation were considered complete once the yeast stopped consuming sugar (<5 g/L) and were subsequently pressed separately with a small bladder press at one bar for two minutes into glass carboys. Wines were filtered and bottled into 750mL glass bottles, sealed with a natural cork and stored in the CCOVI wine cellar (17.5°C and 74.5% humidity)

5.2.5 Sample Analysis: *B. cinerea* Trial

The parameters tested for juice and wine samples and the method/instrumentation used, in parentheses, were as follows: soluble solids for grape and must samples (Abbe refractometer model 10450) and using a degree Brix hydrometer for fermentation time course samples, pH (model SB70P, SympHony, VWR), titratable acidity (was determined by titration with 0.1 mol/L of NaOH to an endpoint of pH 8.2), acetaldehyde (Megazyme K-ACHYD enzyme kit), acetic acid (Megazyme K-ACET enzyme kit), amino acid nitrogen (Megazyme K-PANOPA enzyme kits), ammonia nitrogen (Megazyme K-AMIAR enzyme kit), fructose and glucose (Megazyme K-FRUGL enzyme kit), gluconic acid (Megazyme K-GATE enzyme kit), glycerol (Megazyme K-GCROL enzyme kit), lactic acid (Megazyme K-LATE enzyme kit), and malic acid (Megazyme K-LMALR enzyme kit), ethanol in juice (Megazyme K-ETOH enzyme kit), ethanol in wine and ethyl acetate by gas chromatography (Hewlett-Packard 6890 series gas chromatograph equipped with a flame ionization detector (FID) and a split/split-less injector), separations were carried out with a DB®-WAX (30 m, 0.25 mm, 0.25 µm) GC column, model 122-7032, (Agilent Technologies Incorporated) with helium as the carrier gas at a flow rate of 1.5 mL/min). Each winemaking replicate sample was tested in duplicate.

5.2.6 VOC & VFA Analysis: *B. cinerea* Trial

Methods for VOC and VFA analysis are outlined in data chapter four (based on the methods of Botezatu et al., 2016) with no modifications. In summary, samples were analyzed using an Aglient (Mississauga, ON, Canada) 7890A gas chromatograph coupled to a 5975C mass selective detector (MSD) equipped with a Gerstal MPS2 XL autosampler (Linthicum Heights, MD, USA). Internal standards were d_{11} ethyl hexanoate and octanal-d_{16}.

5.2.7 Data Processing: *B. cinerea* Trial

The analytical data software (Chemstation, MSD E.02.00.493 by Agilent Technologies) was used to extract the quantifying ions, and the ratio of the standard over the internal standard was plotted against each analyte concentration to fit a quadratic equation where the intercept was set to zero. Triplicate spiked samples were prepared and analyzed after every 20 wines samples to calculate the recovery.

5.2.8 Descriptive Analysis: *B. cinerea* Trial

Wines were bottled in 750 mL glass bottles, and descriptive analysis took place 18 months after bottling. After a preliminary bench tasting (n=5) that determined the treatments (control vs *Bot*10%) were different from each other, a descriptive analysis was conducted on all six wines (triplicate fermentation replicates of each treatment). Methods are outlined in data chapter four with the following modifications:

Consensus terminology, language training, scoring wines on the scale and reference standards were developed over two three-hour training sessions. During the first two training sessions, all wines were presented to the panelists. The final descriptor list with additional terms in parentheses is as follows: red fruit- dried (strawberry, raspberry, cranberry), fruit- black (cassis, blackberry), vegetal (canned green vegetable), coffee, candied cola (cola candies), medicinal,

mushroom (earthy), spice (black pepper, allspice), dirty (wet leaves and humus) and dusty for aroma modalities and red fruit- dried (strawberry, raspberry, cranberry), fruit- black (cassis, blackberry), vegetal (canned green vegetable), spices (black pepper, allspice), medicinal and dark chocolate for flavour modalities. Basic tastes and in-mouth sensations are as follows: bitterness, acidity (sourness), heat, astringency and length of finish. After the descriptors were agreed upon by the panel, reference standards were presented for all aroma descriptors and the following training sessions optimized the standards to agree with the descriptors present in the wine. Reference standards were made according to recipes outlined in *Table 5.1* with 2013 Cabernet franc appassimento style base wine (24.5°Brix starting sugar concentration). Following training, panelists evaluated six wines in duplicate over two sessions using a complete randomized block design. Descriptive analysis data was collected using Compusense™ software (Guelph, ON, Canada). The order of presentation of the samples was randomized. Sessions were completed with a one-hour break in between session one and two. There were forced three-minute breaks between wines to prevent fatigue.

5.2.9 Consumer Preference Test: *B. cinerea* and CN1 Yeast Trial

Recruitment and implementation of consumer preference test was done at Compusense (Guelph, ON, Canada) in their sensory lab. Recruited panelists met the following inclusion criteria:

Participants must be 19 years of age or older, with equal distribution of males and females. Participants were divided into two groups for data collection: frequent red wine drinkers and infrequent to moderate drinkers of wine (both red and white) excluding red wine avoiders, as determined by an alcohol consumption questionnaire. A total of 153 participants were recruited for this study, and they were instructed to taste the wines and rate them on a

standard nine-point hedonic scale, and then two additional questionnaires were presented to the panelists. A self-administered computerized questionnaire was used to collect data on Apple™ iPads. Panelists were presented with three ballots: the first was a nine-point hedonic scale (*Figure 5.1*) that participants used to rate their liking after each sample was tasted. The next two ballots were completed at the end of the tasting. In order to measure self-rated wine expertise, the first ballot asked the question "How would you rate your wine expertise?" and the possible five answers were as follows: "I am a wine connoisseur", "I am very knowledgeable about wine", "I am somewhat knowledgeable about wine", "I know a little bit about wine", "I don't know anything about wine". In order to measure wine involvement, the final ballot stated "An appassimento/Amarone wine is made from grapes that are dried" and the possible five answers were as follows: "I drink this type of wine all the time", "I drink this type of wine quite often", "I sometimes drink this type of wine" and "I rarely drink this type of wine".

Samples were as follows: Wines from "study one", control (EC1118 28.0°Brix, 0% *B. cinerea* infection), *Bot*10% (EC1118 28.0°Brix, 10% *B. cinerea* infection) and wine from "study two", Yeast Trial (CN1 27.5°Brix), all appassimento-style wines made from Cabernet franc grapes from two different yeast strains (*S. cerevisiae* EC1118 and *S. bayanus* CN1) in 2013. All wines were made from grapes that were sourced from the same vineyard and dried in the same chamber. The wines were served monadically using a balanced William's design. The product was stored and evaluated at room temperature., The wines were decanted into one-litre carafes 60 minutes before serving. To each panelist, 25mL of each sample was served in a 210mL wine glass, labelled with a three-digit blinding code. Samples were poured into the wine glasses 15 minutes before serving. During the tasting, consumers were encouraged to consume unsalted

soda crackers and room temperature distilled water during a 60-second forced break between samples. Data was collected with Compusense® Cloud Software.

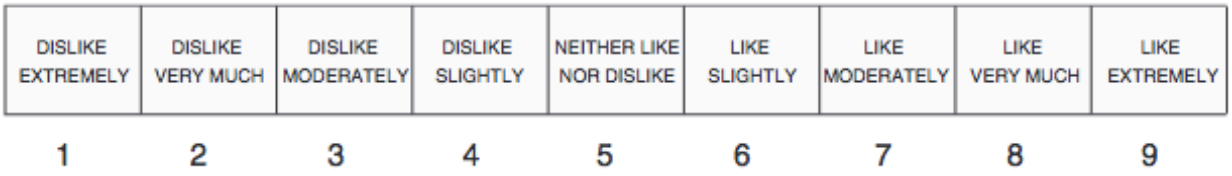


Figure 5.1: Nine-point hedonic scale used to rate wine liking for consumer preference analysis

5.2.10 Statistical Analysis: *B. cinerea* and CN1 Yeast Trial

Analysis of Variance (ANOVA) including two-way ANOVA [factors: Tasting replicate, winemaking replicate and tasting replicate*winemaking replicate interaction] and three-way ANOVA [f=Tasting replicate, judge, wine and tasting replicate*judge, tasting Replicate*wine and judge*wine interactions] for descriptive analysis results and one-way ANOVA (f=clustered liking scores), Chi-Squared (contingency table) were performed using XLSTAT software version 2018.6 (Addinsoft, Paris, France), at 95% confidence interval (p<0.05) for consumer segmentation results. Attributes that differed were analyzed by Tukey's honest significant difference (HSD) post-hoc tests. Principal component analysis (PCA) (observations/variables were chemical compounds, supplementary variables were winemaking treatments, no rotation, PCA type: Pearson (n), type of biplot: correlation biplot/ coefficient=automatic) was performed with XLSTAT software version 2018.6 (Addinsoft, Paris, France).

Table 5.1: Complete list of reference standards and terms used for descriptive analysis for control and Bot10% made from partially dehydrated grapes.

Aroma	Includes Terms	Reference Composition
Note: Unless otherwise stated, all standards prepared in 50mL of neutral base wine		
Red fruit- Dried	Strawberry, raspberry, cranberry	Three cooked strawberries + five raisins
Fruit- Black	Cassis, blackberry	Ribena* concentrated juice +o ne drop "artificial ripe blackberry"† + one drop "natural black currant / cassis"†
Vegetal	Canned green vegetable	One teaspoon of canned green beans in brine‡
Coffee	Coffee bean	Five drops of base wine that was steeped with one coffee bean
Candied Cola	Cola candies	Two cola candies cut into pieces
Medicinal	Cherry menthol	50 mL of base wine that was steeped with one cherry cough candy§
Mushroom	Earthy	Two teaspoons canned mushrooms in brine‡
Spicy	Pepper, allspice	Four crushed allspice balls + three shakes black pepper
Dirty	Wet leaves and humus	50 g dried plant material (primarily leaves) sourced from two centimetres below soil surface
Dusty		Weed stems

All standards were prepared using EC1118 24.5°Brix wine as base wine unless otherwise indicated.

Wine Awakenings Kit: Niagara Falls, ON, Canada, http://www.wineawakenings.com, 1-877-595-5678

* Compliments© brand Lucozade Ribena Suntory Ltd., 2 Longwalk Road, Stockley Park, Uxbridge, United Kingdom; sourced in Canada from Sobeys Inc.

† http://www.wineawakenings.com, 1-877-595-5678

‡ Compliments© brand, Empire Company Limited, 115 King Street, Stellarton, NS, Canad; sourced from Sobeys Inc.

§ Halls© brand, Mondeléz International, 100 Deforest Avenue, East Hanover, NJ, United States of America; sourced from Dollarama Inc.

5.3 Results

5.3.1 B. cinerea Identification

5.3.1.1 Must Analysis

The must from the three categories of berries were analyzed for soluble solids (°Brix), glycerol and gluconic acid. The soluble solids were significantly higher in berries that were infected with *B. cinerea* (red and sporulating). The same trend was observed in glycerol concentration (*Table A5.6*). The approximate amount of juice extracted from the infected berries was less than the healthy berries (*Table 5.2*).

Table 5.2: Colour descriptions and approximate amount of juice in each of the berry categories.

Category	Colour	Approximate amount of juice (mL)
Black (healthy)	pink	50
Red (internal infection)	dark gold	20
Sporulating	light gold	7

5.3.1.2 Grape juice plated on PDA plates and Tray Assay

Black berries: Six plates were cultured with black berries, three undiluted and three at ten times dilution. Only one of the ten times dilution replicates were not confirmed with the presence of *B. cinerea* (*Figure A5.11*).

Red berries: All six plates were confirmed with the presence of *B. cinerea* (*Figure A5.12*).

Sporulating berries: All six plates were confirmed with the presence of *B. cinerea* (*Figure A5.13*).

For the tray assay, all ten berries were clean on “Day 0” (*Figure 5.2*). By “Day 8”, all ten berries in each of the red berry and sporulating berry categories were confirmed with the presence of *B. cinerea*. Only one of the ten black berries were confirmed as *B. cinerea* infected (*Figure 5.3*).

Figure 5.2: Timepoint "Day 0" of tray assay for B. cinerea identification.

Figure 5.3: Timepoint "Day 8" of tray assay for B. cinerea identification.

5.3.2 Fermentation Kinetics

Fermentations progressed without difference amongst treatments. Small error bars (standard deviation) show that the winemaking replicates consumed sugar at approximately the same rate (*Figure 5.4*). After 168 hours (7 days), the wines were fermented to dryness (<5g/L residual sugar).

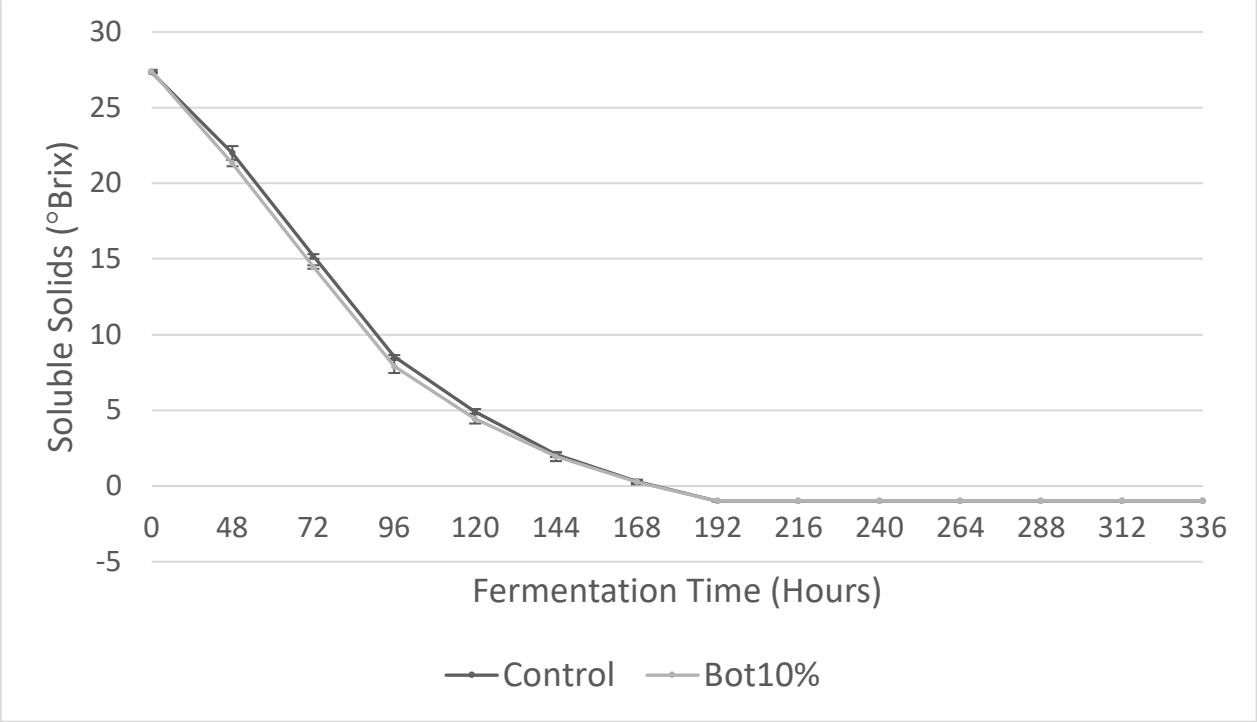


Figure 5.4: Fermentation kinetics for Control and 10% B. cinerea infected: Cabernet franc wines made from partially dehydrated grapes.

Each point represents the mean from triplicate fermentation with standard deviation shown as error bars.

5.3.3 Must and Wine Analysis:

During the drying, the sugars concentrated in the grapes to reach the target starting sugar concentration of approximately 28.0°Brix (*Table 5.3*). For most metabolites in the must, control and *Bot*10% are similar, except for glycerol and gluconic acid. Small differences in metabolites can be attributed to cluster variation.

Control and *Bot*10% wines differed significantly in the following metabolites: acetic acid, glucose, glycerol, gluconic acid and primary amino nitrogen, with *Bot*10% having significantly higher concentrations in all listed metabolites, while tartaric acid is significantly lower in control wines (*Table 5.3*).

Table 5.3: Must and wine analysis of physio-chemical characteristics and metabolites measured for two treatments: Control and 10% B. cinerea infected.

Fermentations were conducted in triplicate and each sample was tested in duplicate for all metabolites other than soluble solids. Data represents the mean value ± standard deviation of duplicate measurements per sample (three winemaking replicates per treatment). Asterisks indicate significant difference (alpha=0.05) with Student's *t*-test.

Physio-Chemical Characteristic/Metabolite	**Treatment**	**Must**	**Wine**
Soluble solids (°Brix)	Control	27.6 ±0.2	-
	*Bot*10%	28.1 ±0.1	-
pH	Control	3.65 ±0.02	3.97 ±0.3
	*Bot*10%	3.66 ±0.01	4.01 ±0.02
Titratable acidity (g/L)	Control	4.8 ±0.0	6.7 ±0.0*
	*Bot*10%	4.7 ±0.1	6.4 ±0.0*
Malic acid (g/L)	Control	2.5 ±0.1	2.2 ±0.1
	*Bot*10%	2.3 ±0.1	2.1 ±0.1
Lactic acid (g/L)	Control	0.05 ±0.00	<0.03
	*Bot*10%	0.05 ±0.00	<0.03
Acetic acid (g/L)	Control	<0.02	0.28 ±0.00*
	*Bot*10%	<0.02	0.35 ±0.00*
Glucose (g/L)	Control	132 ±5	<0.07*
	*Bot*10%	128 ±4	0.09 ±0.01*
Fructose (g/L)	Control	145 ±10	0.11 ±0.01
	*Bot*10%	142 ±5	0.15 ±0.02
Reducing sugar (g/L)	Control	278 ±11	0.17 ±0.01
	*Bot*10%	270 ±9	0.24 ±0.02
Ethanol (% v/v)	Control	0.004 ±0.000	16.4 ±0.2
	*Bot*10%	0.004 ±0.000	16.4 ±0.2
Acetaldehyde (mg/L)	Control	<16	108 ±10
	*Bot*10%	<16	113 ±8
Ethyl acetate (mg/L)	Control	n/d	53 ±2
	*Bot*10%	n/d	59 ±3
Glycerol (g/L)	Control	0.4 ±0.0	11.5 ±0.4*
	*Bot*10%	1.2 ±0.1	12.7 ±0.4*
Gluconic acid (g/L)	Control	0.14 ±0.01	0.23 ±0.02*
	*Bot*10%	0.29 ±0.02	0.34 ±0.01*
Ammonia nitrogen (mg/L)	Control	7 ±1	<6
	*Bot*10%	7 ±0	<6

Primary amino nitrogen (mg/L)	Control	91 ±3	51 ±2*
	*Bot*10%	85 ±0	66 ±1*

5.3.4 Analysis of Variance: Volatile Organic Compounds and Volatile Fatty Acid Compounds

A one-way ANOVA was conducted on the VOC and VFA compound data ($p<0.05$) (*Table 5.4*) to identify treatment differences.

Several ethyl esters were measured. Ethyl hexanoate concentration was significantly higher in control. The concentration of ethyl isobutyrate was significantly higher in the control treatment, and the same trends were observed for ethyl butyrate. Ethyl 2-methyl butyrate and ethyl octanoate were not significantly different in control and *Bot*10% treatments.

Concentration of 2-phenylethanol was not different, but hexanol concentrations were higher in the control treatment. Isoamyl acetate was significantly higher in *Bot*10%.

There were no significant differences between treatments for hexanoic or octanoic acids.

Table 5.4: Concentrations of VOCs and VFAs in Control and 10% B. cinerea infected and sensory threshold values. Results are the mathematical average of two representative wine bottles and two sample replicates of each treatment (eight data points per result) ± standard deviation.

Treatment	mg/L	Volatile Organic Compound (μg/L)							
	2-Phenyl Ethanol	Ethyl isobutyrate	Ethyl butyrate	Ethyl isovalerate	Ethyl octanoate	Ethyl 2-methyl Butyrate	Isoamyl acetate	Hexanol	Ethyl hexanoate
Sensory Threshold	140†	15†	20*	3†	580^	18†	30†	8000†	14•
Control	159.1± 13.3[a]	231.3± 46.3[a]	279.0± 13.9[a]	72.0± 11.2[a]	589.4± 18.6[a]	63.3± 7.8[a]	318.5± 13.9[b]	2870.4± 87.2[a]	635.70± 21.2[a]
*Bot*10%	147.5± 16.1[a]	186.2± 26.8[b]	266.2± 5.5[b]	64.5± 9.0[a]	574.6± 51.1[a]	55.8± 4.6[b]	344.7± 22.6[a]	2440.35± 111.9[b]	577.35± 20.2[b]

Treatment	Volatile Fatty Acid (μg/L)	
	Hexanoic Acid	Octanoic Acid
Sensory Threshold	420†	500†
Control	1898.6±77.1[a]	2025.1±110.9[a]
*Bot*10%	1751.8±76.8[a]	1943.8±74.2[a]

Analysis of variance (ANOVA) with mean separation by Fisher's Least Significant Difference (LSD; $p < 0.05$). Lowercase letters within the same starting sugar concentration yeast indicate differences using Fisher's $LSD_{0.05}$.

† Ferreira et al., 2000 (determined in 11% ethanol/water solution with 7 g/L glycerol and 5 g/L tartaric acid at pH 3.4)

* Guth et al. (1997) (determined in 10% ethanol/water solution)

^Etiévant, 1991

•López de Lerma et al., 2012

5.3.5 Descriptive Analysis

A preliminary bench tasting revealed that the treatments (control and *Bot*10%) were different from each other, while the winemaking replicates were considered somewhat similar to each other. Thus, the descriptive analysis proceeded without blending winemaking replicates. Rather, all three winemaking replicates were assessed by the panel.

In order to determine if the data that was collected for six wines (control and *Bot*10% and their respective triplicate winemaking replicates) could be collapsed to represent two treatments, a preliminary two-way analysis of variance (ANOVA) was conducted for each treatment [factors = tasting replicate and winemaking replicate and tasting replicate*winemaking replicate interaction]. For control, none of the attributes were significant (*Table A5.7*). The same trends were observed for *Bot*10% *(Table A5.8).*

Thus, the attribute intensity data for the triplicate winemaking replicates for each treatment (representing six separate wines) were collapsed into two: control and *Bot*10% for analysis purposes. The three-way ANOVA [factors = tasting replicate, panelist, winemaking replicate and tasting replicate*judge, tasting replicate*wine, judge*wine interactions] for control and *Bot*10% reveal significant differences between the wines in only one attribute: dried red fruit aroma (higher for *Bot*10%) *(Table A5.9).* This is visually demonstrated in Figure 5.5 spider plot.

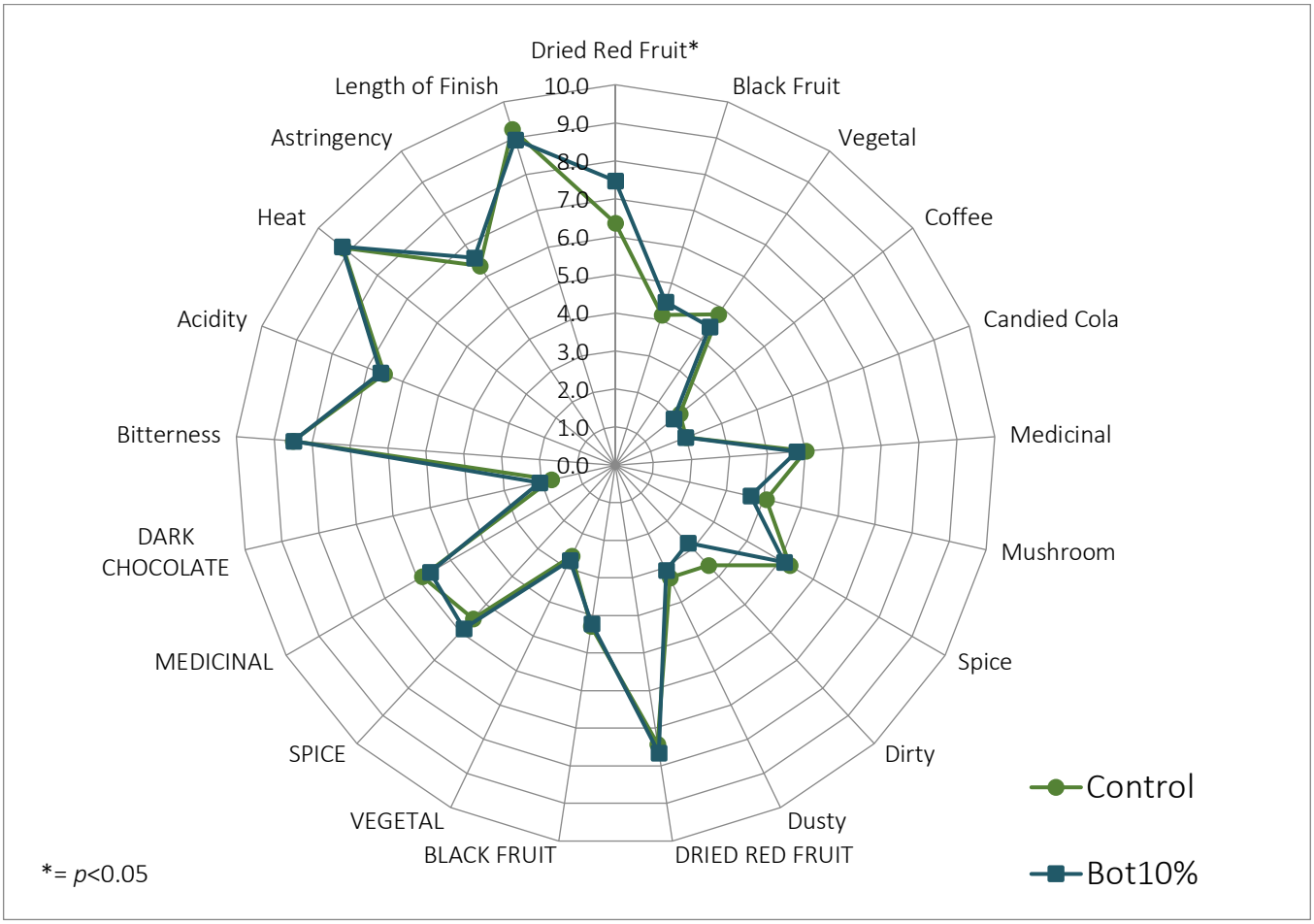


Figure 5.5: Spider plot of means of intensity ratings for all attributes generated by the panel for Control and 10% B. cinerea infected Cabernet franc wines made from partially dehydrated grapes.

Aroma attributes are indicated in lowercase letters, flavour attributes in capital letters. Each point represents average intensity ratings for triplicate fermentations. Statistical differences were determined by analysis of variance (ANOVA) with mean separation by Fisher's Least Significant Difference (LSD; p< 0.05).

5.3.6 Principal Component Analysis

A principal component analysis (PCA) (*Figure 5.6*) was conducted, and all winemaking replicates are plotted with the attributes generated by the panelists. The PCA explains 58.35% of the variation on two factors which were retained. Most of the attributes are positively loaded on F1, while coffee aroma, mushroom aroma, medicinal flavour and candied cola aroma are positively loaded on F2. The winemaking replicates don't follow any kind of trend; rather, all

replicates are positioned on three quadrants of the biplot. “Control replicate three” and “*Bot*10% replicate three” are positioned in the middle of the plot, suggesting they are poorly defined by the model, while “control replicate one” is associated with vegetal and dirty aroma. The small angles between attributes represents correlations between the attributes. For example, heat and dried fruit flavour are positively correlated to each other. Attributes on opposite sides of the biplot are negatively correlated (for example, candied cola aroma and mushroom aroma), and attributes at a 90° angle from each other are not correlated (for example, dirty aroma and black fruit flavour). “*Bot*10% replicate one” and “*Bot*10% replicate two” are positioned on opposite sides of the plot.

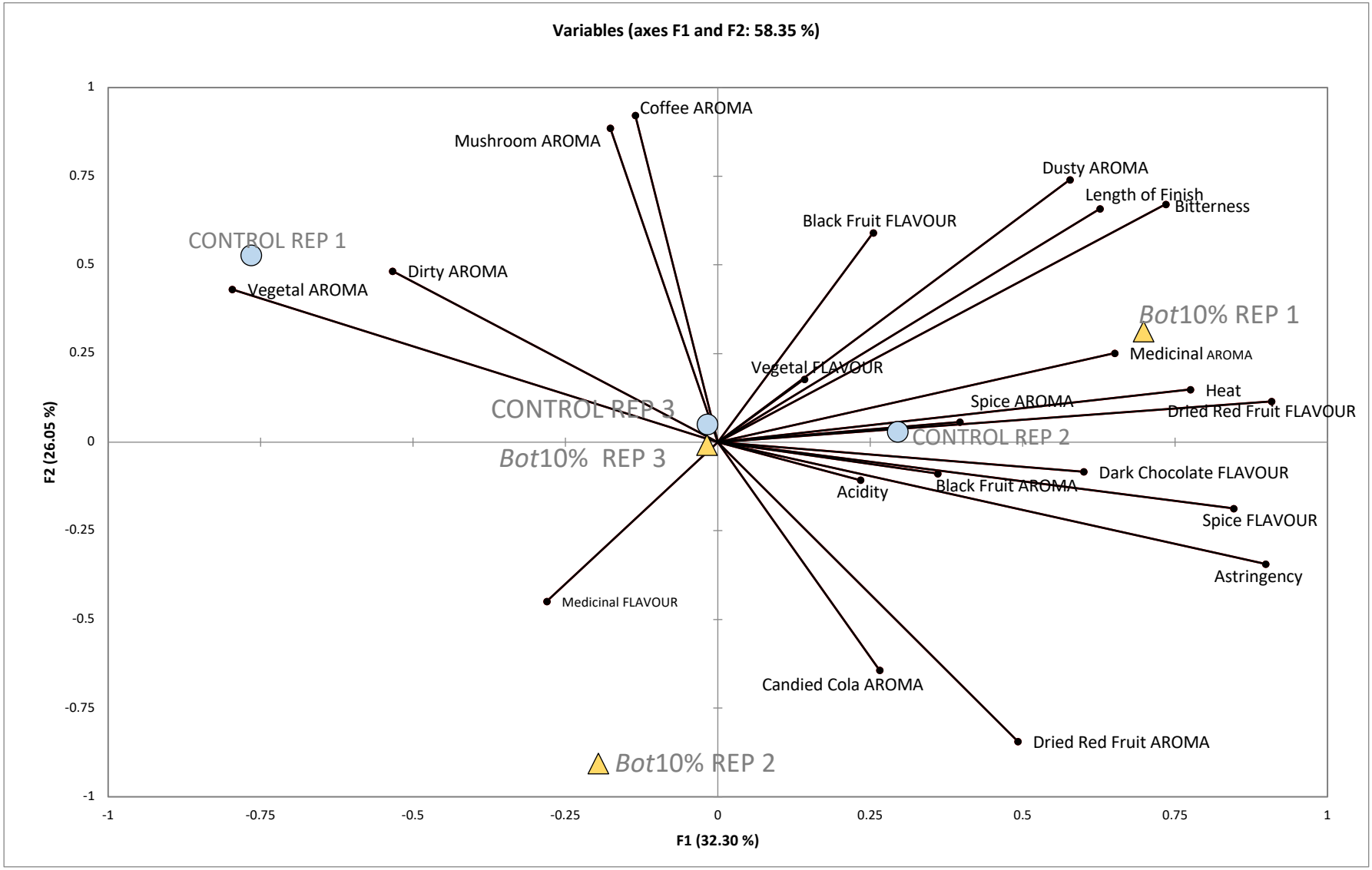


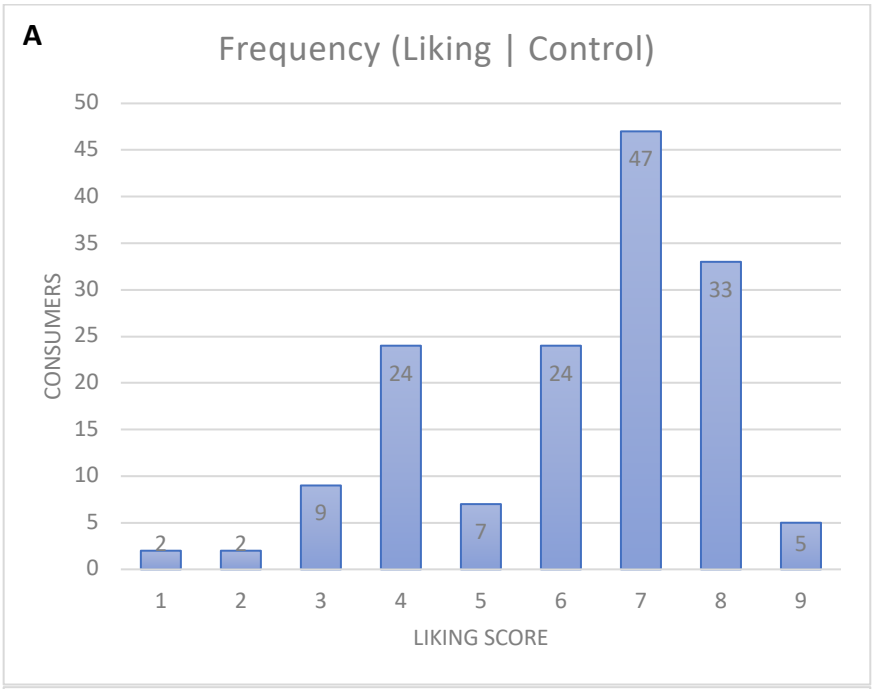
Figure 5.6: PCA sensory map of Control and 10% B. cinerea infected Cabernet franc wines made from partially dehydrated grapes including winemaking replicates and descriptive attributes from descriptive analysis.

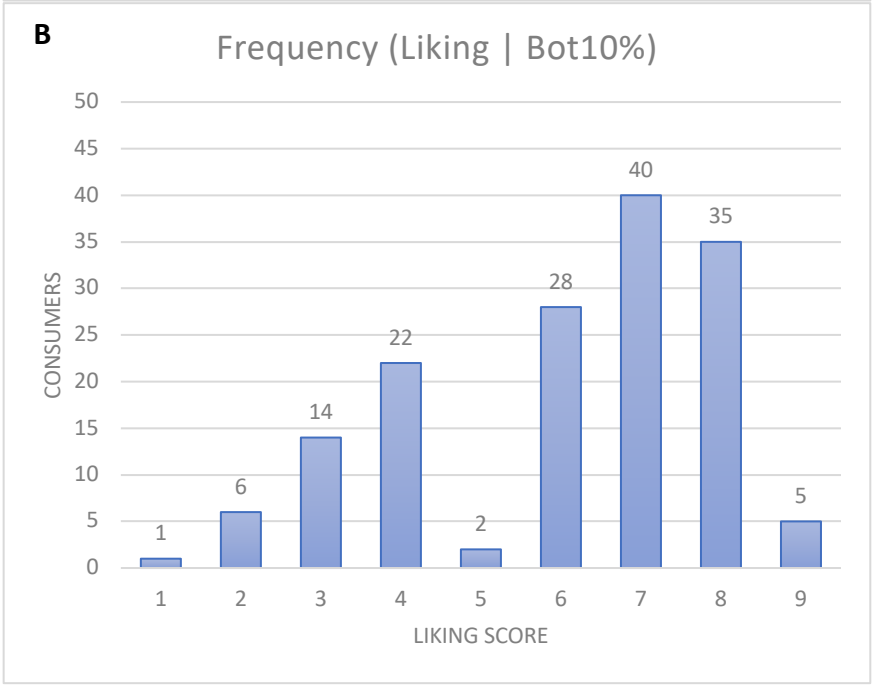
5.3.7 Consumer Preference

Wines (control, *Bot*10% and CN1 yeast trial) were rated on a nine-point hedonic scale (*Figure 5.1*). No significant difference was reported amongst wines. Each wine was rated above six (like slightly) on average on the scale. The distribution of liking scores is given in *Figure 5.7*.

Demographic information was collected (*Figure 5.8; Figure 5.9*), along with self-rated wine expertise and wine involvement data. The majority of respondents self-classified as being somewhat knowledgeable about wine, about one quarter of participants know a little bit about wine, and even fewer (15%) consider themselves highly wine knowledgeable. Only 2% of respondents self-rated as knowing very little about wine (*Figure A5.14*). The majority of participants rarely or never consume appassimento-style wines, while approximately one-third of participants sometimes drink this type of wine. A small proportion (7%) of participants drink this wine regularly (*Figure A5.15*).

An ANOVA in which liking scores for each wine were the dependent variables and age, sex, self-rated wine expertise and wine involvement (collapsed data, see below) were the independent variables was conducted (data not shown). The liking score results are as follows: control: 6.2, *Bot*10%: 6.1 and CN1 yeast trial: 6.4. This analysis showed no effect from the independent variables (p-value=0.16); we decided to investigate this null result further by considering consumer segmentation methods.


A
Frequency (Liking | Control)
CONSUMERS
LIKING SCORE
50
45
40
35
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
2
2
9
24
7
24
47
33
5


B
Frequency (Liking | Bot10%)
CONSUMERS
LIKING SCORE
50
45
40
35
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
1
6
14
22
2
28
40
35
5

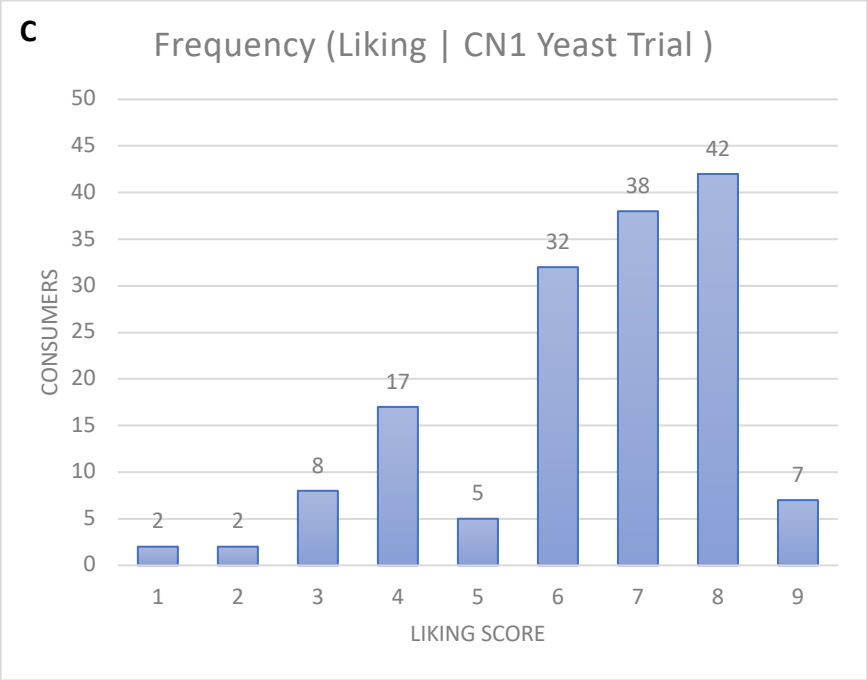


Figure 5.7A-C: Distribution of liking scores for A.) Control B.) 10% B. cinerea infected and C.) CN1 S. bayanus Yeast Trial.

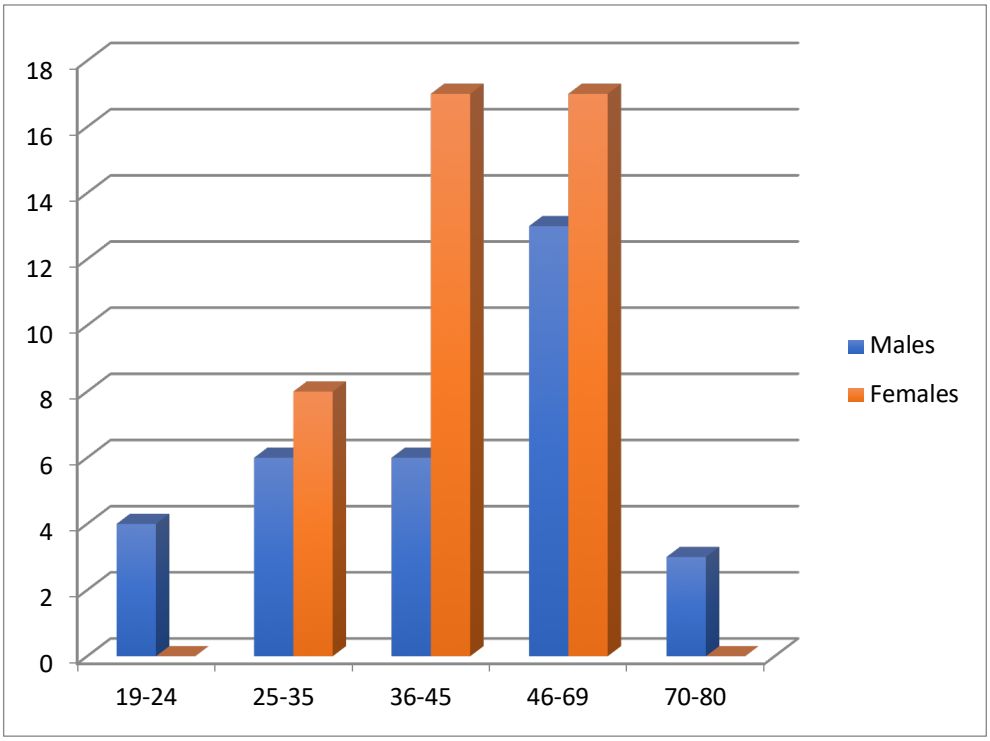


Figure 5.8: Demographic data for group one: Frequent red wine drinkers, represented by sex.

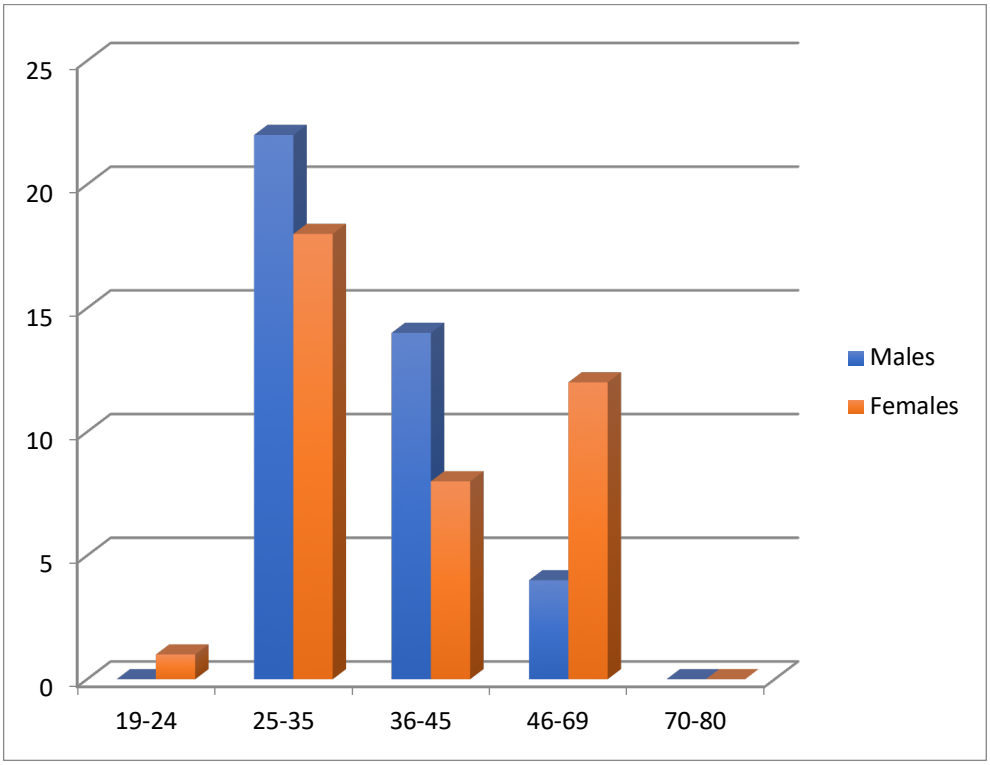


Figure 5.9: Demographic data for group two: Infrequent to moderate wine drinkers, represented by sex.

5.3.8 Consumer Segmentation:

Agglomerative hierarchical clustering (dissimilarity proximity, Ward's method, Euclidean distance) was performed on the consumer preference data to identify trends in the consumers based on wine liking scores (*Figure 5.10*). Three clusters emerged from this dendrogram and were identified as "everything likers" (highest liking scores across all wines), "CN1 likers" (highest liking score for CN1 wine) and "CN1 dislikers" (highest score for control and *Bot*10%, all fermented with EC1118, or conversely, especially low scores for CN1 wines) based on liking scores. Collected demographic information was then linked to the clusters to classify the participants. Each cluster was then compared for sex, age, frequency of consumption, self-rated wine expertise and wine involvement. Clusters were examined using one-way analysis of variance (ANOVA) for the quantitative variables and Chi-Squared for qualitative variables (*Table*

5.5). For the above-mentioned analyses, we collapsed some of the data. For self-rated wine expertise data, the first two ("I do not know anything about wine" and "I know a little bit about wine") and last three categories ("I am somewhat knowledgeable about wine", "I am very knowledgeable about wine" and "I am a wine connoisseur") were collapsed to increase statistical power due to the low number of respondents in some of the categories. The collapsed categories have been renamed "low" and "medium/high" self-rated wine expertise participants (*Table 5.5*).

The same strategy was applied to age demographics, as the first (19-24) and last (70-80) age categories were collapsed to increase statistical power due to the low number of participants in those categories. For analysis purposes for wine involvement data, we collapsed the first two ("I never drink this type of wine" and "I rarely drink this type of wine") and last three categories ("I sometimes drink this type of wine", "I drink this wine quite often" and "I drink this type of wine all the time") to increase statistical power due to the low number of respondents in the options. The collapsed categories have been renamed to "less frequent" and "more frequent" wine involvement participants (*Table 5.5*).

Cluster one has the highest number of participants (n=73) and contains an almost equal number of males and females, with most participants aged from 46-80. More than half (55%) of these participants are frequent red wine drinkers, and 81% of cluster one is considered to have a medium to high level of self-rated wine expertise, the highest level of expertise amongst all clusters. This group has the highest incidence of wine involvement in Amarone/appassimento Cluster two (n=50) is also composed of an almost equal number of males and female, with most participants aged 19-35. This group is the youngest of the three clusters. This group is mostly

infrequent to moderate drinkers of both red and white wine. This group is composed of an almost equal number of participants in each of the two self-rated wine expertise categories, and their involvement in this wine style is quite low (only 32% of participants have more frequent consumption of this wine style). Cluster three is the smallest group (n=30), with almost equal number of males and females, and contains the highest percentage of older participants (46-80 years) than any other group. The frequency of consumption category contains almost equal participants. Similar to cluster two in both self-rated wine expertise and wine involvement, most participants of this cluster have medium to high levels of self-rated wine expertise, and most participants are less frequent consumers of this wine style.

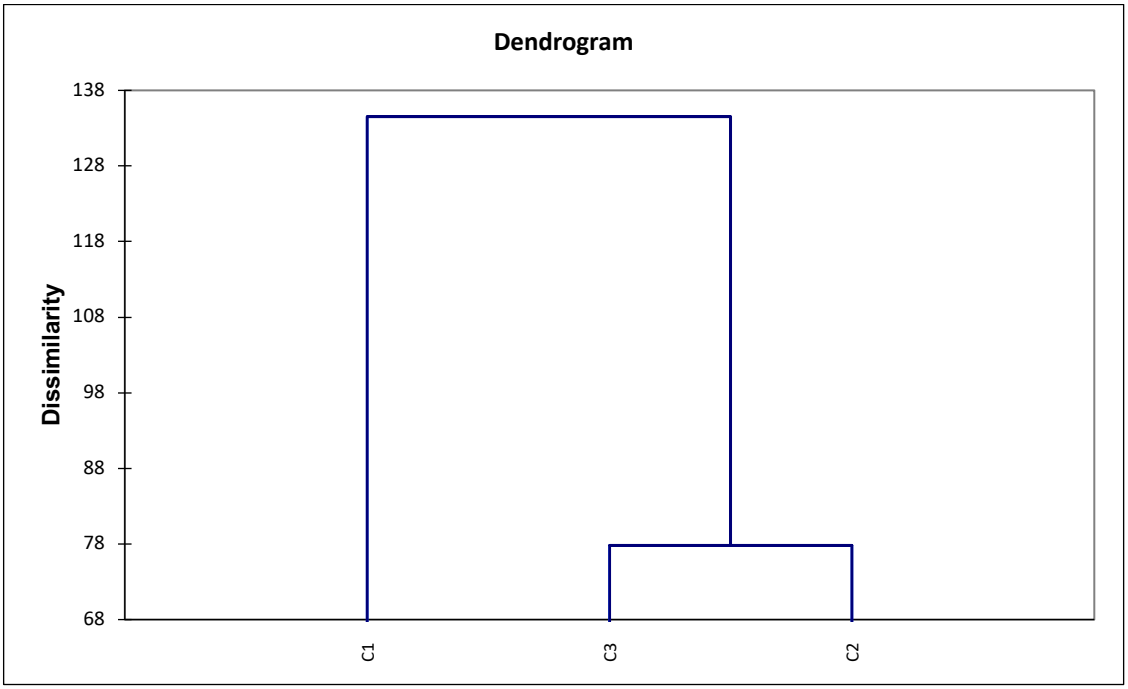


Figure 5.10: Dendrogram from cluster analysis of wine liking scores (agglomerative hierarchical clustering: dissimilarity proximity, Ward's method, Euclidean distance).

Table 5.5: Characteristics of Wine Liking Segments.

	Group One (n=73) "everything likers"	Group Two (n=50) "CN1 likers"	Group Three (n=30) "CN1 dislikers"
Average Liking Scores[a]			
Botrytis Control (0%)	7.2	4.7	7.3
Botrytis Trial (10%)	7.2	4.9	6.8
CN1 Yeast Trial	7.3	6.8	3.5
Sex[b] (NS)			
Male (n=72)	48%	46%	47%
Female (n=81)	52%	54%	53%
Age (years)[c]			
19-35 (n=59)	30%	52%	36%
36-45 (n=45)	30%	36%	17%
46-80 (n=49)	40%	12%	47%
Frequency of Consumption[d] (NS)			
Infrequent to Moderate (n=79)	45%	60%	53%
Frequent (n=74)	55%	40%	47%
Self-Rated Wine Expertise[e]			
Low (n=43)	19%	40%	30%
Medium to High (n=110)	81%	60%	70%
Wine Involvement[f] (NS)			
Less Frequent (n=97)	58%	68%	70%
More Frequent (n= 56)	42%	32%	30%

[a]ANOVA = All 3 clusters differ significantly in liking scores (Fisher's LSD), P=<0.0001

[b]Chi-Squared = 0.047, df=2, P=0.997, no significant difference in sex

[c]Chi-Squared 15.741=df= 4, P=0.002, clusters differ significantly in age

[d]Chi-Squared = 2.664, df=2, P=0.267, no significant difference in frequency of consumption

[e]Chi-Squared = 6.434, df=2, P=0.0.40, clusters differ significantly in self-rated wine expertise

[f]Chi-Squared = 2.102, df=2, P=0.350, no significant difference in wine involvement

5.4 Discussion

This study aimed to investigate the impact of the pathogenic fungus *B. cinerea* at a controlled rate (10%) on dry red wine made from partially dehydrated grapes. By controlling the amount of *B. cinerea* infected grapes in the fermentation, it was hypothesized that the chemical and sensory profile of the wines would differ, perhaps in many descriptors, which could result in a wine that is more complex than one without infected berries. The formation of the desirable form of *B. cinerea*, is favoured during drying based on the conditions present in the chamber (namely drying breezes), and the stand-up fans placed strategically in the drying barn may have provided such conditions. When wine grapes are infected with *B. cinerea*, the hyphae of the mould cause microscopic injuries on the surface of berries, causing dehydration (Thakur, 2018). In the case of this study, berries were already undergoing a dehydrative process while drying in the barn, so the infected grapes were further withered. Grapes impacted by *B. cinerea* undergo significant physiological changes and concurrent dehydration which results in must that is high in sugar, acid and glycerol (Paronetto and Dellaglio, 2011), which may result in sensorial changes. The impact of botrytized grapes on sweet white wines has been well-established (Sarrazin et al., 2007; Campo et al., 2008; Genovese et al., 2007; Bailley at al., 2009). Studies that have examined the impact of controlled *B. cinerea* infection of dry red wines made from partially dehydrated grapes have focused only on Italian Amarone (Fedrizzi et al., 2011b; Tosi et al., 2012), and further evaluation of the impact of *B. cinerea* on similar wines produced in Ontario, Canada is yet to be elucidated.

5.4.1 Fermentation Kinetics:

There were no differences between treatments during fermentation. Sugar was consumed at the same rate. Although residual sugar levels were higher for *Bot*10% wines, dryness was still

achieved (<5 g/L residual sugar). Fermentation of botrytized wines can be difficult, as challenging conditions such as high starting sugar content and the presence of atypical microbial communities on the botrytized grapes (Magyar and Soós, 2016). Thus, selected starter cultures are recommended (Magyar and Soós, 2016), and were utilized in this study. There was no evidence of a stuck fermentation at any time.

5.4.2 Wine and Must

The outcome of the physio-chemical characteristics and metabolite analysis of the must and wine was expected, as markers for *B. cinerea* are previously identified in literature (Magyar and Soós, 2016), namely high glycerol and gluconic acid concentrations (produced by oxidation of the aldehyde function of glucose) (Ribéreau- Gayon et al., 2006) were reported. The relative concentrations and ratios of these compounds are indicative of the growth phase of the infection, as well as whether *B. cinerea* develops as grey mould or noble rot (Aleu and Collado, 2001). Interestingly, Amarone musts from botrytized grapes have a concentration of gluconic acid between 1.0 and 5.0 g/L (Azzolini et al., 2013), values much higher than reported in this current study. This is dependent on many factors, the most obvious being the duration of grape drying, as the percentage of fruit showing infection usually increases in relation to the duration of storage (Jackson, 2008). The shorter drying times practiced in this study are likely responsible for this difference.

The difference in tartaric acid concentrations can be attributed to the degradation of main organic acids from *B. cinerea* infection (Allonzini et al., 2013). There were no differences in the malic acid concentration in the must or wine.

The results of the primary amino nitrogen values are in conflict with current literature, as low concentration of yeast-assimilable nitrogen is often associated with *B. cinerea* infection (Magyar and Soós, 2016). Primary amino nitrogen levels were higher in *Bot*10% wines. However, this study agrees with other findings of elevated levels of acetic acid that are correlated with the presence of *B. cinerea* infection (Ky et al., 2012), as well as in wines made from partially dehydrated grapes (Kelly et al., 2018). Further, changes in volatile acidity may be related to metabolic activity of undesirable bacteria and yeasts that have gained access to damaged, infected berries (Zapparoli et al., 2018). The acetic acid concentrations in the must between treatments, however, were not different.

5.4.3 VOC and VFA Analysis

Although there were significant differences in some of the VOCs measured in control and *Bot*10% wines, the concentrations were relatively close. All VOCs and VFAs measured above reported thresholds except for hexanol (both control and *Bot*10%) and ethyl octanoate (only *Bot*10%).

In relevant literature, the compounds associated with the presence of *B. cinerea* are 1-octen-3-ol, described as a mushroom-like aroma with a low sensory threshold (LeGuerche et al., 2006), benzaldehyde (Genovese et al., 2007; Azzolini et al 2013; Tosi et al, 2012), lactones and sotolon [4,5-dimethyl-3-hydroxy-2(5H)-furanone], a honey-like aroma (Teissedre and Donèche 2013). Indeed, the panelists from this study's descriptive analysis reported a perceived mushroom aroma, but the associated compound was not measured in this study. In agreement with Tosi et al., (2012), where aroma compounds of healthy and botrytized Amarone wines (50% botrytized: 50% healthy in the fermentation) were compared, there is a reduction of compounds arising

from yeast metabolism; ethyl esters and isoamyl acetate in the wines that are affected by *B. cinerea*. Differences in ester components between botrytized and healthy wines are likely due to higher esterase activity or the depletion of nitrogen in botrytized wines (Negri et al., 2017; Teissedre and Donèche 2013), which give wines a fruit flavour and aroma. This is an example of fungal metabolism destroying aromatic compounds. A study (Fedrizzi et al., 2011b) that examined that impact of Amarone wine fermented with 20% and 40% *B. cinerea* infected grapes reported significant changes amongst the healthy and the botrytized fermentations, specifically noting decreases in fatty acids and increases in fruity acetates (such as isoamyl and 2-phenyl acetate) in botrytized wines. Some compounds (ethyl phenylacetate) increased depending on the percentage of infected grapes, as well.

Other compounds associated with the presence of *B. cinerea* are as follows: ethyl phenylacetate (honey), methionol (cooked potato), 4-terpineol (balsamic earthy note), 3-keto-alpha-ionol (hints of tobacco) (Azzolini et al., 2013). Although these compounds were not measured, they may have contributed to the aromatic and flavour profile of the wines in this study.

Interestingly, wines in this study made from partially dehydrated berries were somewhat differentiated by fruit-smelling compounds (esters). These differences, however, were not nuanced enough to modulate the flavour of the wines. The similarities in the concentrations of volatiles supports the results of the descriptive analysis. These wines were not sensorially differentiated, and their volatile constituents are relatively close in concentration.

5.4.4 Descriptive Analysis:

Despite chemical and volatile compound differences in the must and wines, there was limited evidence that the inclusion of 10% *B. cinerea* infected grapes made an organoleptic impact on the wines. Only dried red fruit aroma was a significant attribute between the two wines. Further, the PCA biplot shows separation amongst winemaking replicates; even though the ANOVA determined that there were no significant differences between the winemaking replicates. In this model 58.35% of the variation is explained, and there is no logical relationship in the positioning of the winemaking replicates. Perhaps the 10% *B. cinerea* inclusion was not robust enough to differentiate the wines, while a higher percentage may have resulted in a difference in more attributes. It is also possible that the abilities of the individual assessors to discriminate between the products was not sensitive enough (Kemp et al., 2009), given the similarities in the results. However, panel performance can be assessed. Two desirable qualities in a trained panelist are: repeatability, the ability to score the same product consistently for a given attribute and reproducibility, the ability to score products the same, on average, as the other panel members (Rossi, 2001). The descriptive analysis was conducted in duplicate and therefore data from tasting session one and tasting session two (panelist*tasting replication interaction) can provide evidence for repeatability. Examples of repeatability are in appendix (*Figure A5.17*A-C,E), and this measure of performance tends to vary with attribute. When rating the only significant attribute, dried red fruit aroma, panelists demonstrate good repeatability as scores are relatively close between tasting replicates. Reproducibility can also be assessed by relating the scores of each individual judge to the average, represented as a black line across the figure. Medicinal flavour is an attribute that significantly differed in ratings between tasting

reps (HSD, p<0.05). Some panelists rated medicinal flavour quite differently between tasting replicates, representing poor repeatability. The average is indicated here as well, and most panellists were not rating medicinal flavour the same, on average, as the other panel members. Better performance with regards to reproducibility is illustrated in bitterness ratings (*Figure A5.17*E). Dark chocolate flavour was included to provide an attribute from the flavour category, but also to illustrate how individuals rated the different treatments (*Figure A5.17*D). With respect to training, it is suggested that the impact is an increase in discrimination ability of the individual and of the panel (Labbe et al., 2004). Certainly, the panel could have benefitted from additional training which may have resulted in better discrimination amongst these fairly heterogeneous products. In a study that sensorially examined botrytized Amarone wines (at 29% infection) to healthy wines (Zappoli et al., 2018), there were perceived differences between the wines, with botrytized wines having correlations to attributes such as “muddy”, “sherry-cognac” and “mushroom”. This suggests that an increase in percentage of infected berries may have differentiated the wines in this study. This research therefore provides foundational information for further studies regarding *B. cinerea* infection rates in appassimento-style wines.

It was hypothesized that botrytized dry wines made from partially dehydrated grapes may be more complex. When considering complexity as a marker for quality that is defined as “persistent” and “with many aromas” (Meillon et al., 2010), the results of this study cannot confirm complexity as a result of botrytizing grapes. Although many attributes were generated, it is not possible to differentiate the wines at this level of infection. Complexity however, may be defined by other measures. An attribute that may persist in-mouth after expectorating is the

sensation of bitterness (Sokolowsky and Fischer, 2012). This oral sensation is perceived differently by individuals and can be influenced by many factors including gender and age, for example, and has been indicated as a driver for liking (Pickering et al., 2010). Control and *Bot*10% wines were both rated high in bitterness (average intensity rating score of 8.5 for both), as well as length of finish (average intensity rate score of 9.2 for control and 9.0 for *Bot*10%) which may suggest that complexity comes from the grape drying, rather than the presence of botrytized grapes. With respect to liking scores, it is prudent to consider the intersection of complexity and preference. Köster and J. Mojet (2007) suggested that complexity and hedonic liking are not related linearly. Thus, the level of complexity and product liking are positively correlated until an optimal level (unique to everyone) is reached, after which it decreases (Meillon et al., 2010). Considering that bitterness can illicit negative responses in tasting participants and impact liking (Lesschaeve and Noble, 2005) and that bitterness is experienced differently by individuals (Pickering et al., 2010), perhaps the bitterness in the wines contributed to liking differences within the clusters.

Although descriptive analysis is a highly valuable tool, when applied alone it provides no information regarding whether a particular wine is more appealing than another. In some cases, an inference can be drawn that a particular attribute is undesirable and that a wine with a higher rating for this attribute is likely to be less appreciated, but this is not a strong basis for decision making (Lim, 2011). Thus, consumer preference was included in order to support the descriptive analysis and make further conclusions about the wines.

5.4.6 Consumer Preference Report:

The results of the consumer preference study indicate no global difference in hedonic liking scores amongst the three wines tasted. All wines were rated above six, an indication of consumer acceptability (Moskowitz and Sidel, 1971). The nine-point hedonic scale is accepted as a simple and effective measuring tool to predict consumer acceptance (Lim, 2011), as well as an indication of inferred preference (Wichchukit and O'Mahoney, 2015). Thus, the inclusion of *B. cinerea* affected grapes at 10% did not impact liking, nor did the use of different yeasts (EC1118 and CN1) for wines made from partially dehydrated grapes. Through clustering analysis, participants were segmented into groups based on liking, offering more insight into the patterns within the liking scores.

Interestingly, the liking scores within the clusters are driven more by yeast differences rather than the inclusion of *B. cinerea*. There is currently no available literature that clusters liking scores for appassimento-style wines, and this research will contribute this to the body of knowledge. Of all the factors contributing to differences amongst clusters, age was significant. This has been observed across literature (King et al., 2012; Bruwer et al., 2011; Thach and Olsen, 2006), where age has contributed to preference and consumption of certain wine styles. It should be noted that generational cohorts are one of the least understood marketing dynamics (Bruwer et al., 2011) and that the intersection of other factors are important to understanding consumer behaviour. For example, since this is such a broad category that relies on the shared experiences of a group of people, considerations such as geographic, demographic, psychological and behavioural variables within the broad stroke of generations are of great importance for effective marketing strategy implementation (Howell, 2012). It was

the youngest group in this study that preferred the wine made with CN1 yeast. This wine is significantly different in aroma and flavour from wine made with EC1118 (data chapter four). Thach and Olsen (2006) report the driving factor for wine consumption for millennials (born between 1977 and 2000) is taste. Perhaps the yeast-derived differences that promote fruit flavour, for example, align with purchasing behaviour and preferences of this age group.

The other factor that generally associates strongly with liking is self-rated wine expertise. Participants with "medium to high" wine knowledge total 72% of the participants. Wine knowledge has been implicated as superior to frequency of consumption, age or gender when explaining wine choice (Lockshin et al. 2006), and positions consumers with greater ability and acuity to differentiate attributes in the wine (Francis and Williamson, 2015), as highly knowledgeable consumers focus more on the intrinsic sensory properties of wine rather than external cues (King et al., 2012). Self-rating, though, can potentially be inflated. For example, when asked to self-rate wine expertise, those with high and mid-level knowledge accurately rate themselves, but those with the least wine knowledge tend to rate themselves across a range of expertise level (Corkindale & Welsh, 2005). This suggests that self-rating is not an accurate method to collect wine knowledge data. The cluster with the largest proportion of "medium to high" wine knowledge participants is cluster one, "everything likers". This cluster also contained the highest proportion of participants in the "more frequent" wine involvement category. Those with higher levels of expertise may be more likely to be involved with wine of various styles. Perhaps those who rate themselves as having above average wine knowledge are simply wine enjoyers. Wine knowledge may drive which wine styles are preferred, as novices and experts tend to differ in terms of purchasing behaviour (Ballester et al., 2008). It is

possible that those with high knowledge and involvement like the atypical characteristics of appassimento-style based on previous experiences and well-defined previous ratings of such wines (Ballester et al., 2008).

Participants were asked to self-rate their involvement with appassimento/Amarone wine to understand their perceived relevance to this particular wine style (Lockshin and Spawton, 2001). 37% of participants reported “more frequent” involvement, but this had no impact on liking scores, both in the global data nor amongst the clusters. Purchasing behaviour and wine consumption can be predicted by certain identifiers such as wine expertise, sensory ability and wine knowledge, along with wine involvement (Pickering et al., 2014; Cox, 2009). Wine involvement is a motivational and goal-directed emotional state that drives purchasing decisions (Lockshin et al., 2006) and may be expressed by a wide range of behaviours described in a statement below:

You love to go to wine-tastings and your friends, like you, all have well-stocked wine cellars. You spend a lot of free time reading magazines for wine-aficionados and increasing your already extensive collection. Your fondest memory of a vacation is that wine-tasting tour that you and your spouse took of France, Italy and Spain, six years ago. You would rather talk about wine vintages and comparative strengths and weaknesses of French and US wines than any other subject in the world. (Dholakia, 2001).

Wine involvement was not a differentiating factor in the clustering of participants based on liking scores. This was an excepted result due to the nature of appassimento-style wine, as it is unique, a value-add product (generally more expensive) and high-involvement consumers tend to use key attributes like price to drive their wine choice behaviour (Lockshin et al., 2006).

5.5 Conclusions:

The inclusion of grapes infected with 10% *B. cinerea*, a naturally occurring pathogenic grape fungus, had minimal impact on dry wines fermented with partially dehydrated grapes. Analysis of chemical parameters, volatile constituents, sensory attributes and consumer preference yielded little differences between the wines. Cabernet franc grapes were successfully dried to 28.0°Brix in the drying chamber, and *B. cinerea* was visually identified after assaying the grapes to confirm its presence, which provided visual cues for sorting.

Chemical parameters such as gluconic acid, glycerol and acetic acid are higher in botrytized must and wine and titratable acidity is higher in control wine, but all other metabolites were not statistically different. Most volatile compound concentrations were reported above sensorial threshold values, but the differences between the two wine treatments were generally negligible. The correlation to sensorial differences is inconclusive, as the wines were not differentiated sensorially, except for one attribute, dried red fruit. A consumer preference analysis revealed that the wines from *B. cinerea* trial and CN1 yeast trial were preferred equally by the participants. When consumers were segmented into clusters based on their liking scores for these wines, it was the different yeasts used for primary fermentation, rather than the presence of *B. cinerea*, that defined the clusters. Sex and self-rated wine expertise were significant factors that associated with liking scores within each cluster, but all other demographics were not significant.

This study provides valuable wine quality information of wines made from partially dehydrated grapes with the controlled inclusion of *B. cinerea*. Cool climate viticultural grape growing regions that experience climate uncertainty may benefit from this winemaking

technique and understanding the impact on *B. cinerea* on the wine composition can assist in the optimization of this winemaking style. This research can inform post-harvest winemaking decisions, such as yeast choice and sorting assessments for the production of dry wine from grapes that have been dehydrated. Further, this is the first time that wines produced with the locally isolated *S. bayanus* yeast, CN1, have been compared to the commercial *S. cerevisiae* EC1118 through the lens of consumer preference. Similar global liking scores suggests that CN1 is preferred just as much as the widely-used EC1118 and may be a candidate for commercialization, however it should be noted that when consumers are clustered, one of the clusters (with the oldest age demographic) was defined by the lowest liking ratings of CN1.

Consideration of these results may inform future studies that perhaps include higher percentages of *B. cinerea* infected grapes included in wine fermentation to further investigate the potential impact on this wine style.

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5.7 Appendix

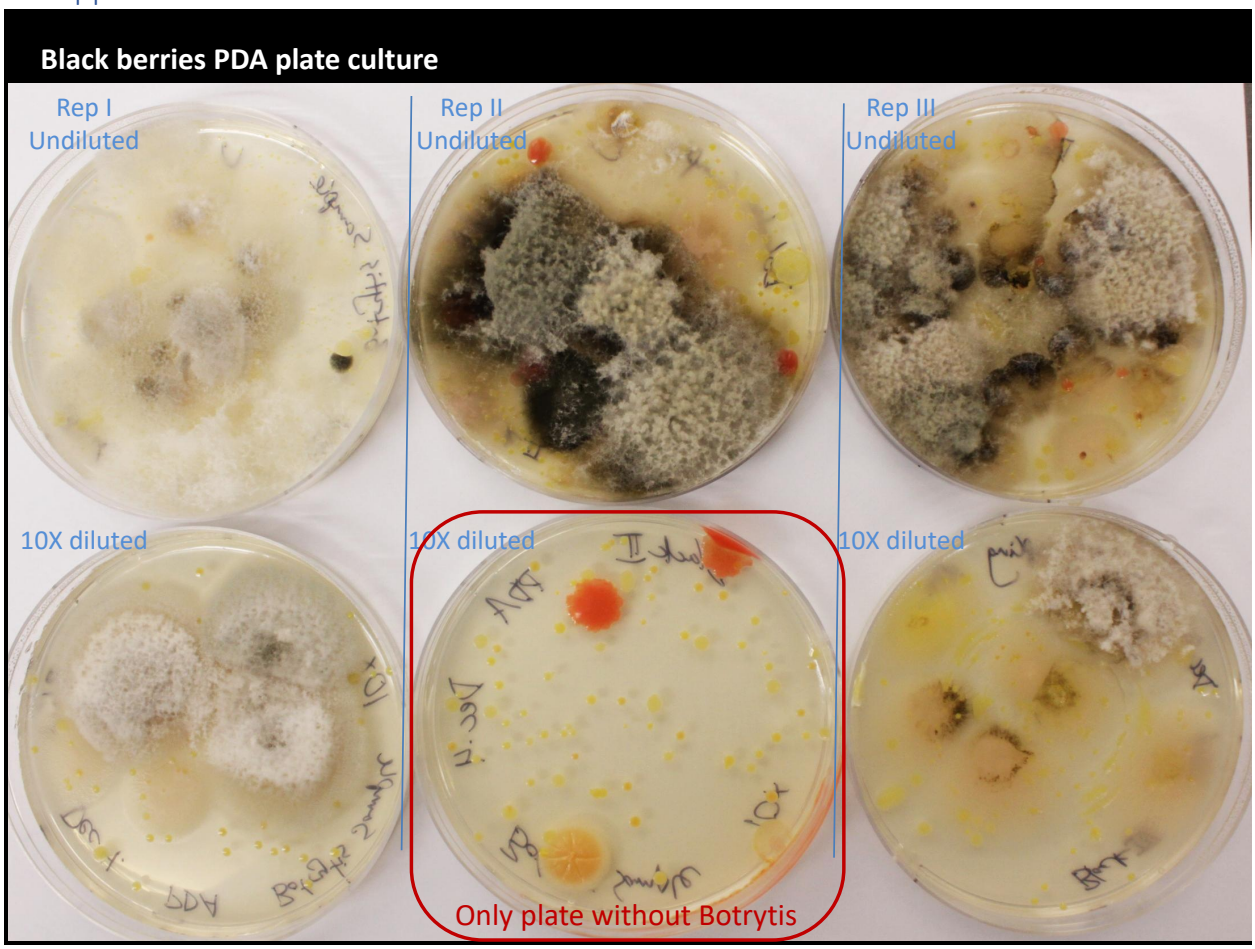


Figure A5.11: Black berry cultures on PDA plate, undiluted and 10x diluted, each in triplicate.

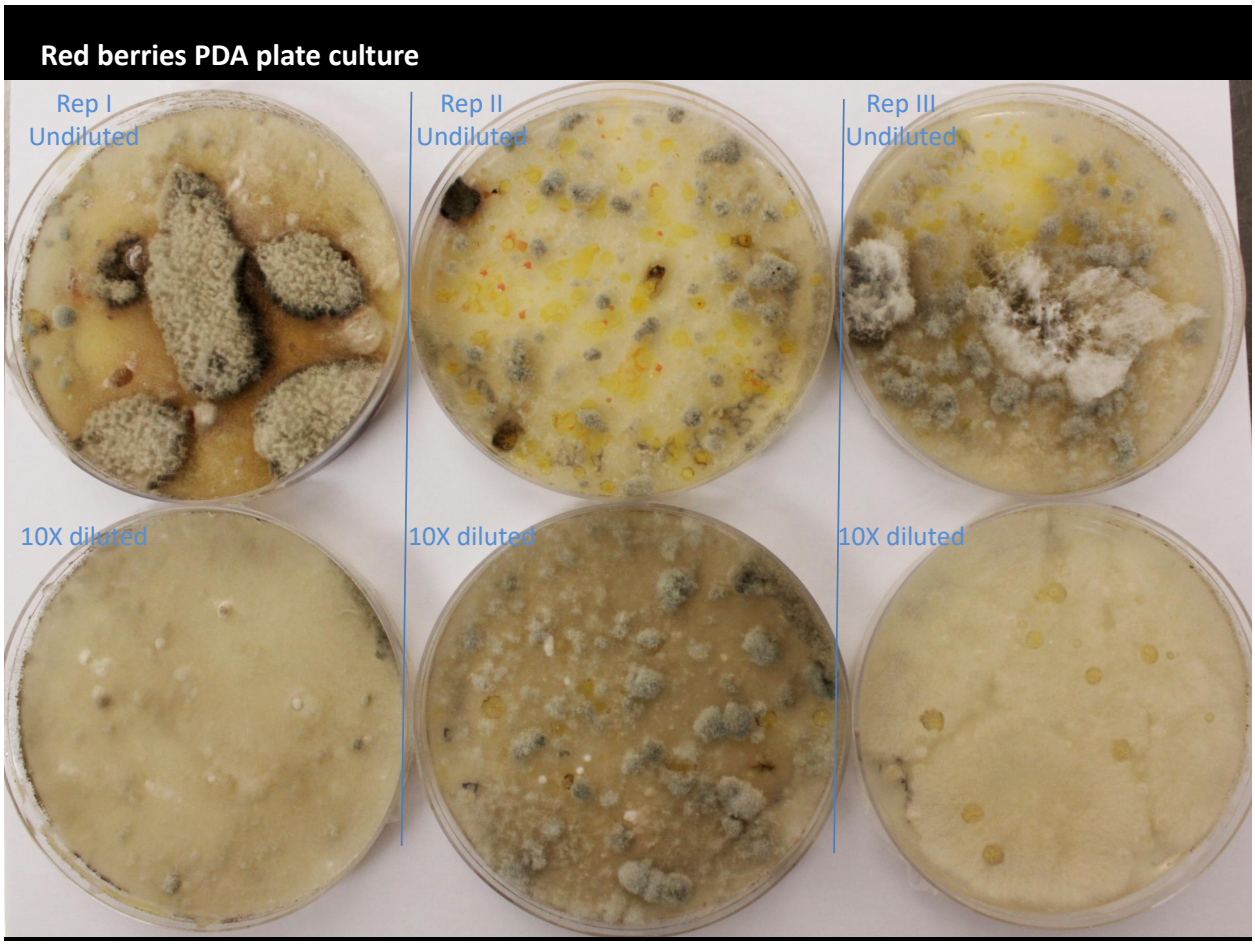


Figure A5.12: Red berry cultures on PDA plate, undiluted and 10x diluted, each in triplicate.

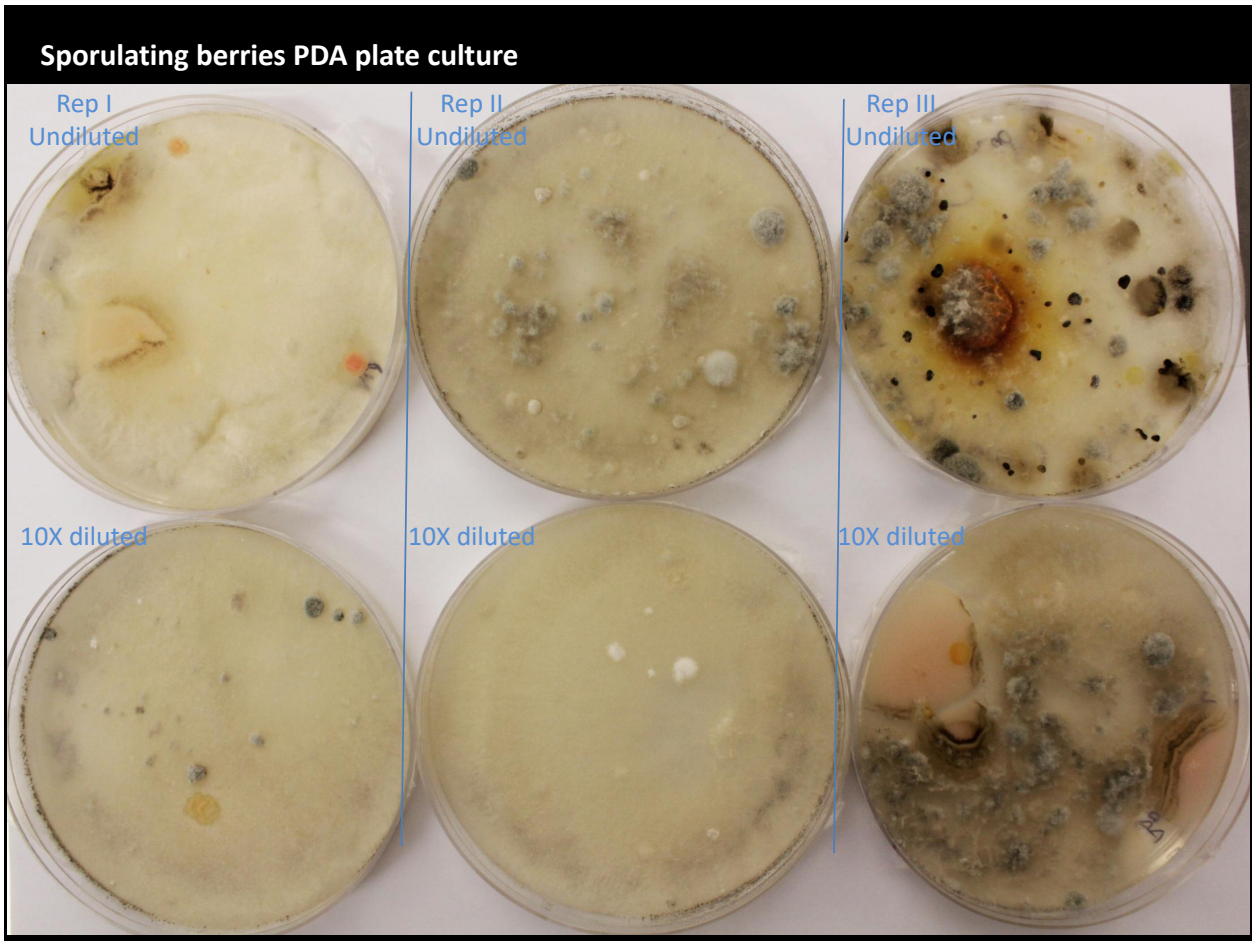


Figure A5.13: Sporulating berry cultures on PDA plate, undiluted and 10x diluted.

Table A5.6: Initial and post-incubation must values tested in Cabernet franc berries of three experimental categories.

Parameter/Metabolite	Berries @ Initial	Berries @ Post-Incubation	Category
Soluble solids (°Brix)	27.9	20.9	Black (healthy)
	31.3	17.2	Red (internal infection)
	34.2	22.8	Sporulating
Glycerol (g/L)	0.1	3.3	Black (healthy)
	9.3	12.1	Red (internal infection)
	11.1	14.3	Sporulating

*Table A5.7: Control, 2-way ANOVA [Factors: Tasting replicate, winemaking replicate and tasting replicate*winemaking replicate interaction] (p<0.05).*

Attribute		Tasting Replicate	Winemaking Replicate	Tasting Replicate * Winemaking Replicate
Dried Red Fruit AROMA	F-Value	0.415	1.422	0.792
	p-Value	0.521	0.248	0.457
Black Fruit AROMA	F-Value	0.062	0.061	0.432
	p-Value	0.804	0.940	0.651
Vegetal AROMA	F-Value	1.733	0.392	0.292
	p-Value	0.192	0.677	0.748
Coffee AROMA	F-Value	0.057	0.188	0.216
	p-Value	0.811	0.829	0.806
Candied Cola AROMA	F-Value	0.280	0.308	0.333
	p-Value	0.59	0.736	0.718
Medicinal AROMA	F-Value	0.395	0.327	0.604
	p-Value	0.532	0.722	0.549
Mushroom AROMA	F-Value	0.917	0.227	0.456
	p-Value	0.341	0.797	0.635
Spice AROMA	F-Value	1.467	0.388	0.477
	p-Value	0.230	0.680	0.623
Dirty AROMA	F-Value	2.177	0.312	0.187
	p-Value	0.144	0.733	0.830
Dusty AROMA	F-Value	1.271	0.012	0.074
	p-Value	0.263	0.988	0.929

Dried Red Fruit FLAVOUR	F-Value	1.014	0.839	0.217
	p-Value	0.317	0.436	0.805
Black Fruit FLAVOUR	F-Value	0.321	0.027	0.150
	p-Value	0.573	0.973	0.861
Vegetal FLAVOUR	F-Value	0.019	0.051	0.002
	p-Value	0.891	0.950	0.998
Spice FLAVOUR	F-Value	1.129	0.153	0.080
	p-Value	0.292	0.859	0.923
Medicinal FLAVOUR	F-Value	0.989	0.039	0.137
	p-Value	0.323	0.961	0.872
Dark Chocolate FLAVOUR	F-Value	2.282	0.267	0.543
	p-Value	0.135	0.767	0.584
Bitterness	F-Value	0.069	0.161	0.876
	p-Value	0.793	0.852	0.421
Acidity	F-Value	1.579	0.148	0.103
	p-Value	0.213	0.862	0.902
Heat	F-Value	0.381	0.964	0.237
	p-Value	0.539	0.386	0.789
Astringency	F-Value	0.003	0.815	1.297
	p-Value	0.956	0.447	0.280
Length of Finish	F-Value	0.051	0.361	0.008
	p-Value	0.822	0.698	0.992

*Table A5.8: Bot10%, 2-way ANOVA [Factors: Tasting replicate, winemaking replicate and tasting replicate*winemaking replicate interaction] (p<0.05).*

Attribute		Tasting Replicate	Winemaking Replicate	Tasting Replicate
Dried Red Fruit AROMA	F-Value	0.054	0.929	1.665
	p-Value	0.817	0.400	0.196
Black Fruit AROMA	F-Value	0.419	0.320	0.543
	p-Value	0.520	0.727	0.584
Vegetal AROMA	F-Value	0.212	0.364	1.187
	p-Value	0.647	0.696	0.311
Coffee AROMA	F-Value	0.099	0.261	0.498
	p-Value	0.754	0.771	0.610
Candied Cola AROMA	F-Value	0.000	0.462	0.323
	p-Value	0.997	0.632	0.725
Medicinal AROMA	F-Value	0.115	0.165	1.015
	p-Value	0.736	0.848	0.367
Mushroom AROMA	F-Value	0.030	1.320	1.559
	p-Value	0.862	0.273	0.217
Spice AROMA	F-Value	2.648	0.113	0.351
	p-Value	0.108	0.894	0.705
Dirty AROMA	F-Value	2.054	0.208	0.098
	p-Value	0.156	0.813	0.907
Dusty AROMA	F-Value	1.382	1.225	0.122
	p-Value	0.244	0.300	0.885

Dried Red Fruit FLAVOUR	F-Value	0.306	0.582	0.459
	p-Value	0.582	0.561	0.634
Black Fruit FLAVOUR	F-Value	0.005	0.181	0.041
	p-Value	0.943	0.835	0.959
Vegetal FLAVOUR	F-Value	0.114	0.484	1.116
	p-Value	0.737	0.618	0.333
Spice FLAVOUR	F-Value	0.865	0.226	0.180
	p-Value	0.356	0.798	0.836
Medicinal FLAVOUR	F-Value	1.437	0.130	0.169
	p-Value	0.235	0.878	0.844
Dark Chocolate FLAVOUR	F-Value	0.763	0.121	0.115
	p-Value	0.385	0.886	0.891
Bitterness	F-Value	0. 121	1.666	0.181
	p-Value	0.728	0.196	0.835
Acidity	F-Value	0.103	0.402	0.045
	p-Value	0.749	0.670	0.956
Heat	F-Value	0.018	0.393	0.250
	p-Value	0.892	0.677	0.780
Astringency	F-Value	0.175	0.075	0.064
	p-Value	0.677	0.928	0.938
Length of Finish	F-Value	0.161	1.236	0.026
	p-Value	0.689	0.297	0.974

Table A5.9: Output of 3-way ANOVA [Factors=Tasting replicate, Judge, Wine] and interactions amongst factors (p<0.05).

Attribute		Tasting Replicate	Judge	Wine	Tasting Replicate* Judge	Tasting Replicate *Wine	Judge*Wine
Dried Red Fruit AROMA	F-Value	0.096	**8.383**	**6.245**	0.733	0.551	0.888
	p-Value	0.758	< 0.0001	0.014	0.717	0.459	0.561
Black Fruit AROMA	F-Value	1.163	**24.042**	0.953	0.597	0.243	0.568
	p-Value	0.283	< 0.0001	0.331	0.841	0.623	0.864
Vegetal AROMA	F-Value	0.575	**6.445**	0.507	0.245	2.190	0.180
	p-Value	0.450	< 0.0001	0.478	0.995	0.142	0.999
Coffee AROMA	F-Value	0.003	**13.128**	0.495	0.978	0.309	1.097
	p-Value	0.959	< 0.0001	0.483	0.474	0.579	0.369
Candied Cola AROMA	F-Value	0.371	**21.583**	0.000	0.414	0.360	0.625
	p-Value	0.543	< 0.0001	0.989	0.956	0.549	0.818
Medicinal AROMA	F-Value	0.096	**14.801**	0.311	0.868	0.996	0.779
	p-Value	0.757	< 0.0001	0.578	0.581	0.320	0.671
Mushroom AROMA	F-Value	1.616	**18.803**	0.939	0.807	0.817	0.955
	p-Value	0.206	< 0.0001	0.335	0.642	0.368	0.496
Spice AROMA	F-Value	**7.460**	**10.481**	0.220	1.267	0.220	1.057
	p-Value	0.007	< 0.0001	0.640	0.247	0.640	0.403
Dirty AROMA	F-Value	**6.484**	**7.278**	2.585	0.763	0.072	1.005
	p-Value	0.012	< 0.0001	0.111	0.687	0.788	0.449
Dusty AROMA	F-Value	**5.090**	**12.191**	0.244	1.060	0.007	0.577
	p-Value	0.026	< 0.0001	0.622	0.400	0.936	0.857

Dried Red Fruit FLAVOUR	F-Value	0.225	**18.367**	0.468	0.935	2.955	1.307
	p-Value	0.636	< 0.0001	0.495	0.515	0.088	0.224
Black Fruit FLAVOUR	F-Value	0.503	**38.357**	0.048	0.785	0.841	1.456
	p-Value	0.479	< 0.0001	0.826	0.665	0.361	0.151
Vegetal FLAVOUR	F-Value	0.210	**12.493**	0.134	0.763	0.030	0.887
	p-Value	0.648	< 0.0001	0.715	0.687	0.863	0.562
Spice FLAVOUR	F-Value	**4.932**	**16.071**	1.330	**2.559**	0.026	1.769
	p-Value	0.028	< 0.0001	0.251	**0.005**	0.873	0.061
Medicinal FLAVOUR	F-Value	**9.976**	**37.453**	0.652	1.580	0.071	1.496
	p-Value	0.002	< 0.0001	0.421	0.107	0.790	0.135
Dark Chocolate FLAVOUR	F-Value	0.054	**10.611**	1.146	0.455	**4.638**	1.517
	p-Value	0.816	< 0.0001	0.287	0.936	**0.033**	0.128
Bitterness	F-Value	0.005	**8.994**	0.001	0.545	0.294	0.510
	p-Value	0.945	< 0.0001	0.975	0.881	0.589	0.904
Acidity	F-Value	2.959	**17.797**	0.139	0.948	1.022	0.684
	p-Value	0.088	< 0.0001	0.710	0.502	0.314	0.764
Heat	F-Value	0.376	**20.849**	0.021	1.733	0.815	0.319
	p-Value	0.541	< 0.0001	0.885	0.068	0.369	0.985
Astringency	F-Value	0.133	**15.457**	0.426	1.134	0.233	0.605
	p-Value	0.716	< 0.0001	0.515	0.340	0.630	0.834
Length of Finish	F-Value	0.584	**24.065**	0.869	2.118	0.037	1.258
	p-Value	0.446	< 0.0001	0.353	**0.021**	0.847	0.253

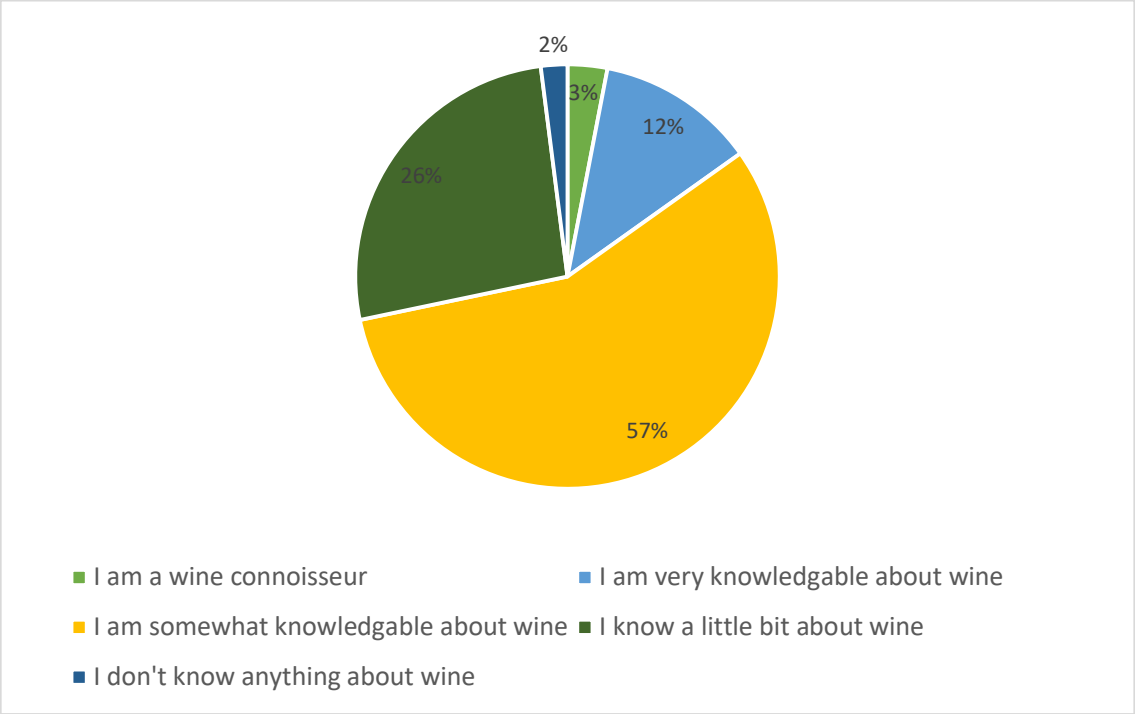


Figure A5.14: Self-rated wine expertise, collected during consumer preference study (n=153).

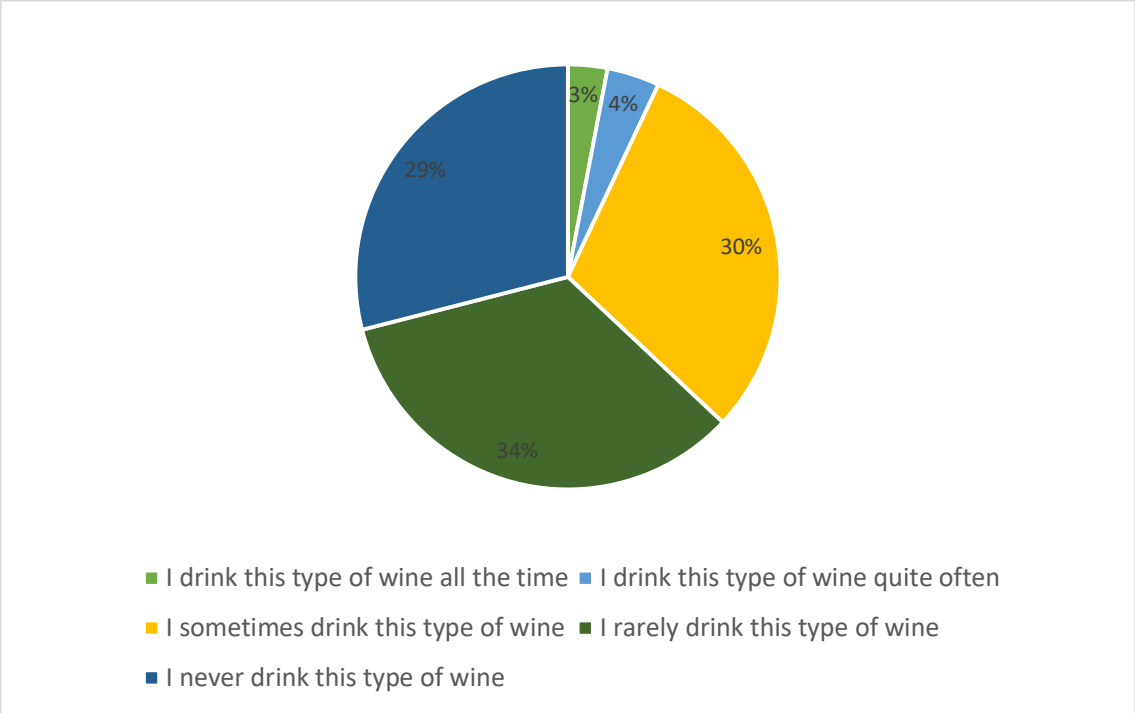
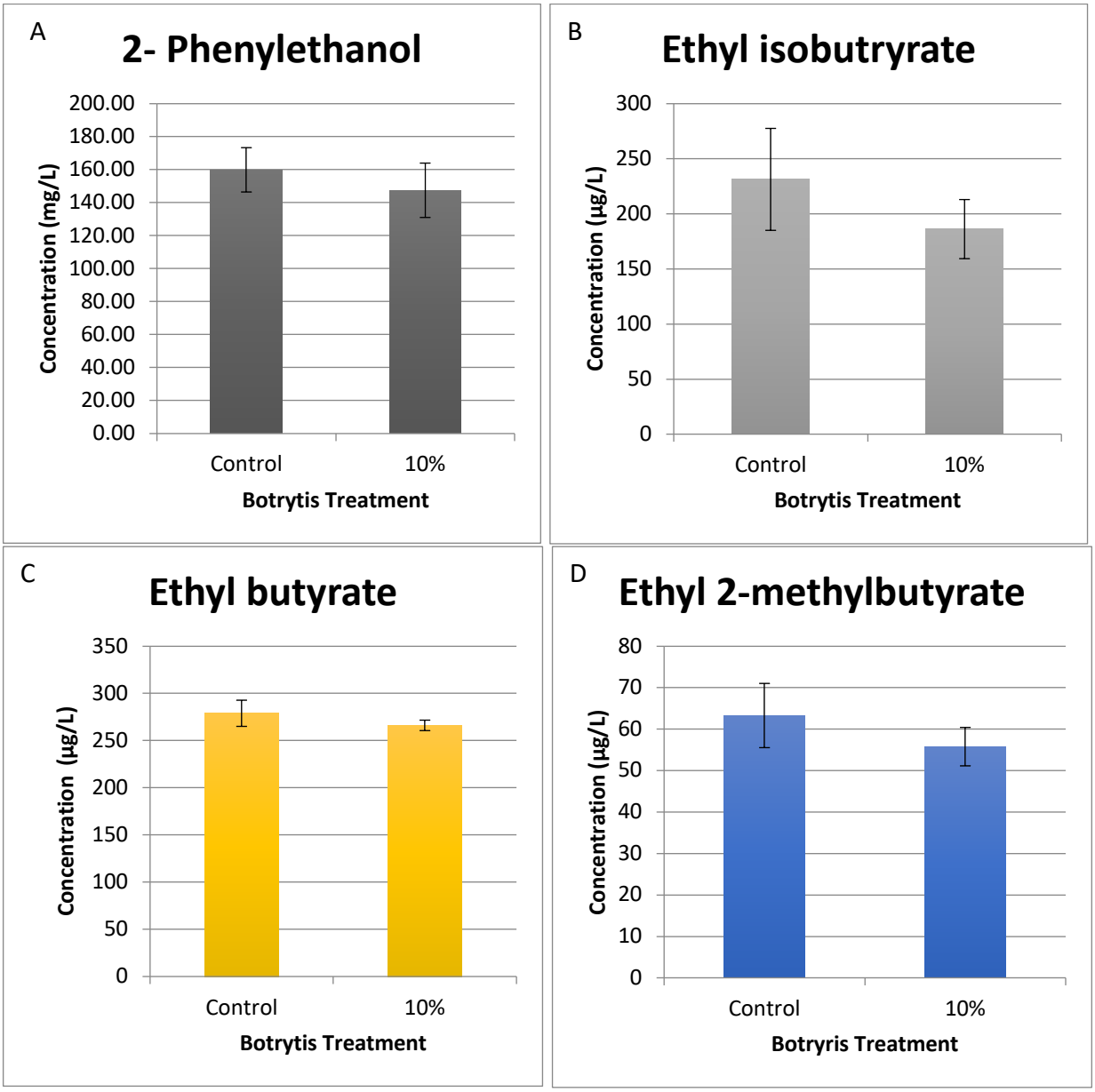
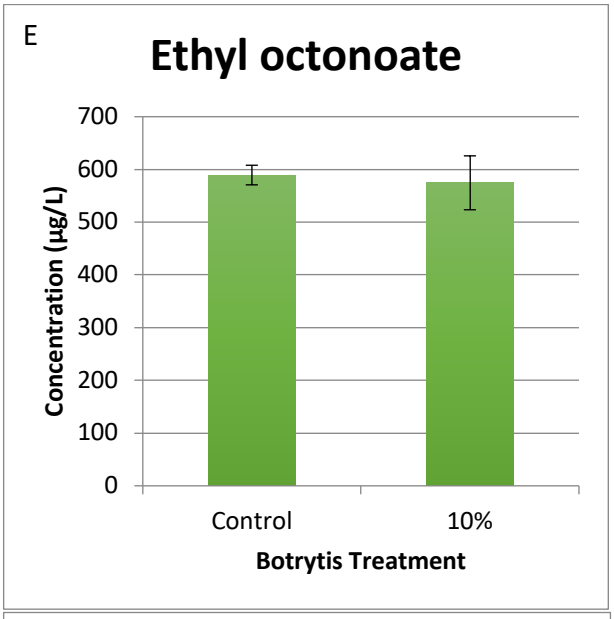
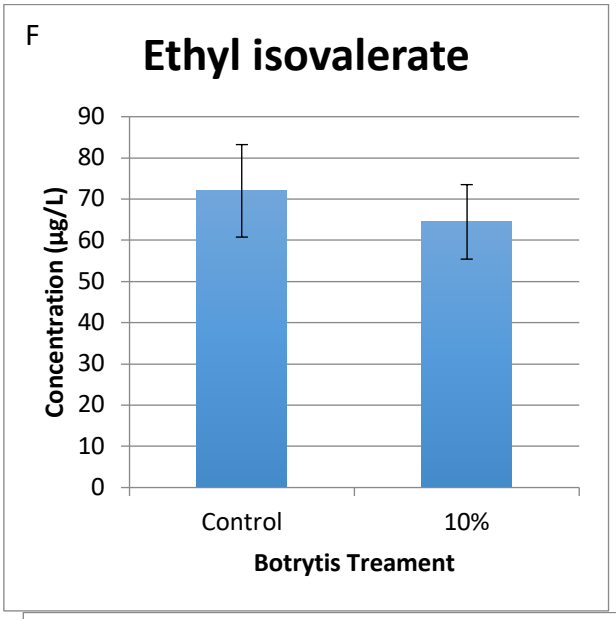
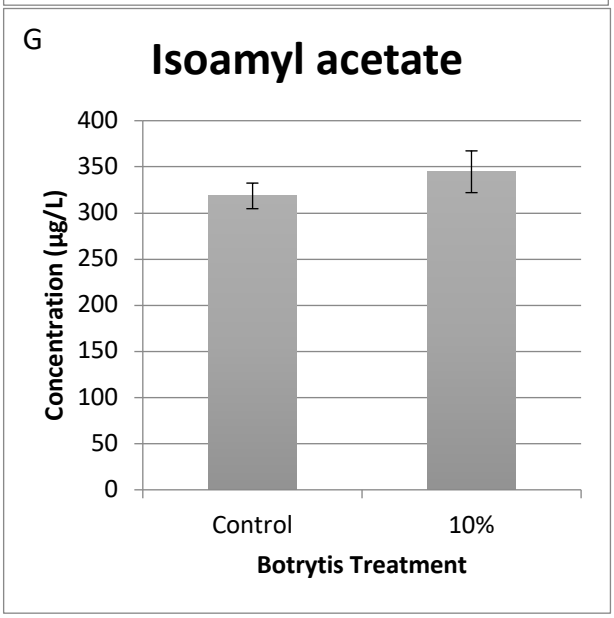


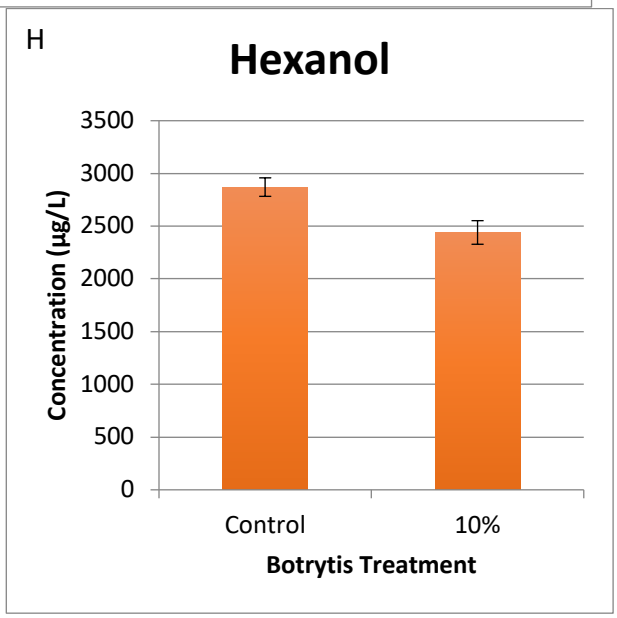
Figure A5.15: Self-rated wine involvement: appassimento/Amarone, collected during consumer preference study (n=153).


A
2- Phenylethanol
Concentration (mg/L)
200.00
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Control
10%
Botrytis Treatment
B
Ethyl isobutryrate
Concentration (µg/L)
300
250
200
150
100
50
0
Control
10%
Botrytis Treatment
C
Ethyl butyrate
Concentration (µg/L)
350
300
250
200
150
100
50
0
Control
10%
Botrytis Treatment
D
Ethyl 2-methylbutyrate
Concentration (µg/L)
80
70
60
50
40
30
20
10
0
Control
10%
Botryris Treatment


E
Ethyl octonoate
Concentration (µg/L)
700
600
500
400
300
200
100
0
Control
10%
Botrytis Treatment


F
Ethyl isovalerate
Concentration (µg/L)
90
80
70
60
50
40
30
20
10
0
Control
10%
Botrytis Treament


G
Isoamyl acetate
Concentration (µg/L)
400
350
300
250
200
150
100
50
0
Control
10%
Botrytis Treatment


H
Hexanol
Concentration (µg/L)
3500
3000
2500
2000
1500
1000
500
0
Control
10%
Botrytis Treatment

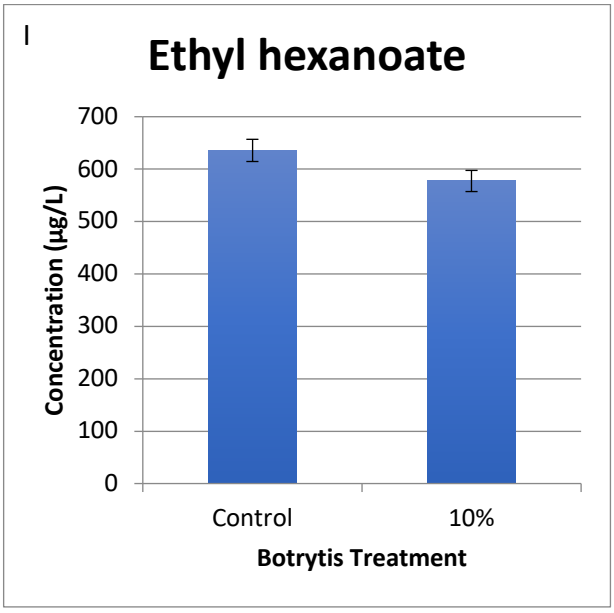
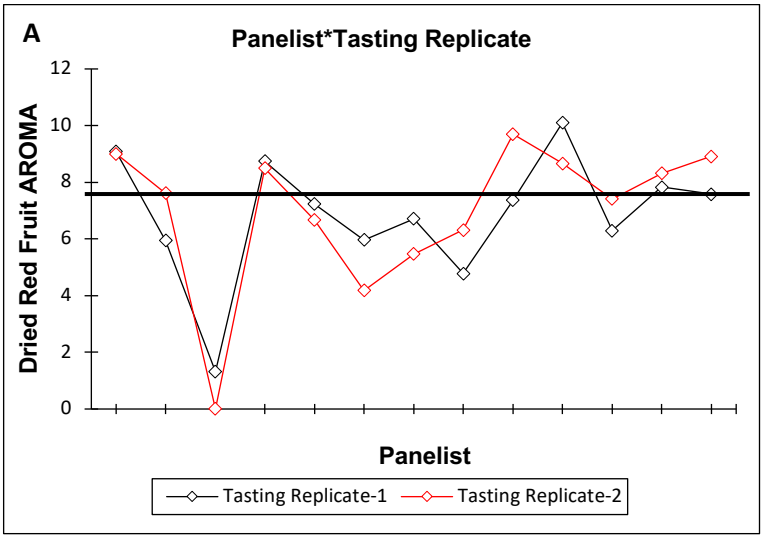


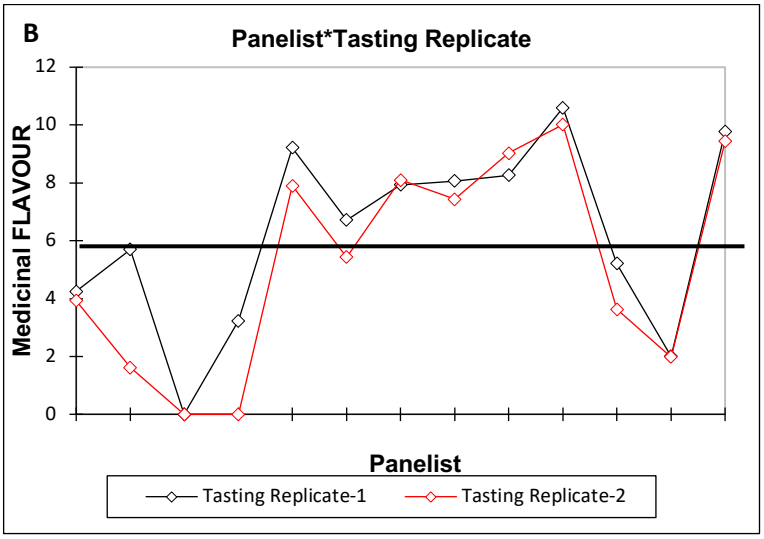
Figure A5.16: Concentrations (µg/L) of 2-phenylethanol (mg/L) (A), ethyl isobutyrate (B), ethyl butyrate (C), ethyl 2-methylbutyrate (D), ethyl octanoate (E), ethyl isovalerate (F), isoamyl acetate (G), hexanol (H), ethyl hexanoate (I) for wines.

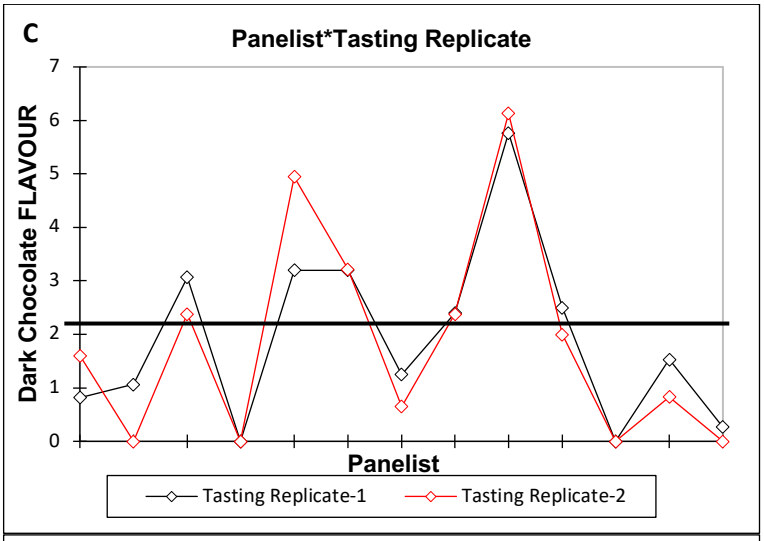
Table A5.10: Means of all intensity ratings of attributes for each wine, including replicates, from descriptive analysis.

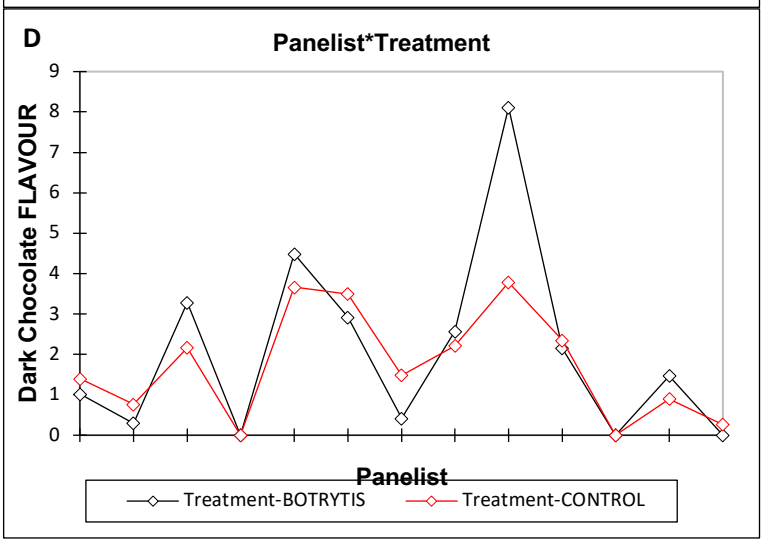
Treatment	Dried Red Fruit	Black Fruit	Vegetal AROMA	Coffee AROMA	Candied Cola AROMA	Medicinal AROMA	Mushroom AROMA	Spice AROMA	Dirty AROMA
Control Rep 1 Average	5.6	4.2	5.4	2.4	1.7	4.7	4.5	5.0	4.1
Control Rep 2 Average	7.0	3.9	4.6	2.1	2.3	5.5	3.7	5.2	3.1
Control Rep 3 Average	6.5	4.2	4.4	2.0	1.9	4.9	4.0	5.7	3.6
CONTROL AVERAGE	**6.4***	**4.1**	**4.8**	**2.2**	**2.0**	**5.0**	**4.1**	**5.3**	**3.6**
*Bot*10% Rep 1 Average	7.3	4.9	4.0	2.2	2.0	5.1	3.9	5.3	3.0
*Bot*10% Rep 2 Average	8.2	4.5	4.3	1.7	2.4	4.6	2.6	5.1	2.9
*Bot*10% Rep 3 Average	6.9	4.0	4.9	1.9	1.6	4.6	4.4	4.9	2.4
***Bot*10% AVERAGE**	**7.5***	**4.5**	**4.4**	**1.9**	**2.0**	**4.8**	**3.6**	**5.1**	**2.8**
	Dusty AROMA	Dried Red Fruit FLAVOUR	Black Fruit FLAVOUR	Vegetal FLAVOUR	Spice FLAVOUR	Medicinal FLAVOUR	Dark Chocolate FLAVOUR	Bitterness	Acidity
Control Rep 1 Average	3.2	6.7	4.4	2.6	5.3	5.7	1.5	8.3	6.3
Control Rep 2 Average	3.3	7.6	4.3	2.8	5.8	6.0	1.7	8.8	6.6
Control Rep 3 Average	3.3	7.9	4.2	2.6	5.4	5.8	2.0	8.4	6.7
CONTROL AVERAGE	**3.3**	**7.4**	**4.3**	**2.7**	**5.5**	**5.9**	**1.7**	**8.5**	**6.5**
*Bot*10% Rep 1 Average	4.0	8.2	4.6	2.6	6.2	5.4	2.1	9.3	6.4
*Bot*10% Rep 2 Average	2.4	7.2	4.1	2.5	5.8	5.9	1.8	7.7	6.4
*Bot*10%	2.8	7.6	4.0	3.2	5.6	5.5	2.2	8.5	7.0

Rep 3 Average									
***Bot*10% AVERAGE**	**3.1**	**7.7**	**4.2**	**2.8**	**5.9**	**5.6**	**2.1**	**8.5**	**6.6**
	Heat	Astringency	Length of Finish						
Control Rep 1 Average	8.6	5.6	8.8						
Control Rep 2 Average	9.9	6.9	9.7						
Control Rep 3 Average	8.9	6.4	9.2						
CONTROL AVERAGE	**9.2**	**6.3**	**9.2**						
*Bot*10% Rep 1 Average	9.5	6.8	9.5						
*Bot*10% Rep 2 Average	8.8	6.5	8.2						
*Bot*10% Rep 3 Average	9.4	6.4	9.2						
***Bot*10% AVERAGE**	**9.2**	**6.6**	**9.0**						




B
Panelist*Tasting Replicate
Medicinal FLAVOUR
12
10
8
6
4
2
0
Panelist
Tasting Replicate-1
Tasting Replicate-2


C
Panelist*Tasting Replicate
Dark Chocolate FLAVOUR
7
6
5
4
3
2
1
0
Panelist
Tasting Replicate-1
Tasting Replicate-2


D
Panelist*Treatment
Dark Chocolate FLAVOUR
9
8
7
6
5
4
3
2
1
0
Panelist
Treatment-BOTRYTIS
Treatment-CONTROL

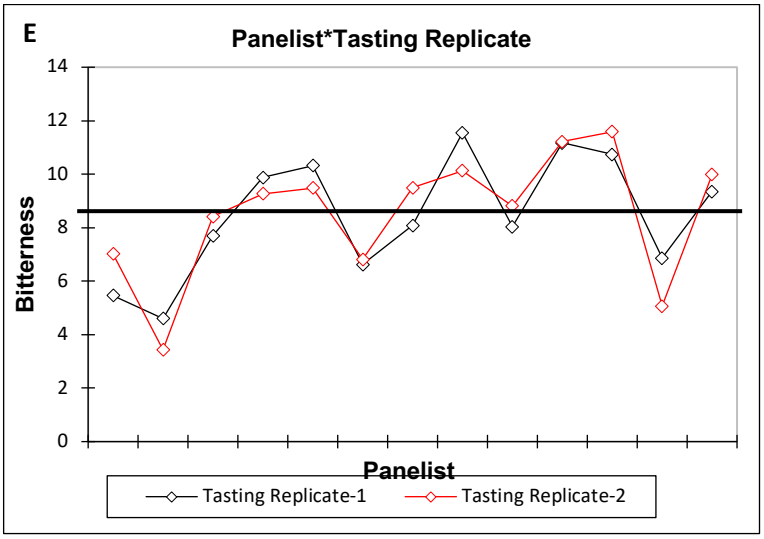


Figure A5.17,A-E: Panel assessment data for repeatability and reproducibility for (a.) dried red fruit aroma (b.) medicinal flavour (c.), (d.) dark chocolate flavour and (e.) bitterness.

Chapter 6 General Discussion and Conclusions

6.1 Introduction

The Ontario wine industry is an important agricultural sector that provides revenue to the economy through tourism, jobs and farming opportunities. However, the stability of the industry is at risk due to a changing climate that impacts grape quality due to extreme weather events. Optimizing viticultural and oenological practices to produce high-quality wines that best suit our cool climate is of critical value in order to adapt to climate change, but also to increase consumer acceptance of cool climate wines and compete in a crowded global marketplace. One practice that is gaining momentum within the Ontario wine industry is the production of appassimento-style wines; this is, wines that are produced from partially dehydrated grapes. This technique is a valuable mitigation tool, as it allows for grapes continue ripening off the vine in spite of regional environmental conditions. It represents a sustainable innovation to the threats associated with extreme weather events that pose a risk to production. Meeting the apparent consumer demand for full-bodied, ripe-flavoured red wine in Ontario could represent a growth opportunity for the Niagara region, even in less than optimal vintage conditions where grapes may not reach optimal maturity before the cold weather arrives. When innovative techniques are used, some of the benefits that are associated with diversifying a wine portfolio include expansion of style ranges and blending opportunities. The drying process, however, can potentially negatively impact the organoleptic profile of resultant wines due to the accumulation of oxidation compounds, as well as the development of off-odours and flavours in high sugar fermentation. Fermentation decisions like yeast selection for primary fermentation can assist in overcoming faults. Thus, understanding the impact of yeast and sugar

concentration is pertinent to optimizing this wine style. The first two chapters of this thesis report findings from two years of winemaking data, where our yeast of interest (*S. bayanus* CN1) is applied to appassimento-style winemaking. It was hypothesized, based on previous research with this yeast, that there would be a reduction in oxidation compounds like acetic acid, acetaldehyde and ethyl acetate. However, it was unknown what starting sugar concentration would be optimal for this yeast strain to ferment wine to dryness. The first winemaking year provided useful preliminary information on this yeast as it applies to this wine style. These considerations were reflected in the second year of winemaking, as parameters were optimized to better suit the style and starting sugar concentration was lowered. It was hypothesized that yeast choice and starting sugar concentration are important variables for the production of metabolites and sensory outcomes. The next chapter further characterized the wines made from partially dehydrated grapes by examining the volatile composition and conducting an in-depth sensorial analysis. It was hypothesized that there would be differences in the abundance of volatiles as a result of different sugar concertation and yeast strain, and that these differences would be detectable sensorially. The last chapter of this thesis outlines the impact of including grapes infected with *Botrytis cinerea* into fermentation of partially dehydrated grapes, a practice that is traditionally employed for Amarone production (Tosi et al., 2012). It was hypothesized that the inclusion *B. cinerea* grapes at 10% infection rate would impact the wines sensorially and chemically. All of these studies and subsequent results contribute to a deeper understanding of regional wines made from partially dehydrated grapes in Ontario, Canada.

6.2 Chapter 2 Characterization of *Saccharomyces bayanus* CN1 for Fermenting Partially Dehydrated Grapes Grown in Cool Climate Winemaking Regions

The main objectives of this data chapter were to (i) identify our yeast of interest, (ii) determine its fitness for making wine from partially dehydrated grapes, and (iii) more fully understand the impact of high sugar fermentation on red wine composition, colour, and sensory quality. The results of this study provided some framework for the utilization of *S. bayanus* CN1 in the niche of appassimento style winemaking. Prior work with this autochthonous yeast for Icewine fermentation yielded promising results, as there was a reduction in acetic acid when compared to a commercially available *S. cerevisiae* strain, K1-V1116. This agrees with existing literature on *S. bayanus* yeast (Eglinton et al., 2002). Although the taxonomy of *S. bayanus* is continually changing due to its nearly identical similarity to *S. uvarum* (Sulo et al., 2017), GenBank sequence comparisons of β-tubulin and *COX*II mitochondrial gene regions identified this yeast as *S. bayanus*. CN1 yielded an incomplete sugar transformation when the starting sugar concentration was 28.0°Brix, an important finding of this study. In agreement with Erasmus et al., (2004), a positive correlation between increased acetic acid production and high starting sugar concentration was indicted, a potentially problematic by-product of fermentation of high sugar wines like Icewine and Amarone. Consideration for this is included in the legislated limits are imposed on these wines. When compared to commercial yeast, not only were the oxidation compounds reduced in CN1 wines, there was an increase in glycerol content in these wines. Glycerol has been indicated as a compatible solute for hyperosmotic stress response in *S. cerevisiae*, accompanied by acetic acid production to maintain redox balance. The concentrations of these compounds provide insight onto this autochthonous yeast's mechanism for responding to osmotic stress. Preliminary sensorial differences were confirmed

amongst wines made with different yeasts and at different starting sugar concentrations. Colour differences were also indicated.

This study lays the groundwork for further investigation of the potential of *S. bayanus* CN1 yeast for winemaking from partially dehydrated grapes in Ontario. Perhaps a range of starting sugar concentrations would be beneficial to indicating the upper fermentation limit of CN1. The preliminary sensorial work was promising, as it specified that wines made from both yeast strains were perceptibly differentiated from each other, which gives rise to more in-depth descriptive analysis.

6.3 Chapter 3 Investigation of *Saccharomyces bayanus* CN1 Yeast Strain for Winemaking from Partially Dehydrated Grapes in Cool Climate Viticultural Areas

The aim of this study is to further define the parameters under which this yeast is best suited to appassimento style winemaking by i) assessing optimal processing conditions by fermenting partially dehydrated grapes at varying starting sugar concentrations and different yeast strains, and ii) assessing them chemically. In order to examine this, local Cabernet franc grapes were dehydrated to three target starting sugar concentrations: 24.5°Brix, 26.0°Brix and 27.5°Brix and compared to a control, processed immediately after picking (21.5°Brix). These grapes were vinified with *S. bayanus* CN1 or a commercial strain, *S. cerevisiae* EC1118. Fermentation kinetics at each starting sugar concentration were the same, except for the high sugar CN1 fermentation (27.5°Brix), which took an additional three days. As expected, acetic acid, ethyl acetate and acetaldehyde concentration were lower in CN1 wines, while glycerol was higher. Ethanol was the same for all ferments between yeast strains except 24.5°Brix. All wines

fermented to dryness, suggesting that 27.5°Brix is the upper sugar limit for CN1, as 28.0°Brix wines contained higher concentration of residual sugar, too high for this typically dry wine. This was the first time this yeast has been applied to appassimento winemaking over a range of four starting sugar concentrations, as well as the first time the upper limit of this yeast has been defined. While it is expected that grape drying can result in an elevated concentration of some compounds (Bellincontro et al., 2016), and that CN1 can assist managing the potentially problematic compounds (Kelly et al., 2018), further characterization of this yeast and wine style is required. Given the positive results yielded in this study, and the fitness for this wine style, the next step was to examine how the wines were different and describe their organoleptic profile through descriptive analysis. Further, measuring the abundance of volatiles that contribute to the profile will provide a deeper understanding of this local yeast's impact on wines made from partially dehydrated grapes.

6.4 Chapter 4 Sensorial and Volatile Analysis of Wines Made from Partially Dehydrated Grapes: An Ontario Case Study

During drying, wine grapes concentrate sugars. Flavour and aroma compounds are also concentrated as a consequence of the drying process; some favourable, some undesirable. In Ontario, Canada, the use of postharvest grape withering for wine production may assist in mitigating challenges associated with climate change, as grapes are dried after they are picked in a protected environment. Little is known about the sensorial and chemical profile of Ontario wines made in this style, and this study aimed to contribute to this. Further, the utilization of an indigenous yeast that has yet to be characterized sensorially has been included in this study. Cabernet franc wines that were fermented with two yeast strains (EC1118 and CN1) over a

range of starting sugar concentrations were analyzed sensorially, and volatile organic compounds (VOCs) and (VFAs) were measured. This study aims to i) assess the impact of yeast strain and ii) starting sugar concentration on the aroma and flavour profile of wines made from partially dehydrated grapes. While it is accepted that variation in the concentrations of aroma and flavour compounds can be classified by their drying time (López de Lerma et al., 2012), the impact of CN1 yeast at different starting sugar concentrations remained to be elucidated. It was hypothesized that these variables would have both and sensorial and chemical impact.

Starting sugar concentration and yeast strain selection for primary fermentation were found to differentiate control wines from wines made with partially dehydrated grapes based on both sensory and chemical profile. Descriptive analysis yielded a range of terms that are appropriate to both Cabernet franc wines and wines made from partially dehydrated grapes and no treatment-related faults were specified. Unexpectedly, terms associated with oxidation compounds did not appear on the list of attributes although significant differences in the responsible metabolites were indicated.

Increased complexity (longer length of finish and more describing attributes) is associated with wines fermented at the highest starting sugar concentration. Further, high starting sugar concentration wines contain higher concentrations of compounds like ethyl hexanoate, ethyl isovalerate, ethyl octanoate, 2-phenylethanol and hexanol, all varying with yeast strain. These findings agree with current literature on appassimento-style wines. (Loizzo et al., 2013; Marquez et al., 2013). CN1 wines had highest concentrations of ethyl isobutyrate and 2-phenylethanol, characteristics of *S. bayanus* yeast strains that have been established in

literature (Eglinton et al., 2000; Gil et al., 1996). However, not all compounds present in high concentrations were correlated to the relevant descriptors.

The most impactful organoleptic outcome occurred when the starting sugar concentration was highest, at 27.5°Brix. At 27.5°Brix, yeast-derived differences were most evident, while at other starting sugar concentrations, the wines fermented with different yeasts were less pronounced. This combination of starting sugar concentration and yeast derived differences is of value to the wine industry, as it suggests a drying target that will result in wines that are complex and with the use of different yeasts, regionally differentiated. This is the first time that CN1 yeast used to ferment partially dehydrated grapes to produce Ontario appassimento wine has been characterized sensorially. Although these wines have been characterized sensorially, this information cannot be extrapolated to infer consumer preference. A consumer preference study will therefore further inform the impact of yeast strain choice on wines made with partially dehydrated grapes. Additional considerations for the development of this wine style will be outlined in the next chapter.

6.5 Chapter 5 Impact of *Botrytis cinerea*-Infected Grapes on Quality Parameters of Wine Made from Partially Dehydrated Grapes

Historically, fermentation of partially dehydrated grapes for sweet wine production includes grape clusters infected with *Botrytis cinerea*, a pathogenic fungus that positively impacts aroma and flavour (Lorenzini et al., 2013; Paronetto and Dellaglio, 2011). This fungus occurs in two forms; the desirable noble rot and the devastating grey rot. The fermentation of dry wines made from partially dehydrated grapes like Amarone also include grapes that are infected, but the influence on quality is variable and uncertain (Tosi et al., 2013) due to the reliance on

favourable conditions for the growth of noble rot. It has been posited that the controlled rate of inclusion of grapes infected with *B. cinerea* may positively contribute to Ontario appassimento style wine quality by impacting its sensory profile and chemical composition. The aim of this study is to understand the impact of 10% *B. cinerea* infection on high sugar wine (compared to wines made with 0% *B. cinerea* infection) made from partially dehydrated grapes by assessing i) chemical differences ii) volatile composition iii) sensory profile and iv) consumer preference of wines. The results of this study indicate that the inclusion of grapes infected with 10% *B. cinerea* had minimal impact on dry wines fermented with partially dehydrated grapes. Expected results like increased concentrations of gluconic acid and glycerol (Magyar and Soós, 2016) were indicated in the *B. cinerea* infected wines, but the differences ended there. Fermentation kinetics were similar, as were volatile concentrations and even descriptive analysis only yielded one term (dried red fruit aroma) that was different between the two wines. A consumer preference test (n=153) revealed that the wines (0% *B. cinerea*, 10% *B. cinerea,* and 27.5°Brix CN1 wine from our previous study) were preferred equally by the participants. When consumers were segmented into clusters based on their liking scores for these wines, it was the yeast differences, rather than the presence of *B. cinerea*, that defined the clusters. Sex and self-rated wine expertise were significant factors that drove liking scores within each cluster, but all other demographics were not significant.

This study provides useful information on wines made from partially dehydrated grapes and the impact that *B. cinerea* has on its profile. When included in a fermentation at a rate of 10%, wines do not differ from wines made with only healthy dried grapes. This can lend useful information to the Ontario wine industry, as it may help dictate sorting decisions and save

personnel time. Further, this is the first time that wines with the locally isolated *S. bayanus* yeast, CN1, has been compared to the commercially used *S. cerevisiae* EC1118 through the lens of consumer preference. The null result of no preference amongst wines suggests that CN1 is preferred just as much as the widely-used EC1118 and may be a candidate for commercialization. Consideration of these results may inform future studies that perhaps observe higher percentages of *B. cinerea* infected grapes included in fermentation.

6.6 Overall Relevance

This project provides new insight into the optimization of appassimento-style wine in Ontario, Canada. Red wine production is considered to more sensitive to the threat of climate change due to the marginal suitability of some varietals in our cool climate. Consumption and purchasing trends in Ontario indicate preference for red wine, with more sales from the international market than the domestic market (Statista, 2017). The apparent consumer demand for red wine is sometimes misaligned with the wines produced in cool climates, and there is a desire for fuller-bodied wines that comes from ripe fruit. Lack of fully ripening berries combined with lack of consistency from year to year can negatively impact the reputation of wineries due to varying quality. Optimizing the process of appassimento-style winemaking can assist the industry in achieving the wines that consumers desire, while providing a growth opportunity for the region: providing sustainable solutions to ongoing problems associated with climate change, and diversifying wine portfolios, as well. Further, an autochthonous yeast that reduces potential quality problems associated with this style offers a regional signature to this wine style, as local grapes are vinified with local yeast. Characterizing this yeast has yielded results that indicate differences in the wine from the commercially used yeast. Application of

this wine production style may assist industry personnel with mitigating the challenges that make cool climate winemaking so challenging.

6.7 Future Directions

This project is directed at further understanding an economically important wine style for the Ontario, Canada wine industry which benefits from innovative techniques to fully realize the potential of the region. This style, however, needs the support of VQA to garner proper credibility in the marketplace. Assigning a designated name to this wine style (like Icewine, for example) may assist producers in marketing the process by which wines are made. Further, enforcing more stringent rules will result in consistent quality. Developing wines that are consistently high-quality because they have adhered to strict regulations will begin the process of establishing a positive reputation in Ontario regarding this wine style. For example, temperature plays an important modulating role in the drying process, and temperatures that are too high generally result in wines with higher treatment-related faults. Perhaps a “cap” on drying temperature (in a controlled environment) may assist with improving wine quality. The dehydration chambers may also be regulated in ensure consistent quality. There are also blending opportunities for wines made with partially dehydrated grapes. Selecting a percentage to blend in to a table wine may change the sensory profile and increase consumer acceptance without increasing price per bottle by too much. Assigning labelling rules and regulations regarding blending would be suitable, as well.

A prominent wine critic suggested that green characteristics of under ripe grapes will only be exacerbated by the drying process, an anecdote that has been disproven through research.

Offering quality wines of this style may reverse that opinion. Disseminating the research to support these promising findings is important, as well.

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