EFFECT OF MIXED SACCHAROMYCES CEREVISIAE STRAINS
ON CHARDONNAY WINE COMPOSITION

by

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ABSTRACT

Chardonnay is the global standard for white wine. Traditional Chardonnay wine is fermented using natural microbial flora, while current Chardonnay wines are fermented using an individual *Saccharomyces cerevisiae* strain. Wine makers are looking for possibility of producing wines with enhanced complexity that has disappeared from current wines due to the use of commercially available yeast starter cultures across the world (Howell et al., 2006). Conducting controlled fermentation using multiple species or strains of yeasts can be one of the available capacities to provide superior Chardonnay wines.

Selected yeasts should have certain technological characteristic as requirements for industrial wine production. In this study, two novel individual Burgundian *S. cerevisiae* strains (C2 and C6) and mixtures of these strains (M1, M2, M3 and M4) were used to produce wine. First, the genetic fingerprinting and killer phenotype of the Burgundian yeasts were discovered. Secondly, the enological characteristics of the Burgundian yeasts were examined. Finally, Chardonnay must was fermented at 16 °C and 20 °C with the *S. cerevisiae* strains. The volatile compounds of the resulting wines were identified and quantified using gas chromatography-mass spectrometry (GC-MS) and compared. The odour active values (OAVs) were calculated for each volatile compound to estimate their sensory contribution of volatile compounds to the overall aroma of the wine samples.

The Burgundian strains C2 and C6 had unique genetic fingerprints and showed a positive killer phenotype. The enological characterization of the Burgundian strains showed that they are enologically equivalent to the available commercial strains.
No significant (p ≥ 0.05) difference existed in the production of 18 volatile compounds by wine yeast strains across the two fermentation temperatures. Principal Component Analysis (PCA) of the volatile compounds and estimated sensory profiles of Chardonnay wines indicated that the individual and mixed Burgundian strains were more similar to one another than the industrial strains. The Burgundian strains collectively produced pleasant aromas with OAVs above the sensory threshold. In conclusion, the potential exists to support the hypothesis that Chardonnay wines, when fermented with mixed Burgundian strains, produce a unique complex aromatic profile which is different from those obtained from commercially available yeast strains.
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DEDICATION

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1 INTRODUCTION

1.1 The Chardonnay grape varietal

Chardonnay is a green skinned grape variety which is used for the production of white wine. Although Chardonnay is most often called a French cultivar, the actual place of origin of the Chardonnay grape has been a matter of discussion for many years. The results of a parental study on more than 300 grape cultivars showed that the parents of the Chardonnay grape and fifteen other wine grapes with long history in northeastern France, including Gamay noir, Aligoté, and Melon, were Pinot noir and Gouais blanc. This study also showed that the parents of Chardonnay were widely scattered in northeastern France in the Middle Ages (Bowers et al., 1999).

Later, the development of wine companies and markets around the world caused the evanescence of local cultivars and the advent of cultivars which are grown worldwide; consequently, the diversity of wine grapes cultivars suffered depletion. Chardonnay is a good example of grapes which are grown in almost all winemaking regions of the new world (Pouget, 1998). Chardonnay is a tough cultivar and can survive in diverse environments, while it is best known as a cool climate cultivar (Kenny and Harrison, 1992). The Chardonnay grape is used for production of many classic wines in the world; it can produce a variety of different styles, flavours and tastes. For these reasons, Chardonnay is considered as the king of white grape varieties and also as the most common white grape for the production of white wines. Furthermore, Chardonnay wines are very popular and maybe the most popular one among the today’s white wines. The adaptation of Chardonnay grapes to nearly all wine making areas plays an important role in the popularity of Chardonnay wine.
1.2 Chardonnay wine

Based on available evidence, Chardonnay is a white wine grape originating from northeastern France, specifically the Burgundy region. The Burgundy region is regarded as the origin of complex wines mostly produced from old cultivars of *Vitis vinifera* (Bowers et al., 1999). Although Chardonnay wine is originally from the Burgundy region of France, different new world countries such as America, Canada, South Africa, New Zealand, Australia and some South American countries produce Chardonnay.

Chardonnay is best known for its richness, golden color, and delicate and distinct aromas such as lemon, melon, citrus, pineapple, grass, and vanilla. Chardonnay is greatly appreciated in both the old and new world for these reasons and have came to represent a global white wine standard.

Customers’ demands for Chardonnay wines with complex aroma and flavour increases as the popularity of Chardonnay wine progresses. Wine makers are currently looking for ways to enhance the flavour and appeal of wines by investigation and application of traditional fermentation approaches; they strongly investigate possibilities to produce wines with favorable complexity which has disappeared from current wines due to the use of commercially available single yeast starter cultures across the world. Conducting controlled fermentation using multiple species or strains of yeasts can be considered as one of the available options in wine research and biotechnology to provide superior Chardonnay wines (Howell et al., 2006).

From a local point of view, wine industry in the British Columbia (B.C.) is growing and developing, corresponding to market demands and business conditions. In recent years, the production of Chardonnay wines in B.C. has been considerably enhanced in both quantity and quality (Cliff and Dever, 1996). Attributes of the wines
depend on different factors like soil, climate, and winemaking process. Chardonnay wines from different regions have distinctive characters. Chardonnay wines from Chablis, Maconnais and Napa are known to have ripe apple, melon, and pineapple characters, respectively (Robinson, 1986). On the other hand, wines from other regions have different styles and qualities. The wine profile changes by grape maturity (Callao et al., 1991), fermentation techniques, and barrel aging (Sefton et al., 1993). The wine industry in B.C. is relatively young. Advanced wine making techniques are being applied to develop B.C. Chardonnay wine’s flavour and aroma (Hennie van Vuuren, 2008, personal communication). Some studies have been done on the sensory and compound profile of B.C. Chardonnay wine (Cliff and Dever, 1996).

1.3 Wine fermentation: History and technology

The final product of the fermentation of grape juice is wine, which is a highly complex mixture of compounds largely defined by its appearance, aroma, flavour and mouth feel properties (Swiegers et al., 2005). The history of wine making relates to the history of civilization and the development of culture. It was discovered that the oldest large-scale wine making site was in Iran at the Haji Firuz Tepe in northern Zagros Mountains around 5400-5000 BC (Pouget, 1988; Alleweldt and Dettweiler, 1994). An Oxford historian considers that the earliest wine was produced in the Caucasus and Mesopotamia at 6000 BC. More evidence refers to wine production in5000 BC in Egypt and Phoenicia; wine was being made in Greece and Crete by 2000 BC. Wine production subsequently arrived at the Mediterranean and then Balkan States, northern Europe and finally Britain. Eventually, European travelers brought wine to the new world in the sixteenth century (Robinson, 1994). Wine production has passed a long journey coming to
today’s lifestyle and cultures across the world. Wine has a critical function in the business of countries with annual wine production of more than 26 billion liters (Barnett, 1998).

Winemaking is considered as one of the first applications of biotechnology. Indigenous yeasts occurring on the skin of the ripe grape berries and in the winery conducted the traditional fermentations (Mateao et al., 2001). The indigenous yeasts are from different strains and species; the various indigenous yeasts form different by products (Henick-Kling, 1988). *S. cerevisiae* is the principal yeast species in wine fermentations but other yeast species have been found to be present at the beginning of fermentations (Heard and Fleet, 1986). The non-*S. cerevisiae* yeasts are mainly suppressed by the progress of fermentation and accumulated alcohol (Saurez and Inigo, 1990).

Traditional wine making depends heavily on the natural growth and fermentation activity of the indigenous yeasts (Reed and Tilak, 1988). The benefits of natural fermentations have been a matter of controversy. In natural fermentations, yeast species such as *Kloeckera, Hansenula, Hanseniaspora, Candida*, and *Pichia* succeed during the first few days of the fermentation and then *S. cerevisiae* conducts the fermentation to its completion. This procession can provide wines with a more complex favorable aroma. On the other hand, it is not possible to control natural fermentations; so wines may have inconsistent quality. Moreover, spontaneous fermentations can be very slow (Heard and Fleet, 1985).

The use of selected yeasts results in a better quality wine than wine produced using traditional spontaneous fermentations (Regodon, 1997). A single strain of *S. cerevisiae* can inhibit the growth of undesirable natural microbial flora and produce
excellent wines (Fleet and Heard, 1993). Controlled wine fermentations have other benefits, such as increased speed and a steady rate of fermentation. Most importantly, it is possible to make wines with consistent quality.

Traditional wine making relies on natural microbial flora, while controlled wine fermentation needs to be inoculated by a specifically cultivated strain or strains of *S. cerevisiae*. The method of Pasteur and the Danish group at the Carlsberg Institute was primarily applied to isolate pure cultures of yeasts. As a result, pure culture slants were produced and distributed among wineries (Reed and Tilak, 1988). These cultures are called “selected pure cultures” and were commonly used by mid-century in the winemaking technology of the US, Canada, Australia, South Africa and New Zealand. These cultures are currently accessible from the Davis culture collection in California and from other collections of wine institutes across the world. Wine makers distribute “selected pure cultures” anaerobically through a serial process from a smaller container to larger one using 1:10 volume ratio. This procedure allows wine makers to gain the benefits from non-limited available cultures from different collections and directly control the distribution process (Reed and Tilak, 1988).

In 1962, the research department of E and J Gallo winery decided to produce commercial bulk yeast (Thoukis et al., 1963). This project resulted in the production of compressed wine yeast for the first time. Compressed wine yeasts were easily spoiled due to high moisture content of approximately 70%. This issue caused the enhanced production of wine yeast in form of “active dry yeast”. In the mid-1960s, “active dry wine yeasts” were broadly accepted and applied in the US and Canada and then in Australia and South Africa. “Active dry wine yeasts” were admitted to Europe 10 years later and gradually accepted in Spain and South America. The main advantages of “active
dry yeasts” include their accessibility in a ready-to-use form and their great durability when stored in vacuum packages (Reed and Tilak, 1988). Currently, a hundred active dry wine yeast strains are available (Vazquez et al., 2001).

In today’s wine making, the favored approach is the use of yeast strains which have been specifically cultivated to ensure the production of wines with desirable characteristics. Therefore, commercial wine yeast strains must be selected to have suitable enological characteristics for industrial wine making. The wine experts widely accept that selected wine yeasts should demonstrate killer phenotype, efficient alcohol production according to the quantity of the sugar in the grape must, good speed of fermentation, growth at high and low temperatures, glycerol production, low volatile acidity production, high tolerance to alcohol, sulphur dioxide resistance, low production of hydrogen sulfide, low foaming, low acetaldehyde production, and limited higher alcohols production (Eschenbruch, 1974; Gardner et al., 1993; Giucidi and Zambonelli, 1992; Henschke and Jiranek, 1993; Lemaresquier et al., 1995; Radler, 1993; Rauhut, 1993; Romano and Suzzi, 1993; Shimizu, 1993; Tipper and Schmitt, 1991; Pretorius, 2000).

Microorganisms convert sugars and other components in grape must to ethanol, carbon dioxide, and hundreds of secondary end-products. Therefore, all of these components collectively make the final wine flavour and aroma of wine (Nykänen, 1986; Lambrechts and Pretorius, 2000). Yeasts are major contributors to wine quality compared to other microorganisms, including bacteria and fungi. Alcoholic fermentation is principally directed by yeasts. The vital and fundamental compounds of wine flavour are produced during the alcoholic fermentation (Fleet, 1993).
The pleasures of wine are largely ascribed to its flavour. The flavour of wine is a sensorial conception. Perceived flavour is the result of interaction between chemical compounds of the wine and taste and smell senses of an individual. Flavour of wine includes volatile and non-volatile compounds, which are responsible for aroma and taste, respectively. Although the final perception of wine depends on various factors, the chemical profile of the wine is one of the most important influential factors on sensory perception of wine (Thorngate, 1997).

The chemical composition of wine and subsequently production of the flavour compounds depends on the grape variety, geographical and grape-growing circumstances, the grape’s microflora, fermentation temperature, winemaking practices, and importantly wine yeast strains (Cole and Noble, 1997).

Customers spend money on wines with a desirable sensory experience. They expect to feel pleasure through drinking an exclusive and complex wine (Bisson et al., 2002). Most recently, they are interested in wines produced using environmentally friendly practices. Consequently, the major challenge of today’s wine makers is to fulfill the consumers’ demand and introduce wines at satisfying quality and price (Swiegers et al., 2005).

The alcoholic fermentation should be controlled to produce premium wines with preferred properties. Inoculation of grape must with selected wine yeasts is known as one of the most important progresses in technology of winemaking (Pretorius, 2000).

Although commercial active dry wine yeasts have been used for many years to control alcoholic fermentation, the end result after some years was mostly similar wines all over the world. Inoculation of controlled wine fermentations by indigenous or natural
wine yeasts which have been specifically isolated from famous winegrowing areas might be a good strategy to solve this problem (Swiegers and Pretorius, 2005).

This strategy allows wine makers to take advantages of controlled fermentations inoculated by indigenous wines yeast strains.

1.4 Characteristics of wine yeast strains

Active dry wine yeasts were produced and applied in winemaking in the United States since mid-1960s and then spread all over the world. More than a hundred different commercial active dry wine yeasts are now available in the market. The 246,423,000 hectoliter (hL) of wine is produced per annum and the rate of yeast consumption is 10-20 g/hL. The available capacity for production of selected dry yeasts is roughly about 500 tons per year (Vazquez et al., 2001)

Different wine yeast strains have a different effect on the fermentation process and wine flavour and aroma. Selected wine yeasts are evaluated based on the defined desirable and undesirable characteristics of wine yeasts. None of the commercially available wine yeasts have all of the desirable characteristics. In the selection of natural wine yeasts we should consider the most important characteristics.

Desirable properties of wine yeasts are classified in five different categories, fermentation properties, flavour characteristics, technological properties, metabolic properties and health implications (Pretorius, 2000); all of these properties can be simply considered as enological properties of the wine yeast strains.
1.4.1 Desirable fermentation properties of wine yeast strains

The fermentation attributes which mainly affect the fermentation performance include “rapid initiation of fermentation, high fermentation efficiency, growth at low and high temperatures, and high ethanol tolerance “(Pretorius, 2000).

1.4.1.1 High fermentation efficiency

The principal factor in the selection of wine yeasts is the ability of yeast to convert more than 98% of the sugar content of grape must to alcohol and carbon dioxide at an even rate and without producing off flavours. Stuck fermentation happens when wine yeast cannot conduct the fermentation to the end and high levels of non-fermented sugar are available (Henschke and Jiranek, 1993). Physiological conditions of the yeast and the physicochemical and nutrient characteristics of the grape collectively determine the lag phase, speed and power of sugar conversion to alcohol, tolerance to restrictive factors, and duration of fermentation (Henschke, 1997).

Fermentation temperature can adjust the rate of fermentation. The fermentation temperature should be decreased when fermentation progresses fast. Raising the fermentation temperature is required to speed up stuck fermentations. Supplementation with vitamins, aeration and reinoculation are the other corrective practices for slow fermentations (Henschke and Jiranek, 1993).

Wine yeast strains must be able to conduct the fermentation at the optimum temperature. White wine yeast strains need to handle the fermentation at 10-15 ºC to reduce the volatilization of aromatic compounds to a minimum. Red wine yeast strains are required to direct fermentations at higher temperatures (18-30 ºC) to increase anthocyanin pigments which strongly affect wine quality and cost (Henschke, 1997).
Selection of wine yeast stains with high fermentation proficiency at optimum temperature is really critical in wine making due to the strong association between alcoholic fermentation and quality of wine.

1.4.1.2 High ethanol tolerance

Ethanol can potentially act as a stress factor and cause stuck fermentations, although the production of ethanol is the main target of wine fermentation. Extravagant ethanol production results from high sugar content of grape must from overripe grapes. High sugar content of grape hinders yeast growth. Excessive ethanol obstructs the transfer of solutes like sugars and amino acids; consequently, this restrains yeast growth rate, viability, and fermentation capacity. The physiological fundamentals of ethanol toxicity is complicated and not clearly known, but it seems that ethanol acts mostly by affecting the cohesion and permeability of cell membranes (Boulton et al., 1996).

In addition to ethanol tolerance of wine yeast strains, the other natural and ecological parameters improve the restrictive character of ethanol cooperatively. These factors include high fermentation temperature, limited oxygen, limited nutrients such as nitrogen, lipids and magnesium ions and metabolic byproducts like other alcohols, aldehydes, esters, organic acids particularly octanoic acid and decanoic acid, certain fatty acids and carbonyl and phenolic compounds (Edwards et al., 1990).

Some of the unsaturated long chain fatty acids and sterols make cells last under high ethanol concentrations. These survival factors are produced only when molecular oxygen is available. This phenomenon explains the triumph of commercial starter cultures which are cultured in high levels of oxygen and low glucose concentrations. This type of starter culture is able to last under high ethanol concentrations and introduce their
own survival properties to the next six or seven generations of growth in a characteristic wine fermentation (Boulton et al., 1996).

The ethanol tolerance of selected wine yeast strains should be considered due to the toxicity of ethanol and its effect on slowing fermentation.

1.4.2 Desirable flavour properties of wine yeast strains

Wine yeast strains contribute differently to the flavour of wine depending on the metabolites produced during fermentation. It is desirable that wine yeast strains produce low sulphide and thiol compounds, low volatile acidity, and low amounts of higher alcohols. Conversely, they are expected to produce high amounts of glycerol. Furthermore, the liberation of glycosylated flavour precursors and modified esters activity are considered to be desirable properties of wine yeasts (Pretorius, 2000).

1.4.2.1 Production of sulphide and thiol compounds

Sulphur is widely available in various combinations in the world. Sulphate is an oxidized form of sulphur and sulphide is a reduced form of sulphur. Sulphur has a key role in biology due to its existence in amino acids such as cysteine and methionine and critical co-factors. Microorganisms have two sulphur metabolic pathways; a sulphate absorbent pathway and a sulphate dissimilatory reduction pathway. The sulphate absorbent pathway utilizes sulphate for the production of organic combinations such as cysteine and methionine. The sulphate dissimilatory reduction pathway converts sulphate to sulphone or sulphide without metabolism and mostly by excretion (Kappler and Dahl, 2001). Microbial degradation of amino acids such as cysteine and methionine produce sulphides, thiols, and other volatile sulphur compounds (Dainty et al., 1989;
Wine yeast *S. cerevisiae* can produce some volatile sulphur compounds. The sulphur volatile compounds are important in winemaking due to their sensory effect on the quality of wine. Perception threshold of Hydrogen peroxide (H$_2$S) is 50-80 µg/L; concentrations exceeding this value impairs the quality of wine, producing a rotten egg aroma (Monk, 1986). Some of the other sulphur compounds that have been recognized in wine include methanethiol or methylmercaptan (cooked cabbage aroma), dimethylsulphide, dimethyldisulphide, and dimethyltrisulphide (cabbage, cauliflower, and garlic aromas), and the fruity volatile thiols in wine (passionfruit, grapefruit, gooseberry, guava, and box hedge aromas (Swiegers and Pretorius, 2005; Swiegers et al., 2006).

Wine yeast strains can produce H$_2$S immoderately in wine through assimilatory sulphate pathways during wine fermentation (Swiegers et al., 2005). Unfixed cases can result in impaired wine quality and consequently consumers’ refusal to buy (Henschke and Jiranek, 1991). Copper sulphate is commonly added to wine to remove the undesirable impacts of H$_2$S and mercaptans in accomplished wines. The prompt reaction of copper sulphate removes H$_2$S; however, the addition of copper sulphate to wine is not a desirable practice.

Available sulphur compounds, wine yeast strain, fermentation conditions, and the nutrition profile of the grape must affect the amount of H$_2$S formed during wine fermentation (Henschke and Jiranek, 1991; Spiropoulos et al., 2000).

Wine yeast strains are able to make some volatile sulphur compounds which positively affect the quality of wine. Furfurylthiol is one of these beneficial compounds. The
detection threshold of furfurylthiol is very low (0.4 ng/L) due to its vigorous flavour, one recognized in roasted coffee, meat, wheat bread, and popcorn (Tominaga et al., 2000).

The 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH), and 3-mercaptohexyl acetate (3MHA) are other volatile thiols which are formed by wine yeasts using available precursors in the grape. These volatile thiols have very vigorous flavour and low detection thresholds: 0.8 ng/L (4MMP), 60 ng/L (3MH), and 4 ng/L (3MHA) (Tominaga et al., 1995; Dubourdieu et al., 2006).

Current winemakers face the challenge of restricting or stopping the formation of the unpleasant H₂S and mercaptans and concurrently improving the formation of the desirable volatile thiols. Additionally, the removal of H₂S content of wines using copper sulphate is another big challenge for winemakers due to the simultaneous effect of copper sulphate reducing the amount of favorable thiols. This coincidence happens because the copper ion does not differentiate between the two types of sulphur compositions. Considering this fact, the selection and introduction of wine yeasts that form pleasant amounts of beneficial volatile thiol compounds for wine aroma during fermentation and do not produce H₂S and mercaptans is the conclusive approach (Pretorius, 2000; Pretorius and Bauer, 2002).

Mendes-Ferreira et al. (2002) recently categorized wine yeast strains based on their ability to reduce sulphate as either “non-producers,” “must-composition-dependent producers,” or “invariable producers” of H₂S. Various wine yeast strains form different concentrations of H₂S under similar circumstances, indicating that production of H₂S is partially dependent on genetics (Henschke and Jiranek 1993; Spiropoulos et al., 2000). Production of sulphur compounds and subsequently H₂S is highly associated with
S. cerevisiae metabolism; therefore, production of sulphur compounds by wine yeasts should be assessed in selection of wine yeasts.

1.4.2.2 Production of volatile acidity

Non-volatile acids in addition to volatile acids constitute the overall acidity of a wine. In general, volatile acidity of wine is regarded as the acetic acid concentration (g/L) in wine, while the other present volatile acids in wine are considered in the old-fashioned evaluation method of volatile acidity. The other volatile acids which comprise wine volatile acidity include carbon dioxide (carbonic acid), sulphur dioxide (sulphurous acid), and in lesser quantities lactic, formic, butyric, and propionic acids. Furthermore, sorbic acid should be considered; sorbic acid is added to wine as potassium sorbate to inhibit growth of fungi (Zoecklein et al., 1995).

Assessment of volatile acidity is commonly considered as a measure showing wine spoilage (Zoecklein et al., 1995). A non-faulty produced dry table wine normally contains 0.2 to 0.4 g/L acetic acid (Ribereau-Gayon, 1961). Risk of wine spoilage should be considered when concentration of acetic acid increases beyond this range. High amounts of acetic acid in wine are often produced by acetic acid bacteria which come from bad fermentation approaches. S. cerevisiae produces acetic acid, but usually in the range of 0.2-0.3 g/L in most completed wines (Ribereau-Gayon et al., 2000). The legal limit of acetic acid in different wine styles ranges from 0.9-1.4 g/L. In the United States, white wines and red wines that are produced from grape must with Brix 28° can have acetic acid to 1.5 g/L and up to 1.7 g/L, respectively. The sensorial detection threshold of acetic acid is more than 0.7 g/L, which is higher than the amount of acetic acid produced by S. cerevisiae (Zoecklein et al., 1995).
Different *S. cerevisiae* wine yeast strains produce various amount of acetic acid. As formation of acetic acid by *S. cerevisiae* is associated with acetyl-CoA, variation among strains occurs. Furthermore, environmental factors such as pH, sugar, nitrogen concentration, fermentation temperatures, and the contributions of other microorganisms influence acetic acid production (Zoecklein et al., 1995).

Low concentrations of acetic acid can be beneficial for the complexity of wine (Zoecklein et al., 1995). It is therefore important to select wine yeasts that produce low concentrations of acetic acid.

1.4.2.3 Production of higher alcohols

Higher alcohols are those alcohols with more than two carbons. The common name for higher alcohols is fusel oils. Wine yeast strains synthesize higher alcohols during wine fermentation. The importance of higher alcohols in wine making is due to their contribution to wine flavour and aroma. The most common higher alcohols which have been found in wine making are isoamyl (3-methyl-1- butanol), active amyl alcohol (2-methyl-1-butanol), isobutanol (2-methyl-1-propanol), and n-propyl alcohols (Webb and Ingraham, 1963).

The quantitative and qualitative impacts of higher alcohols on wine flavour are higher than the other types of alcohols; the concentration of higher alcohols in wines varies significantly. Isoamyl alcohol usually constitutes more than 5% of the higher alcohols (Muller et al., 1993).

Higher alcohols concentrations in wines vary from 100 mg/L to more than 500 mg/L (Nykänen, 1986). Higher alcohols can be beneficial for pleasant complexity of wine when higher alcohol content of wine is less than 300 mg/L. Higher alcohols at
concentrations more than 400 mg/L have a destructive impact on the quality of wine (Rapp and Mandery, 1986).

Wine yeasts form higher alcohols during alcoholic fermentation through transamination of amino acids, decarboxylation and reduction of keto acids using the Ehrlich pathway (Webb and Ingraham, 1963). The production of higher alcohols depends on the genetic history of wine yeasts (Lambrechts and Pretorius, 2000). Extrinsic factors such as accessibility of required nutrients for wine yeast (Herandez et al., 2001), fermentation temperature (Ough and Amerine, 1967), and other wine making practices may direct the production of higher alcohols in wine. Ultimately, employing wine yeasts that produce low amounts of higher alcohols is important as higher alcohols constitute the largest portion of the flavour compounds in wines.

1.4.2.4 Production of glycerol

Glycerol is a non-volatile thiol (Scanes et al., 1998). After ethanol and carbon dioxide, glycerol is the main product of alcoholic fermentation. Glycerol formation is started by reduction of dihydroxyacetone phosphate to glycerol-3-phosphate through glycerol-3-phosphatedehydrogenase (GPDH). Then, phosphatase converts glycerol-3-phosphate into glycerol (Hammond, 1997).

Glycerol is a non-volatile compound and does not directly contribute to the wine aroma. Glycerol is a moderately sweet and viscous compound which contributes to the sweetness, body and mouth feel of wine (Ribereau-Gayon et al., 1972; Eustace and Thornton, 1987).

_S. cerevisiae_ produces different amounts of glycerol in different wines ranging from 2 to 15 g/L, while the common range of glycerol production is between 4 and 9 g/L.
The glycerol taste is perceived in white table wine at a concentration of 5.2 g/L and in red table wine at 13 g/L, while the mouthfeel threshold of glycerol is more than 25 g/L (Scanes et al., 1998). Wine composition may affect this threshold, as it has been shown that acidity and amount of ethanol raise the threshold value (Hinreiner et al., 1955).

Formation of glycerol by yeasts is affected by various intrinsic and extrinsic factors (Rankine and Bridson, 1971; Ough et al., 1972; Gardner et al., 1993). The amount of glycerol produced changes by degree of ripeness of grapes (sugar content) and the grape variety (micronutrients) (Ough et al., 1972). Nitrogen content of grape must affect glycerol production. The production of glycerol increases when temperature increases (Rankine and Bridson, 1971; Ough et al., 1972; Gardner et al., 1993); this may explain the higher glycerol content of red wines compared to white wines (Radler and Schutz, 1982). Wine yeast strain, fermentation temperature, and agitation collectively affect glycerol production (Gardner et al., 1993). It is therefore important to select wine yeasts that produce high concentrations of glycerol.

1.4.2.5 Acetaldehyde production

The contribution of aldehydes to the aromatic properties of wine is considerable due to their low perceived threshold (Suomalainen and Lehtonen, 1979). Acetaldehyde constitutes more than 90% of the wine aldehydes (Nykänen, 1986). Acetaldehyde is an intermediate in the glycolytic pathway and is produced from pyruvate during fermentation by *S. cerevisiae* (Lehninger, 1970). Acetaldehyde is a precursor of acetate (Nordstrom, 1966), acetoin (Collins, 1972), and ethanol (Romanoat et al., 1994).
Acetaldehyde is typically produced during alcoholic fermentation and its concentration in various wines ranges from 10 mg/L up to 300 mg/L (Schreier, 1979). Acetaldehyde produces a sour green apple aroma when its concentration is more than its sensory threshold which is 0.5 mg/L (Lambrechts and Pretorius, 2000).

The production of acetaldehyde is affected by aging, grape must, and specifically the sulphur dioxide content of grape must. It has been noted that the level of acetaldehyde in wine rises during aging due to oxidation of ethanol to acetaldehyde. It has been reported that yeasts produce significantly more acetaldehyde when the sulphur dioxide content of grape must is high (Romanoat et al., 1994).

Although various wine yeasts produce acetaldehyde, current evidence shows that *S. cerevisiae* strains form comparatively high amounts of acetaldehyde, ranging from 15 to 50mg/L. In contrast, the production of acetaldehyde by other yeasts like *Kloeckeraapiculata, Candida krusei, Candida stellata* is very low, starting from non-identifiable levels and rising to 40 mg/L (Lopes et al., 1996). Considering the capacity of *S. cerevisiae* yeast strains for production of acetaldehyde and the undesirable aromatic properties of acetaldehyde, low production of acetaldehyde by wine yeasts is regarded as a desirable attribute.

1.4.2.6 Acetate and ethyl ester production

Esters are volatile compounds which significantly contribute to the flavour and aroma of wine. Esters typically contribute to wine aroma and flavour by providing the fruity properties of wine (Herraiz and Ough, 1993). Wine esters are usually classified into different types: The first type of esters originate from acetate, ethanol and higher alcohols. The ethyl-, isobutyl-, isoamyl-, 2-phenethyl-, and hexyl-acetates are in the first
group of esters. Ethyl acetate is predominant among recognized esters in wine. Ethyl acetate is occasionally developed during the malolactic fermentation. The second group of esters comes from ethanol and straight-chain fatty acid precursors. The ethyl esters of hexanoic, octanoic, and decanoic acid are examples of a second type of esters. The second group of esters contributes less to the sensory quality of wine than acetate esters (Zoecklein et al., 1995). Hexyl acetate, ethyl caproate and caprylate (apple-like aroma), isoamyl acetate (banana-like aroma), and 2-phenylethyl acetate (fruity, flowery flavour with a honey note) are the main contributors to the fruity aroma of wine (Fujii et al., 1997).

Different factors such as wine yeasts strains, grape cultivar, grape maturity, pH of grape must, sulphur dioxide and amino acid content of grape must affect ester formation in wine. Production of esters in wine is considerably affected by wine yeast strains. The indigenous yeasts present at the beginning of the fermentation, during the course of fermentation, and during post-fermentation processing can have significant impact on the amount of ester produced. Higher concentrations of ethyl acetate and isoamyl acetate are formed by natural yeast species such as *Hansenula anomala*, and *K. apiculata* the beginning of fermentation (Soles et al., 1982). Therefore, wine yeasts should produce esters which contribute to wine aroma and flavour by providing the fruity properties of wine.

1.4.3 Desirable technological properties of wine yeast strains

Desirable technological characteristics of the selected wine yeast strains include low foam formation, zymocidal (killer) properties, compatibility with malolactic bacteria,
high genetic stability, high sulphite tolerance, flocculation properties, proteolytic activity, and low nitrogen demand (Pretorius, 2000).

1.4.3.1 Low foam production

Foam formation is one of the undesirable technological properties of wine yeasts. Foaming occurs in the initial phase of wine fermentation and reduces real space of the fermenter since around 5% of the capacity of fermenter is assigned for potential foam formation. Foam contains a significant amount of yeast cells which may accumulate or sediment above the grape must on the walls and roof (of a closed tank). In this case, the yeast will no longer participate in the fermentation. Additionally, a black, unpleasant-smelling layer may be caused by yeast on the surface of grape must (Thornton, 1978). Low formation of foam is a valuable attribute for wine yeasts due to limited space available in fermentation tanks in wineries during the vintage.

1.4.3.2 Killer phenotype

The existence of non-infectious, intercellular virus-like particles (VLP) causes killer phenotype in *S. cerevisiae*. These VPLs include two main linear double-stranded ribonucleic acid (dsRNA) types named L and M genomes. The role of the L genome is to encode an RNA-dependent RNA polymerase and the viral coat protein that encapsulates the RNA. The M genome encodes both a proteinaceous toxin (zymocin) and an immunity factor. The toxin is secreted by the zymocidal strains and can kill sensitive strains of the same species (Wickner, 1996).

Killer yeasts secrete proteins which have a fatal effect on sensitive wine yeasts. *S. cerevisiae* wine yeast strains are categorized as killer positive (K⁺), neutral (N),
sensitive (S) or killer negative (K⁻) strains, regarding their ability to produce toxins.

Killer yeasts (K⁺) secrete a proteinaceous toxin; this toxin kills sensitive strains (S) but killer strains are immune to their own toxins. Neutral yeasts are not able to kill sensitive strains and are immune to the toxin of killer strains (Bevan and Makower, 1963).

The 11 groups of killer yeasts are known; three groups (K1, K2, and K3) have been found in *S. cerevisiae*. High temperature and proteases could destroy the K1 toxin. Optimal pH for production of K1 toxin and its stability is between 4.6-4.8 (Woods and Bevan, 1968). Therefore, K1 killers are not important in wine fermentations due to the low pH of grape must. The K2 toxin is stable at pH between 2.8 to 4.8 (Shimizu et al., 1985). No great deal of information is available about K3 killer toxin. Remaining killer types (K4-K11) belong to the other yeast genera and species (Young and Yagiu, 1978).

The K2 killer strains and probably the K3 killers are sources of threats to the wine industry because their toxins kill the sensitive wine strains during fermentation of grape must. The killer yeasts in a wine fermentation inoculated with sensitive strains might dominate and cause problems such as stuck fermentation, high volatile acidity, and H₂S production (Van Vuuren and Jacobs, 1992).

The ability of killer yeast to cause stuck fermentations depends on different elements such as the population of killer yeast at the time of inoculation, the activity of killer toxins, the fermentation capability of the killer yeasts, and the sensitivity level of the wine yeasts to the killer toxin. The consequences of stuck fermentation are longer fermentation time and high residual sugars in wine. Stuck fermentations are one of the most important problems in winemaking due to its adverse effects on product quality and financial losses.
Killer yeasts could be beneficial in winemaking. When killer yeasts are used as commercials starter cultures, they will inhibit the growth of wild sensitive yeasts (Van Vuuren and Jacobs, 1992). A killer positive phenotype is known as a desirable technological attribute in \textit{S. cerevisiae} wine yeasts.

1.4.3.3 Compatibility with malolactic fermentation

One of the important factors in winemaking is the production of well-balanced wines. To produce well-balanced wines the acidity of grape must containing high concentrations of acid must be decreased. L- malic acid and L- tartaric acid are the major organic acids in grape must (Beelman and Gallander, 1979). The bacterial malolactic fermentation (MLF) is conducted to decrease the acidity of wine and produce microbially stable wines. During the MLF, L-malic acid is decarboxylated to L-lactic acid and carbon dioxide; malolactic enzymes (L-malate: NAD$^+$ carboxylyase) catalyze this reaction without the formation of any free intermediates (Caspritz and Radler, 1983). The MLF is preferably conducted by \textit{Oenococcus oeni} (Dicks et al., 1995) after accomplishment of alcoholic fermentation by \textit{S. cerevisiae} wine yeast strains (Lonvaud-Funel, 1995). \textit{O. oeni} is a lactic acid bacterium and restricted by ethanol, low pH, sulphur dioxide, low temperature, fatty acids, decreased nutrient content, competitive interactions with yeast, and bacteriophage infections (Davis et al., 1985). Alternative measures for reduction of wine acidity include carbonate addition, perception, dilution and carbonic maceration; these methods are hard to apply and usually decrease the quality of wine (Gallander, 1977). On the other hand, inoculated lactic acid bacterial starter cultures may grow slowly and unexpectedly in wines, and particularly in Chardonnay wines, which results in
stuck MLF and spoilage of wine (Davis et al., 1985). To accomplish MLF, compatibility of *S. cerevisiae* strains with lactic acid bacteria is a desirable property for wine yeasts.

1.4.4 Desirable metabolic properties of wine yeast strains with health implications

Consumers are looking for safe wines; therefore, metabolites which can be harmful for health must be considered in wine making. Wine yeasts strains are favorable if they produce low sulphite, low biogenic amines and low ethyl carbamate (Pretorius, 2000).

1.5 Technology of yeast usage in winemaking

Different strategies are used for the production of wine yeast strains. It has recently been discovered that major enological properties of wine yeasts are complicated and affected by different genes. The genetic engineering of wine yeasts is a constantly improving strategy in the production of wine yeasts. It is currently known that indigenous strains may perform better in the fermentation of grape must (Regodon et al., 1997, Perez-Coello et al., 1999). This approach requires selection and isolation of the novel yeast strains according to the desirable enological properties (Rainieri and Pretorius, 2000). Mixed cultures of novel yeast strains is another strategy to gain the benefits from the metabolic interactions of yeasts and produce more complex wine. Inoculation of grape musts with novel individual or mixed starter cultures allow winemakers to get the benefit from spontaneous fermentations by novel yeast strains under controlled circumstances.

Some recent studies focused on the use of non-*Saccharomyces* yeast species from the genera *Candida, Kloekera, Hanseniaspora, Zygosaccharomyces,*
*Schizosaccharomyces, Torulaspora, Brettanomyces, Saccharomyces, Pichia* and *Williopsis* in wine fermentation (Jolly et al., 2006). Some studies focused on the impact of the other indigenous *Saccharomyces* strains. As an example, one study by Orlic et al. (2007) specifically evaluates the effect of indigenous *Saccharomyces paradoxus* strains on the fermentation and aroma of Chardonnay wine. In this study, seven indigenous yeast strains of *S. paradoxus* were isolated and identified using molecular and physiological methods, after which their fermentation properties were characterized. *S. paradoxus* strains had good fermentation power and ethanol tolerance compared with the control *S. cerevisiae* strain in the study. Production of higher alcohols by *S. paradoxus* strains was lower than the amount produced by the control *S. cerevisiae* strain. One *S. paradoxus* strain had degradation ability of malic acid up to 40 % which is beneficial for deacidification of wine. Sensory results showed good enological characteristics and a positive impact of *S. paradoxus* strains on the final quality of wine. This study addresses the possibility of the use of *S. paradoxus* strains in winemaking.

Only a few studies address the effect of mixed *S. cerevisiae* strains on wine fermentation. A study by Howell et al. (2006) investigates the effect of mixed *S. cerevisiae* strains on the chemical profile and aromatic properties of wine. Compounds in wines fermented by mixed *S. cerevisiae* strains were different from the chemical profile of the wines that were inoculated with individual *S. cerevisiae* strains. The chemical profile of mixed culture fermentations was not reached by blending the single fermented wines. It is believed that different chemical composition and aromatic properties result from the metabolic interactions of the wine yeast strains. Currently, little information is available about the metabolic interaction of wine yeast strains.
Another recent study by Swiegers et al. (2007) from the Australian Wine Research Institute showed that mixed *S. cerevisiae* strain fermentations produced lower thiol concentrations and enhanced sensory properties in Sauvignon Blanc wines, which was different from the individual yeast strains. This study showed interactions of mixed yeast strains potentially increase fruitiness in white wines, which would respond to consumers’ requests.

### 1.6 Proposed research

The use of selected wine yeast strains to control the microbiological phase of fermentation is one of the most important and beneficial technological advances in winemaking (Rainieri and Pretorius, 2004). Although many commercial wine yeast strains are available to conduct wine fermentation, locally selected yeasts and mixtures of such yeasts are believed to be much more effective in producing more complex wines (Querol et al., 1992).

Use of selected yeasts results in a final product with more consistent quality in comparison with the wine produced via traditional spontaneous fermentations. The application of new locally selected yeasts for controlled fermentation in countries with a winemaking tradition has recently increased (Regodon et al., 1997). Some recent studies show that inoculation of wine fermentations using a mixture of *S. cerevisiae* may produce more complex wines (Howell et al., 2006; Swiegers et al., 2007).

The hypothesis for this research is that mixed *S. cerevisiae* strains isolated from the Burgundy region in France produce more complex Chardonnay wine with exclusive aromatic profiles than single *S. cerevisiae* cultures.

The aim of this study is to characterize two novel Burgundian strains of
S. cerevisiae and determine the effect of mixed Burgundian strains on the flavour and aroma of Chardonnay wine. Six commercially available strains will be included to provide a baseline representing the strains currently in use by the wine industry for production of Chardonnay wine.

New yeasts should have commercially acceptable enological characteristics. In this study, individual industrial and Burgundian S. cerevisiae strains will be enologically characterized and compared to industrial strains. First, genetic fingerprinting and the killer phenotype of the Burgundian yeasts will be investigated. Second, enological characteristics of the Burgundian yeasts such as the growth rate, rate of ethanol production, glycerol and acetic acid production, ethanol tolerance, compatibility with malolactic fermentation, SO₂ production, and foam formation will be measured and statistically compared to find significant differences among yeast strains and across fermentation temperatures.

Furthermore, Chardonnay must will be fermented with the six individual industrial, two novel individual Burgundian and four mixed Burgundian strains to evaluate the metabolic effect of mixed Burgundian S. cerevisiae strains on the aromatic profile of Chardonnay wine. The volatile compounds in the headspace of the resulting wine will be analyzed using gas chromatography-mass spectrometry (GC-MS). All of the compounds will be quantified and statistically examined for significant differences among yeast strains and between fermentation temperatures. Similarities among the yeasts will be explored using multivariate analysis.

Finally, the chemical profile and odour profile of the volatile compounds in Chardonnay wines fermented with individual industrial, individual Burgundian and
mixed Burgundian strains will be created to evaluate effect of yeasts on the complexity of Chardonnay wine.
2 MATERIALS AND METHODS

2.1 Juice

Chardonnay must was obtained from White Salmon Vineyard in California (2008). The Chardonnay must had soluble solids of 24 °Brix. The pH and titratable acidity (TA) of Chardonnay must were 3.46 and 5.76 g/L, respectively. In addition, Yeast Available Nitrogen (YAN) of this must was 132 mg N/L. The juice was stored at -20 ºC prior to use.

2.2 Yeasts

2.2.1 Individual industrial S. cerevisiae strains

Six strains of S. cerevisiae typically used for Chardonnay fermentation were selected as control strains. Individual industrial strains Blanc, Elegance, and Fusion were obtained from Mauri Yeast Australia (Sydney, Australia). The yeast strains CY3079 and ICV-D254 (Lallemand), and X16 (Laffort) were purchased from Scott Laboratories as active dry yeasts. These strains were recommended for white wines especially Chardonnay wine to increase fruity aroma and complexity.

2.2.2 Novel individual and mixed S. cerevisiae strains

The novel S. cerevisiae strains were isolated from a vineyard in Burgundy region, France by Dr. Hennie van Vuuren and named C2 and C6. The novel Burgundian S. cerevisiae strains were mixed in four different ratios as indicated in Table 1, to evaluate the impact of mixed S. cerevisiae strains on the complexity of Chardonnay wine.
Table 1. Ratios of mixed Burgundian *S. cerevisiae* strains used in this study.

<table>
<thead>
<tr>
<th>Mixture Name</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio (C2: C6)</td>
<td>1:1</td>
<td>1:2</td>
<td>1:3</td>
<td>3:2</td>
</tr>
</tbody>
</table>

2.3 Bacteria

The *Oenococcus oeni* strain Lalvin 31 was obtained from Lallemand Inc. (Rexdale, Canada); *O. oeni* is a lactic acid bacterium that was used to conduct the malolactic fermentation.

2.4 Media and culture conditions

All *S. cerevisiae* strains were kept at –80 °C in 15% glycerol/yeast peptone dextrose (YPD). Yeasts were cultured in Difco YPD broth (Becton, Dickinson and Co., Sparks, USA) based on the standard method (Ausubel et al., 1999). Lyophilized *O. oeni* was rehydrated in 50 ml of sterile distilled water for 15 minutes and directly inoculated to fermentations for the malolactic fermentation compatibility assay. Media for the killer phenotype assay is described under killer phenotype assay.

2.5 Genetic and phenotypic characterization

2.5.1 Genetic fingerprinting

Cells of *S. cerevisiae* strains were grown overnight in 5 ml of YPD broth at 30 °C in a rotary wheel. Cells were harvested and DNA was extracted as described by Hoffman and Winston (1987).

Individual yeast strains were genetically fingerprinted by a polymerase chain reaction (PCR) method. The PCR method discriminated yeast strains based on the
amplification of repetitive δ sequences of \textit{S. cerevisiae} genome, which are often associated with the Ty1 transposon (Schuller et al., 2004). The PCR was performed on a MJ Research Peltier Thermal Cycler 200 (Walthman, USA) using the primers δ 2 (5′-GTGGATTTTTATTCCAAC-3′) and δ 12(5′-TCAACAATGGAATCCCAAC-3′).

The 30 μl reaction mixture was prepared with 10 ng of DNA, 1μl Taq polymerase (MBI Fermentase), Taq buffer (10 mM Tris- HCL, 50 mM KCL, 0.08% Nonidet P-40), 25 pmol of each primer, 0.4 mM of each dNTP and 3mM MgCl2. The initial denaturation was done at 95 °C for 2 minutes; then, the reaction mixture was cycled 35 times using the following program: 95 °C for 30 seconds, 43. 2 °C for 1 minute, 72 °C for 1 minute and final extension was done at 72 °C for 10 minutes. The PCR products were separated by electrophoresis on a 1% (w/v) agarose gel and visualized with SYBR safe DNA gel stain (Invitrogen Inc., Burlington, Canada) (Schulleret al., 2004).

2.5.2 Killer factor typing

The killer phenotype of individual Burgundian \textit{S. cerevisiae} strains C2 and C6 was identified in this assay. Information about the killer phenotype of the industrial strains was obtained from industrial yeast manufacturers.

To test the killer phenotype of Burgundian strains C2 and C6, two strains with previously known killer phenotype were selected as control strains; Elegance is a positive killer strain (K⁺), while Maurivin B is a killer negative (K⁻) or sensitive strain.

The killer assay medium was made according to the modified method introduced by van Vuuren and Wingfield (1986). YPD agar medium was buffered with 50 mM dibasic sodium phosphate and the pH was adjusted to 4.1 with citric acid prior to
autoclaving. Filter sterile (0.22 µm) methylene blue (0.0015% w/v) was added to medium.

The C2, C6, K⁺, and K⁻ strains were cultured on YPD agar plates at 30 °C for 72 hours. The three colonies of the K⁻ strain were picked and resuspended in sterile MilliQ water to give 5×10⁸ cells/ml; 300 µl of this suspension was spread on the surface of killer assay plate to form a sensitive lawn and allowed to dry. Colonies of C2, C6, control K⁺, and control K⁻ strains were swabbed and spread as thick line on top of the sensitive lawn. The plate was then incubated at 18 °C for 5 days (adapted from van Vuuren and Wingfield, 1986).

The killer positive strains were surrounded by a clear zone of inhibition ringed in blue. The zone of inhibition indicated that the sensitive strain cannot grow and the blue ring confirms that the sensitive strain is dead.

2.6 Enological characterization

2.6.1 Model of fermentations

All fermentations were performed in biological triplicate using 200 ml Chardonnay must. Freezer stocks of *S. cerevisiae* strains were used to inoculate 5 ml liquid cultures of YPD; *S. cerevisiae* cells were grown overnight in a rotary wheel to stationary phase at 30 °C. Flasks containing 50 ml YPD cultures were subsequently inoculated for each strain at a rate of 5×10⁵ cells/mL and grown aerobically in a shaker bath (180 rpm) at 30 °C for 24 hours. Cells were then harvested by centrifugation (5,000 times gravity for 5 minutes). Harvested cells were washed with sterile MilliQ water and resuspended in the fermentation medium. The 250 ml fermentation bottles containing 200
ml Chardonnay must were inoculated at the rate of $2 \times 10^6$ cells/mL. Two individual Burgundian strains were inoculated into fermentation bottles according to ratios in Table1 for mixed fermentations. Yeast strains were not mixed before the inoculation of the mixed strains fermentations. All fermentation bottles were topped with disinfected (70% ethanol) rubber bungs and water-filled capped gas locks to provide anaerobic conditions. Anaerobic sampling was aseptically performed by removing approximately 1 ml sample through the rubber bung with a 5-inch hypodermic needle (Air-Tite, Virginia Beach, USA) attached to a 3 mL syringe (Becton Dickinson, Franklin Lakes, USA). The samples were then filter sterilized (0.22 µm) and placed into glass vials, which were quickly capped.

The primary fermentations were conducted in triplicate in 200 ml of Chardonnay must at 16 ºC and 20 ºC. Samples from these fermentations were used to assess fermentation kinetics, ethanol production, conversion factors of sugar to ethanol, glycerol, and acetic acid production, and production of volatile compounds. An ethanol tolerance assay was conducted using this model of fermentation, while Chardonnay must was enriched with glucose and fructose as described under ethanol tolerance assay (Section 2.6.5). This model of fermentation was used for sulphur dioxide production assay using synthetic juice as described under sulphur dioxide production assay (Section 2.6.8).

Analysis of volatile compounds in Chardonnay wine was conducted when fermentations were completed; 100 mg/L of potassium metabisulphite was added to wine to prevent oxidation of wine samples. Wine samples were stored at 4 ºC before the volatile compounds were quantified by GC-MS.
2.6.2 Evaluation of fermentation kinetics

Ethanol production and CO₂ evolution throughout the fermentation were monitored to determine the fermentation kinetics; CO₂ evolution was measured by keeping track of fermentation weight loss. Experimental fermentation bottles were weighed before and after taking samples for ethanol production at appropriate time intervals. Sampling was done daily in the early phase of fermentation, every other day in the middle phase, and finally every two days in the final stage.

2.6.3 Quantification of ethanol, glycerol, and acetic acid

The ethanol, glycerol, and acetic acid produced by the yeast strains were quantified with the high pressure liquid chromatography (HPLC) method as outlined in section 2.6.6. Samples taken from fermentations were vortexed, centrifuged, filter sterilized (0.22 µm), and placed in to HPLC glass vials prior to HPLC analysis. Ethanol and glycerol production were monitored over the course of the fermentation at time intervals described in section 2.6.2, while acetic acid production was measured at the end of the fermentation.

2.6.4 Conversion of sugar to ethanol

The sugar in Chardonnay must was analyzed by HPLC using the method outlined in section 2.6.6. The final ethanol and sugar concentrations in the samples of the last day of fermentation were analyzed by HPLC (Section 2.6.7).

After determination of sugar in Chardonnay must before inoculation and amount of sugar and ethanol in samples after fermentations were completed, the conversion
factors for each individual industrial, individual Burgundian and mixed Burgundian strains were calculated using a formula which divides the total ethanol produced (% m/v) by the total glucose and fructose consumed (% m/v) during fermentation of Chardonnay wines at 16 °C and 20 °C.

2.6.5 Ethanol tolerance assay

Ethanol tolerance of the *S. cerevisiae* strains was evaluated by fermenting each strain in biological triplicate in 200 ml of Chardonnay must supplemented to 32% sugar with equimolar amounts of glucose and fructose at 16 °C and 20 °C. Initial amount of ethanol, glucose, and fructose was measured at the beginning of fermentation and then monitored through the end of fermentation when no more fermentative activity was seen. The final amount of glucose, fructose, and ethanol was measured using the HPLC method described in section 2.6.7.

2.6.6 Compatibility with malolactic fermentation

Malolactic compatibility of *S. cerevisiae* strains was assessed by conducting fermentations in biological triplicate in 200 mL of Chardonnay must at 20 °C. The fermentations with *S. cerevisiae* strains were monitored; when the sugar content of the must was depleted and alcoholic fermentation was completed, the fermentations were inoculated with *O. oeni* (strain MBR 31). Malolactic fermentations were monitored by collecting samples every three days until the fermentations were completed (21 days). Concentrations of malic and lactic acids at the first and final point of the malolactic fermentations were measured using HPLC as described in section 2.6.7.
2.6.7 Quantification of compounds using HPLC

An Agilent 1100 high pressure liquid chromatograph (Agilent Technologies, Palo Alto, USA) equipped with an Agilent G1362A refractive index detector (RID) and a Supelcogel C-61OH 30 cm × 7.8 mm column was employed to separate and quantify ethanol, glycerol, acetic acid, glucose, and fructose (Adams and van Vuuren, 2010). The mobile phase was 0.01 % phosphoric acid. Both ethanol and sugar analysis were performed in the HPLC using different flow rates. The column flow and total run time for ethanol quantification were 1.4 mL/minute and 12 minutes, respectively; the column temperature and RID temperate in ethanol assessment were 55 ºC and 35 ºC, respectively. Column flow and total run time for sugar analysis were adjusted to 0.75 mL/minute and 22 minutes. Column temperature and RID were 50º C and 35º C, respectively. Data analyses were conducted using LC- MS Chemstation revision A.09.03 software.

An Agilent 1100 HPLC equipped with an Agilent G1362A refractive index detector (RID) and Macherey – Nagel Nucleogel 300 × 7.8 mm ION 300 OA column (Macherey- Nagel, Duren, Germnay) was used for separating and quantifying malic acid and lactic acid. The mobile phase was 0.00425 M sulphuric acid. Column temperature was held at 50º C for 30 minutes. The RID temperature was 40º C. Data was analyzed using LC- MS Chemstation revision A.09.03 software.

2.6.8 Sulphur dioxide assay

Production of sulphur dioxide by S. cerevisiae strains was assessed by conducting fermentations in biological triplicate in 200 mL of synthetic juice at 16 ºC and 20 ºC. Sulphur dioxide in fermentations was quantified using the “Total SO₂” UV test kit from R-Biopharm (Darmstadt, Germany). Synthetic juice was assayed prior to fermentation to
ensure it was free from sulfite compounds. Synthetic juice was made according to the instructions adapted from [Denayrolles et al. (1995)](http://example.com). The synthetic juice contained 100 g/L glucose, 100 g/L fructose, 4.5 g/L L-malic acid, 0.3 g/L citric acid, 4.5 g/L tartric acid, 2 g/L Ammonium Sulphate, 1.7 g/L yeast nitrogen base without amino acids and ammonium sulphate (Difco). Additionally, 1 mL/L of Tween 80 was added to the synthetic juice. The pH of the synthetic juice was adjusted to 3.2-3.5 using KOH pellets and then filter sterilized (Husnik et al., 2006).

### 2.6.9 Foam production assay

Foam production by *S. cerevisiae* strains was measured at 16 °C and 20 °C using a modified assay from [Regodon et al. (1997)](http://example.com). Yeast cells were prepared for fermentation as described in section 2.6.1. Cells were inoculated into 18×150 mm test tubes containing 10 mL of Chardonnay must. The height of produced foam was measured three times per day and the highest level recorded in millimeters.

### 2.6.10 Growth phenotype assay

The growth of *S. cerevisiae* strains were measured using a Bioscreen C Growth Chamber (Thermo- Labsystems, Franklin, USA) in filter sterile (0.22 µm) White Salmon Chardonnay must. The *S. cerevisiae* strains were grown to stationary phase in 5 mL YPD broth at 30 °C overnight in a rotary wheel. The cells were subsequently inoculated into fresh 5 ml YPD broth at a rate of 2×10⁶ cells/mL and incubated overnight at 30 °C in a rotary wheel. Cells (1×10⁷) were harvested by centrifuge (5,000 × gravity) for 5 minutes and washed with filter sterile (0.22 µm) White Salmon Chardonnay must and resuspended in 200 µL of filter sterile (0.22 µm) White Salmon Chardonnay must. White
Salmon Chardonnay must was inoculated at the rate of $5 \times 10^5$ cells/mL and 150 µL aliquots in technical replicate were transferred into a 100-well Bioscreen C optical plate (Thermo-Labsystems, Franklin, USA). This plate was placed in the growth chamber, where cells were grown for 96 hours with continuous shaking. The optical density (OD) of cells was automatically measured hourly at a wavelength of 600 nm and data was collected by the Biolink-doc software. The log of the collected OD values was plotted versus time to show growth kinetics of the *S. cerevisiae* strains at 16 ºC and 20 ºC.

2.7 Quantification of volatile compounds by Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS headspace analysis was utilized to analyze volatile compounds of Chardonnay wine, according to the method utilized by Danzer et al. (1999). This method was without solid phase microextraction (SPEM) and 20 mL headspace vials containing 3 grams of NaCl and 10 mL of Chardonnay wine were placed into the autosampler of the GS-MS. Wine sample were equilibrated at 85 ºC for 10 minutes with agitation.

An Agilent 6890N gas chromatograph connected to a 5973N Mass Selective Detector (MSD) equipped with a 60 m × 0.25 mm ID, 0.25 µm thicknesses DBWAX fused silica open tubular column (J and W Scientific, Folstom, USA) was used to separate, detect, and quantify volatile compounds at the constant rate of 1.3 mL/minute using ultra high purity helium gas. The headspace sample (1 mL) was then injected. The headspace sample valve was maintained at 100 ºC, while the temperature of the transfer line was kept at 110 ºC. Initial temperature of the GC oven was kept at 40 ºC for 5 minutes and was raised to 100 ºC at a rate of 5 ºC/minute, then increased to 200 ºC at a rate of 20 ºC/minute. The Mass Spectrometer (MS) was set in scan mode with mass range
of 35-400 Atomic Mass Unit (AMU) and each sample was quantified in technical triplicates; 3-Octanol was used as an internal standard.

Volatile compounds were identified by GC-MS. Data was analyzed using Enhanced Chemstation software (MSD Chemstation Build 75, Agilent Technologies, Palo Alto, USA) and peaks were matched against the Wiley7Nist05 library (Wiley and Sons, Hoboken, USA).

Data were analyzed using Enhanced Chemstation software (MSD Chemstation Build 75, Agilent Technologies, Palo Alto, USA) and peaks were matched against the Wiley7Nist05 library (Wiley and Sons, Hoboken, USA).

Analyzed compounds were divided in two groups; i) quantifiable compounds, where their peaks had a signal to noise ratio greater than 10, ii) non-quantifiable detectable compounds where the signal to noise ratios of their peaks were greater than 3 and less than 10.

2.8 Statistical analysis

A two-factor analysis of variance (ANOVA) with replication was conducted to evaluate the effect of yeast strain and temperature on the enological traits including production of ethanol, glycerol, acetic acid, sulphur dioxide, ethanol tolerance, foam formation, and final optical density of yeast strains. Differences among yeast strains were determined using Fisher’s least significant difference (LSD) test (p < 0.05). Differences in enological properties between fermentation temperatures were evaluated using Fisher’s LSD test (p < 0.05). A one-factor ANOVA was conducted to evaluate the effect of yeast strains on malolactic fermentation; the effect of temperature was not investigated as malolactic fermentation was conducted only at one temperature.
Principal Component Analysis (PCA) was conducted on mean enological traits including production of ethanol, glycerol, acetic acid, sulphur dioxide, foam and ethanol tolerance using a correlation matrix. PCA allowed the relationships among the enological traits and yeast strains to be evaluated. PC I, PC II and PC III were calculated for the enological properties, and plots were prepared for PCI versus PC II. Plots of PC II versus PC III and PC I versus PC III were not included in the thesis since the yeast groupings were consistent in the other dimensions. Vector coordinates were scaled as three times the sample coordinates, to allow for more effective plots.

A radar diagram was used to summarize the effect of temperature on enological traits for the mixed Burgundian strains. The mean values were connected to create an enological profile for each of the fermentation temperatures. The radar diagram was created in Excel, by standardizing the enological values to a common scale (0-12). Mean values for the yeast strains for ethanol (Tables 3), glycerol (Table 4), acetic acid (Table 5), ethanol tolerance (Table 6), sulphur dioxide (Table 9) and foam production (Table 10) were multiplied by the following constants respectively: 0.71, 1.43, 20, 0.56, 0.25 and 0.91.

A two-factor ANOVA with replication was conducted to evaluate the effect of yeast strains and temperature on the production of volatile compounds. The effect of temperature and yeast × temperature interaction were not significant (p > 0.05) for any of the volatile compounds (Table 29); therefore, only the main effect for yeast strain was reported. Differences among yeast strains were determined using Fisher’s least significant difference (LSD) test (p < 0.05). Since the temperature effect was not significant, the yeast effect was calculated for the average of the two fermentation temperatures.
PCA using the correlation matrix was conducted on mean concentrations of the volatile compounds averaged across both fermentation temperatures, for the six individual industrial, two individual Burgundian, and four mixed Burgundian strains. PCA assessed the relationships among yeast strains according to their volatile profile. PC I, PC II and PC III were calculated for the volatile compounds, a plot was prepared for PCI versus PC II only. Plots of PC II versus PC III and PC I versus PC III were not reported since the yeast groupings were consistent in the other dimensions. Vector coordinates were scaled as three times the sample coordinates, to allow for more effective plots.

Cluster analysis was conducted to group the yeast strains by the similarities in their volatile compounds. Cluster analysis was conducted on the average of volatile concentrations across the two fermentation temperatures using the average linkage method and Euclidean distances.

Radar diagrams were used to create odour profiles and estimate sensory profiles of the volatile compounds in Chardonnay wines. To create the odour profile, mean odourant concentrations (mg/L) of volatile compounds were calculated for yeast groups [individual Burgundian (n=12), mixed Burgundian strains (n=24)], as determined by GC-MS and averaged across both fermentation temperatures. The odourant concentrations (mg/L) of individual industrial strains (n=6) were used for radar diagrams. The concentrations of volatile compounds were scaled to represent the volatiles at the same plot. Mean values of the volatile compounds 2,3-butanediol, 2-methyl-1-butanol, 3-methyl-1-butanol, n-butanol, 1-hexanol, isobutanol, phenylethanol, propanol, ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl laurate, ethyl acetate,
hexyl acetate, isoamyl acetate, acetaldehyde, and acetic acid were multiplied by the following constants 1, 1, 0.1, 10, 1, 0.05, 1, 0.2, 5, 40, 10, 40, 200, 0.1, 50, 10, 1 and 3.

The relative impacts of the volatile compounds were represented in the estimated odour profile using odour Active Values (OAV). OAV of individual volatile compounds were calculated by dividing the odour concentration by the odourant threshold (mg/L) (Rothe and Thomas, 1963). The log of OAV was calculated to evaluate the relative sensory contribution of the odourant (Yilmaz, 2001). The water threshold for each odourant was obtained from the literature. The OAV of the individual Burgundian strains, mixed Burgundian strains, and two individual industrial strains were plotted on each radar diagram.

The ANOVA and radar plots were calculated using MS Excel (Redmond USA), the PCA and cluster analysis were conducted using Minitab 15 (State College, USA).
3 RESULTS

3.1 Genetic and phenotypic characterization of wine yeast strains

3.1.1 Genetic fingerprinting differentiated two Burgundian and six industrial strains

Genetic fingerprinting successfully differentiated two Burgundian and six industrial strains based on their differences in the chromosomal regions between δ sequences. Heterogeneous patterns of the δ sequence regions of two individual Burgundian and six individual industrial strains are indicated in Figure 1.

![Genetic fingerprinting](image)

**Figure 1.** Genetic fingerprints of two individual Burgundian and six individual industrial *S. cerevisiae* yeast strains. δ sequence typing of all strains is shown. The 1 kilobase (kb) DNA ladder obtained from Fermentaz was used as standard size.

The individual Burgundian strains C2 and C6 shared four common bands around 350, 450, 750 and 1000 bp (Figure 1); C6 had an additional band around 250 bp which distinguished it from C2. Elegance was differentiated from Fusion with extra bands between 300 and 500 bp. Blanc had common bands with C2 and C6 around 450, 750, and
1000 bp and an extra band from 250 and less to 350 bp. The CY3079 yeast strain had a distinct pattern regarding bands around 350 bp. Similarly, ICV- D254 and ZYMAFLORE X16 had bands in different areas reflecting different patterns of δ sequence regions.

### 3.1.2 Individual Burgundian strains C2 and C6 are killer positive

The killer phenotype was assayed for individual Burgundian strains C2 and C6. After five days incubation of the plate at 18 ºC, the positive control strain (K⁺), Elegance, was surrounded by a blue-ringed clear zone due to the death of the seeded sensitive strain. In contrast, no clear zone was formed around the negative control strain (K⁻), Maurivin B (Figure 2).

![Figure 2](image)

**Figure 2.** Killer phenotype assay of novel individual Burgundian strains C2 and C6. (A) back side of plate and (B) front side of plate. Strains were plated onto a lawn (5 × 10⁸ cells/ml) of sensitive strain Maurivin B strain and grown at 18 ºC. The positive control (K⁺) strain Elegance was surrounded by a clear zone; no inhibition zone was observed around Maurivin B strain (K⁻). The C2 and C6 strains were surrounded by an inhibition zone indicating positive killer activity.

The individual Burgundian strains C2 and C6 were surrounded by an inhibition zone and a dark blue ring, the same as the K⁺ strain. Therefore, both Burgundian strains,
C2 and C6, were killer positive (Figure 2). The positive killer activity is a desirable factor for Burgundian strains to inhibit the growth of sensitive wild yeasts.

The killer phenotypes of industrial strains were not assayed as the information was available from yeast manufacturers. The killer phenotypes of all strains in this study are indicated in Table 2.

**Table 2.** Killer phenotype of *S. cerevisiae* strains used in this study.

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Killer phenotype</th>
<th>Phenotype information obtained from</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elegance</td>
<td>Killer Positive</td>
<td>Maurin Yeast Australia</td>
</tr>
<tr>
<td>Maurivin B</td>
<td>Sensitive</td>
<td>Maurin Yeast Australia</td>
</tr>
<tr>
<td>Individual industrial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blanc</td>
<td>Killer Positive</td>
<td>Maurin Yeast Australia</td>
</tr>
<tr>
<td>Elegance</td>
<td>Killer Positive</td>
<td>Maurin Yeast Australia</td>
</tr>
<tr>
<td>Fusion</td>
<td>Sensitive</td>
<td>Maurin Yeast Australia</td>
</tr>
<tr>
<td>CY3079</td>
<td>Sensitive</td>
<td>Lallemand Wine Inc</td>
</tr>
<tr>
<td>ICV-D254</td>
<td>Killer Positive</td>
<td>Lallemand Wine Inc</td>
</tr>
<tr>
<td>X16</td>
<td>Sensitive</td>
<td>Laffort</td>
</tr>
<tr>
<td>Individual Burgundian</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>Killer Positive</td>
<td>Current Study</td>
</tr>
<tr>
<td>C6</td>
<td>Killer Positive</td>
<td>Current Study</td>
</tr>
</tbody>
</table>
3.2 Enological characteristics of wine yeast strains

3.2.1 All wine yeast strains showed similar fermentation kinetics

The fermentation kinetics of the individual industrial, individual Burgundian and mixed Burgundian wine yeast strains in Chardonnay must were evaluated as shown in Figure 3.

Figure 3. Fermentation kinetics of individual industrial, individual Burgundian and mixed Burgundian *S. cerevisiae* strains in Chardonnay must. (A) Ethanol production during fermentation at 16 °C. (B) CO$_2$ evolution during fermentation at 16 °C. (C) Ethanol production during fermentation at 20 °C. (D) CO$_2$ evolution during fermentation at 20 °C.

The fermentation kinetics of *S. cerevisiae* strains were slightly different at both temperatures (Figure 3). The two individual industrial strains Elegance and X16 had a higher rate of ethanol production than other strains at both 16 °C and 20 °C. However,
the final ethanol concentration was similar to the other strains (Figure 3, A and C). These kinetic patterns were observed for the evolution of CO₂ at 16 °C and 20 °C (Figure 3, B and D).

3.2.2 Ethanol production varied across yeast strains and the two fermentation temperatures

The ethanol production by individual industrial, individual Burgundian and mixed Burgundian yeast stains in Chardonnay wine fermented at 16 °C and 20 °C is indicated in Figure 4.

![Ethanol concentration graph](image)

**Figure 4.** Ethanol concentration of Chardonnay wines fermented at 16 °C and 20 °C with six individual industrial, two individual Burgundian and four mixed Burgundian *S. cerevisiae* strains (n=3). Error bars indicate standard errors.

Some significant (p ≤ 0.05, F = 4.43) differences in ethanol production were found among yeast strains. Yeast strains produced significantly (p ≤ 0.05, F = 779.95) higher amounts of ethanol at 16 °C than 20 °C (Table 3).
Ethanol concentration at 16 °C ranged from 13.86 % (v/v) for Fusion to 14.27 % (v/v) for CY3079. At 20 °C, ethanol production ranged from 12.87 % (v/v) for Fusion to 13.33 % (v/v) for CY3079.

Table 3. Final ethanol concentration (% v/v) of Chardonnay wines fermented at 16 °C and 20 °C with six individual industrial, two individual Burgundian and four mixed Burgundian S. cerevisiae strains (n=3). Strain and temperature effects are shown. A, B indicate strain effect for each temperature. C shows the temperature effect.

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>16 °C A</th>
<th>20 °C B</th>
<th>Temperature effect C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual Industrial Strains</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blanc</td>
<td>14.13</td>
<td>13.26</td>
<td>s</td>
</tr>
<tr>
<td>Elegance</td>
<td>14.21</td>
<td>13.32</td>
<td>s</td>
</tr>
<tr>
<td>Fusion</td>
<td>13.86a</td>
<td>12.87a</td>
<td>s</td>
</tr>
<tr>
<td>CY3079</td>
<td>14.27g</td>
<td>13.33j</td>
<td>s</td>
</tr>
<tr>
<td>ICV-D254</td>
<td>14.04bcde</td>
<td>13.26hi</td>
<td>s</td>
</tr>
<tr>
<td>X16</td>
<td>14.15fg</td>
<td>13.24ghi</td>
<td>s</td>
</tr>
<tr>
<td>Individual Burgundian Strains</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>14.01abcd</td>
<td>13.23fghi</td>
<td>s</td>
</tr>
<tr>
<td>C6</td>
<td>14.02abcd</td>
<td>13.12defgh</td>
<td>s</td>
</tr>
<tr>
<td>Mixed Burgundian Strains</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C2/C6:1/1), M1</td>
<td>13.94abc</td>
<td>13.10cdefgh</td>
<td>s</td>
</tr>
<tr>
<td>(C2/C6:1/2), M2</td>
<td>14.01abcd</td>
<td>13.19defghi</td>
<td>s</td>
</tr>
<tr>
<td>(C2/C6:1/3), M3</td>
<td>14.10cdef</td>
<td>13.10cdefghi</td>
<td>s</td>
</tr>
<tr>
<td>(C2/C6:3/2), M4</td>
<td>14.02abcd</td>
<td>13.06bcde</td>
<td>s</td>
</tr>
<tr>
<td>Range</td>
<td>13.86-14.27</td>
<td>12.87-13.33</td>
<td></td>
</tr>
</tbody>
</table>

A, B The mean values of the biological replicates of each strain are indicated (n=3) at 16 °C and 20 °C. At a given temperature yeast strains with different superscripts are significantly (p ≤ 0.05) different. C's indicates significant difference between the fermentation temperatures for each yeast strain (p ≤ 0.05).

The ethanol concentrations produced by individual and mixed Burgundian strains were intermediate compared to other yeasts at both temperatures. At 16 °C, individual and mixed Burgundian strains varied significantly (p ≤ 0.05) from the industrial strain CY3079. When the fermentation temperature was increased to 20 °C, the individual and
mixed Burgundian strains produced significantly \((p \leq 0.05)\) lower and higher concentrations of ethanol than the individual industrial strains Fusion and CY3079, respectively.

### 3.2.3 Conversion of sugar to ethanol by wine yeast strains

The sugar to ethanol conversion factors for each individual industrial, individual Burgundian and mixed Burgundian *S. cerevisiae* strains were calculated during fermentation of Chardonnay wines at 16 °C and 20 °C (Table 4).

No significant \((p > 0.05, F = 1.06)\) differences existed in conversion factors among yeasts strains at each temperature. The conversion factors of yeasts at 16 °C on average were significantly \((p \leq 0.05, F = 499.99)\) greater than those at 20 °C. At 16 °C, the conversion factors ranged from 0.459 for Fusion and X16 to 0.465 for Elegance, CY3079 and C2. The conversion factors ranged from 0.425 for Fusion to 0.437 for Elegance, when the fermentation temperature was increased to 20 °C.
Table 4. Conversion ratios (ethanol produced / sugar consumed) by six individual industrial, two individual Burgundian and four mixed Burgundian *S. cerevisiae* strains in Chardonnay wines (n=3). Strain and temperature effects are shown. A, B indicate strain effect for each temperature. C shows the temperature effect.

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>16 °C&lt;sup&gt;A&lt;/sup&gt;</th>
<th>20 °C&lt;sup&gt;B&lt;/sup&gt;</th>
<th>Temperature effect&lt;sup&gt;C&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Individual Industrial Strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blanc</td>
<td>0.463&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.431&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ns</td>
</tr>
<tr>
<td>Elegance</td>
<td>0.465&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.437&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ns</td>
</tr>
<tr>
<td>Fusion</td>
<td>0.459&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.425&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ns</td>
</tr>
<tr>
<td>CY3079</td>
<td>0.465&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.436&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ns</td>
</tr>
<tr>
<td>ICV-D254</td>
<td>0.460&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.435&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ns</td>
</tr>
<tr>
<td>X16</td>
<td>0.459&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.431&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Individual Burgundian Strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>0.465&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.426&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ns</td>
</tr>
<tr>
<td>C6</td>
<td>0.462&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.433&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Mixed Burgundian Strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C2/C6:1/1), M1</td>
<td>0.464&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.426&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ns</td>
</tr>
<tr>
<td>(C2/C6:1/2), M2</td>
<td>0.463&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.431&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ns</td>
</tr>
<tr>
<td>(C2/C6:1/3), M3</td>
<td>0.463&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.433&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ns</td>
</tr>
<tr>
<td>(C2/C6:3/2), M4</td>
<td>0.463&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.431&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>0.459-0.465</td>
<td>0.425-0.437</td>
<td></td>
</tr>
</tbody>
</table>

<sup>A,B</sup> The mean values of the biological replicates of each strain are indicated (n=3) at 16 °C and 20 °C. At a given temperature yeast strains with different superscripts are significantly (p ≤ 0.05) different.

<sup>C</sup> ns indicates no significant difference between the fermentation temperatures for each yeast strain (p ≤ 0.05).

3.2.4 Glycerol concentration varied significantly among wine yeast strains and fermentation temperatures

The glycerol production by individual industrial, individual Burgundian and mixed Burgundian yeast strains was quantified in Chardonnay wine fermented at 16 °C and 20 °C (Figure 5).
Figure 5. Glycerol concentration of Chardonnay wines fermented at 16 °C and 20 °C with six individual industrial, two individual Burgundian and four mixed Burgundian *S. cerevisiae* strains (n=3). Error bars indicate standard errors.

Some significant differences in glycerol production were found between yeast strains (p ≤ 0.05, F = 67.44) and fermentation temperatures (p ≤ 0.05, F = 6.02) (Table 5).

Glycerol concentration at 16 °C fell in the range of 4.45 g/L for ICV-D254 to 6.3 g/L for Fusion. Glycerol concentration at 20 °C ranged from 4.58 g/L in wine inoculated with ICV-D254 to 6.11 g/L in wine fermented with Fusion. The glycerol concentrations in wines fermented by individual Burgundian and mixed Burgundian strains fit into the middle of the range and were significantly (p ≤ 0.05) different from the glycerol concentration in wines fermented with ICV-D254 and Fusion strains, which produced the lowest and highest amounts of glycerol, respectively. The glycerol concentrations in wines fermented with individual Burgundian and mixed Burgundian strains were significantly (p ≤ 0.05) higher than glycerol concentration in wines fermented by individual industrial strains which produced low levels of glycerol.
Blanc produced significantly higher amount of glycerol at 20 °C than 16 °C, while M1 produced significantly lower amount of glycerol at 20°C than 16 °C. No significant difference existed in glycerol production by other yeast strains between two fermentation temperatures.

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>16 °C A</th>
<th>20 °C B</th>
<th>Temperature effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Individual Industrial Strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blanc</td>
<td>4.99e</td>
<td>5.71j</td>
<td>s</td>
</tr>
<tr>
<td>Elegance</td>
<td>5.43efg</td>
<td>5.43defgh</td>
<td>ns</td>
</tr>
<tr>
<td>Fusion</td>
<td>6.30k</td>
<td>6.11k</td>
<td>ns</td>
</tr>
<tr>
<td>CY3079</td>
<td>4.78b</td>
<td>4.85b</td>
<td>ns</td>
</tr>
<tr>
<td>ICV-D254</td>
<td>4.45a</td>
<td>4.58a</td>
<td>ns</td>
</tr>
<tr>
<td>X16</td>
<td>5.67i</td>
<td>5.69ij</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Individual Burgundian Strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>5.48fghi</td>
<td>5.44efgh</td>
<td>ns</td>
</tr>
<tr>
<td>C6</td>
<td>5.49ghi</td>
<td>5.30cde</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Mixed Burgundian Strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C2/C6:1/1), M1</td>
<td>5.55ij</td>
<td>5.30cde</td>
<td>s</td>
</tr>
<tr>
<td>(C2/C6:1/2), M2</td>
<td>5.43efg</td>
<td>5.50gh</td>
<td>ns</td>
</tr>
<tr>
<td>(C2/C6:1/3), M3</td>
<td>5.35defg</td>
<td>5.49fgh</td>
<td>ns</td>
</tr>
<tr>
<td>(C2/C6:3/2), M4</td>
<td>5.53hij</td>
<td>5.54h</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>4.45-6.3</td>
<td>4.58-6.11</td>
<td></td>
</tr>
</tbody>
</table>

A, B indicate strain effect for each temperature. C shows the temperature effect. 

Table 5. Final glycerol concentrations (% v/v) of Chardonnay wines fermented at 16 °C and 20 °C with six individual industrial, two individual Burgundian and four mixed Burgundian S. cerevisiae strains (n=3). Strain and temperature effects are shown. A, B indicate strain effect for each temperature. C shows the temperature effect.

The mean values of the biological replicates of each strain are indicated (n=3) at 16 °C and 20 °C. At a given temperature yeast strains with different superscripts are significantly (p ≤ 0.05) different. s and ns indicate significant and no significant difference between the fermentation temperatures for each yeast strain (p ≤ 0.05).
3.2.5 Acetic acid concentration varied significantly among wine yeast strains and fermentation temperatures

The acetic acid production by individual industrial, individual Burgundian and mixed Burgundian yeast stains was quantified in Chardonnay wine fermented at 16 °C and 20 °C (Figure 6). Some significant differences in acetic acid production were found between yeast strains (p ≤ 0.05, F =7.32) and fermentation temperatures (p ≤ 0.05, F =4.37) (Table 6).

Acetic acid concentration ranged from 0.095 g/L for Blanc to 0.181 g/L for Elegance at 16 °C. When the fermentation temperature was increased to 20 °C, acetic acid concentration ranged from 0.077 g/L for Blanc to 0.178 g/L for CY3079.

![Graph showing acetic acid concentration for different yeast strains at 16 °C and 20 °C.](image)

**Figure 6.** Acetic acid concentration of Chardonnay wines fermented at 16 °C and 20 °C with six individual industrial, two individual Burgundian and four mixed Burgundian *S. cerevisiae* strains (n=3). Error bars indicate standard errors.

The individual Burgundian strain C2 produced significantly higher amount of acetic acid than C6 at 16 °C, while no significant difference existed between C2 and C6 in terms of acetic acid production at 20 °C. The individual Burgundian strain C6 and
mixed Burgundian strains fit into the middle range of acetic acid production at 16 °C and were significantly (p ≤ 0.05) different from Blanc and Elegance. This pattern was not detected at 20 °C, as individual Burgundian strains, M1 and M2 fit into the low end and M3 and M4 were toward the high end of the range acetic acid production.

Elegance, C2, C6 and M1 produced significantly (p ≤ 0.05) higher amounts of acetic acid at 16°C than 20 °C, while Fusion and CY3079 produced significantly (p ≤ 0.05) lower amounts of acetic acid at 16°C than 20 °C.

Table 6. Final acetic acid concentrations (% v/v) of Chardonnay wines fermented at 16 °C and 20 °C with six individual industrial, two individual Burgundian and four mixed Burgundian S. cerevisiae strains (n=3). Strain and temperature effects are shown. A,B indicate strain effect for each temperature. C shows the temperature effect.

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>16 °C A</th>
<th>20 °C B</th>
<th>Temperature effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Individual Industrial Strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blanc</td>
<td>0.095 a</td>
<td>0.077 a</td>
<td>ns</td>
</tr>
<tr>
<td>Elegance</td>
<td>0.181 h</td>
<td>0.138 d e</td>
<td>s</td>
</tr>
<tr>
<td>Fusion</td>
<td>0.103 a</td>
<td>0.137 e d</td>
<td>s</td>
</tr>
<tr>
<td>CY3079</td>
<td>0.153 e fg</td>
<td>0.178 g</td>
<td>s</td>
</tr>
<tr>
<td>ICV-D254</td>
<td>0.166 g h</td>
<td>0.171 f g</td>
<td>ns</td>
</tr>
<tr>
<td>X16</td>
<td>0.117 a b c</td>
<td>0.112 b c d</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Individual Burgundian Strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>0.165 f g h</td>
<td>0.097 a b</td>
<td>s</td>
</tr>
<tr>
<td>C6</td>
<td>0.132 b c d e</td>
<td>0.086 a b</td>
<td>s</td>
</tr>
<tr>
<td><strong>Mixed Burgundian Strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C2/C6:1/1), M1</td>
<td>0.116 a b c</td>
<td>0.085 a b</td>
<td>s</td>
</tr>
<tr>
<td>(C2/C6:1/2), M2</td>
<td>0.119 a b c d</td>
<td>0.112 b c d</td>
<td>ns</td>
</tr>
<tr>
<td>(C2/C6:1/3), M3</td>
<td>0.142 c d e f g</td>
<td>0.149 e f</td>
<td>ns</td>
</tr>
<tr>
<td>(C2/C6:3/2), M4</td>
<td>0.147 d e f g</td>
<td>0.149 e f</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>0.095-0.181</td>
<td>0.077-0.178</td>
<td></td>
</tr>
</tbody>
</table>

A,B The mean values of the biological replicates of each strain are indicated (n=3) at 16 °C and 20 °C.

At a given temperature yeast strains with different superscripts are significantly (p ≤ 0.05) different.

C, s and ns indicate significant and no significant difference between the fermentation temperatures for each yeast strain (p ≤ 0.05).
3.2.6 Ethanol tolerance varied significantly across wine yeast strains and fermentation temperatures

The ethanol tolerance of individual industrial, individual Burgundian and mixed Burgundian yeast stains was quantified in Chardonnay wine fermented at 16 °C and 20°C. Chardonnay must was supplemented to 32% sugar with equimolar amounts of glucose and fructose (Figure 7).

![Ethanol Concentration Graph](image)

**Figure 7.** Ethanol tolerance of six individual industrial, two individual Burgundian and four mixed Burgundian *S. cerevisiae* strains in Chardonnay wines fermented at 16 °C and 20 °C (n=3). Error bars indicate standard errors.

Significant (*p* ≤ 0.05, *F* = 39.86) differences existed in final ethanol concentration produced by yeast strains at each temperature. Elegance, Fusion, CY3079, ICV-D254, C2, C6 and M2 produced significantly (*p* ≤ 0.05, *F*=19.03) different amounts of the ethanol between the two temperatures. Ethanol concentration changed from 15.19 % (v/v) for Fusion to 18.48 % (v/v) for X16 at 16 °C. Final ethanol concentration at 20 °C changed between 15.67-18.74 % (v/v) for ICV-D254 and X16, respectively. Ethanol production at 16 °C by C6 and mixed Burgundian strains M1, M2 and M3 were
toward the middle of the range. Ethanol production by C2, M1 and M3 was intermediate in ethanol production by yeasts at 20 °C, while ethanol production by C6 and M2 was toward the low end of the range. The ethanol production by the mixed Burgundian strain M4 was toward the high end of the spectrum at either temperature.

Table 7. Ethanol tolerance (% v/v) of six individual industrial, two individual Burgundian and four mixed Burgundian *S. cerevisiae* strains in Chardonnay wines fermented at 16 °C and 20 °C (n=3). Strain and temperature effects are shown. A, B indicate strain effect for each temperature. C shows the temperature effect.

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>16 °C</th>
<th>20 °C</th>
<th>Temperature effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Individual Industrial Strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blanc</td>
<td>17.26ghi</td>
<td>17.52gh</td>
<td>ns</td>
</tr>
<tr>
<td>Elegance</td>
<td>18.32jk</td>
<td>16.29b</td>
<td>s</td>
</tr>
<tr>
<td>Fusion</td>
<td>15.19a</td>
<td>16.85f</td>
<td>s</td>
</tr>
<tr>
<td>CY3079</td>
<td>17.37i</td>
<td>16.69ef</td>
<td>s</td>
</tr>
<tr>
<td>ICV-D254</td>
<td>16.60edef</td>
<td>15.67a</td>
<td>s</td>
</tr>
<tr>
<td>X16</td>
<td>18.48k</td>
<td>18.74i</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Individual Burgundian Strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>17.37i</td>
<td>16.62edef</td>
<td>s</td>
</tr>
<tr>
<td>C6</td>
<td>16.66edef</td>
<td>15.94a</td>
<td>s</td>
</tr>
<tr>
<td><strong>Mixed Burgundian Strains</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(C2/C6:1/1), M1</td>
<td>16.90f</td>
<td>16.63edef</td>
<td>ns</td>
</tr>
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<td>(C2/C6:1/2), M2</td>
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<td>15.90a</td>
<td>s</td>
</tr>
<tr>
<td>(C2/C6:1/3), M3</td>
<td>16.52bede</td>
<td>16.85f</td>
<td>ns</td>
</tr>
<tr>
<td>(C2/C6:3/2), M4</td>
<td>17.28ghi</td>
<td>17.54h</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>15.19-18.48</td>
<td>15.9-18.74</td>
<td></td>
</tr>
</tbody>
</table>

A,B The mean values of the biological replicates of each strain are indicated (n=3) at 16 °C and 20 °C. At a given temperature yeast strains with different superscripts are significantly (*p* ≤ 0.05) different. C's and ns indicate significant and no significant difference between the fermentation temperatures for each yeast strain (*p* ≤ 0.05).
3.2.7 Compatibility with malolactic bacteria varied significantly among wine yeast strains

The compatibility of the six individual industrial, two individual Burgundian and four mixed Burgundian \textit{S. cerevisiae} yeast stains with malolactic bacteria was evaluated in Chardonnay wine after the completion of alcoholic fermentation by \textit{S. cerevisiae} wine yeast strains at 20 °C (Figure 8). During malolactic fermentation, the trends of malic acid consumption and lactic acid production were monitored as indicated in Figure 8.

![Figure 8](image-url)

**Figure 8.** Malolactic compatibility of six individual industrial, two individual Burgundian and four mixed Burgundian \textit{S. cerevisiae} strains during malolactic fermentation in Chardonnay wines following alcoholic fermentation (n=3) at 20° C. Consumption of malic acid (g/L)(A) and production of lactic acid (g/L) (B).

No significant (p > 0.05, F = 2.19) differences existed in malic acid content of wines after malolactic fermentation. Chardonnay wines had significantly (p ≤ 0.05, F = 8.92) different concentrations of lactic acid after malolactic fermentation (Table 8, final columns).

The concentration of malic acid after alcoholic fermentation ranged from 4.634 g/L in wine fermented by ICV-D254 to 5.151 g/L in wine fermented by C6. Most of the wines contained significantly (p ≤ 0.05) different concentrations of malic acid after alcoholic fermentation. The initial concentration of malic acid in wines fermented by mixed Burgundian strains was toward the high end of the range.
Table 8. Malolactic compatibility of six individual industrial, two individual Burgundian and four mixed Burgundian *S. cerevisiae* strains in Chardonnay wines following alcoholic fermentation by at 20 °C (n=3). Initial columns indicate concentrations (g/L) of malic acid and lactic acid after alcoholic fermentation. Final columns indicate concentrations of malic acid and lactic acid after malolactic fermentation.

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Malic Acid (g/L)&lt;sup&gt;A&lt;/sup&gt;</th>
<th>Lactic Acid (g/L)&lt;sup&gt;B&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td><strong>Individual Industrial Strains</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blanc</td>
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<td>0.450&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Elegance</td>
<td>4.845&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.442&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fusion</td>
<td>4.650&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.464&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CY3079</td>
<td>5.018&lt;sup&gt;fghi&lt;/sup&gt;</td>
<td>0.442&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ICV-D254</td>
<td>4.634&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.435&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>X16</td>
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<td>0.439&lt;sup&gt;a&lt;/sup&gt;</td>
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<td><strong>Individual Burgundian Strains</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>4.798&lt;sup&gt;ed&lt;/sup&gt;</td>
<td>0.452&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C6</td>
<td>5.151&lt;sup&gt;j&lt;/sup&gt;</td>
<td>0.472&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Mixed Burgundian Strains</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C2/C6:1/1), M1</td>
<td>4.994&lt;sup&gt;efgh&lt;/sup&gt;</td>
<td>0.499&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(C2/C6:1/2), M2</td>
<td>5.042&lt;sup&gt;hi&lt;/sup&gt;</td>
<td>0.497&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(C2/C6:1/3), M3</td>
<td>5.093&lt;sup&gt;ij&lt;/sup&gt;</td>
<td>0.447&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(C2/C6:3/2), M4</td>
<td>5.023&lt;sup&gt;fghi&lt;/sup&gt;</td>
<td>0.507&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>4.634&lt;sup&gt;-&lt;/sup&gt;</td>
<td>0.435&lt;sup&gt;-&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5.151</td>
<td>0.507</td>
</tr>
</tbody>
</table>

<sup>A,B</sup> The mean values of the biological replicates of each strain are indicated (n=3) at 20 °C. Yeast strains with different superscripts are significantly (p ≤ 0.05) different.

The concentration of lactic acid after alcoholic fermentation varied from 0.031 g/L in wine fermented by Blanc to 0.088 g/L in wine fermented by Fusion. The wine fermented by the Fusion contained significantly (p ≤ 0.05) higher concentrations of lactic acid than other wines. The concentration of lactic acid after alcoholic fermentation in wines fermented by individual and mixed Burgundian strains was close to the high end of the spectrum.
No significant (p > 0.05) differences existed in concentration of malic acid among wines after malolactic fermentation. The final concentration of malic acid changed from 0.442 g/L in wines fermented by Elegance and CY3079 to 0.507 in wine fermented by the M4. The final concentration of lactic acid in wines after malolactic fermentation differed significantly (p ≤ 0.05) and changed from 2.928 g/L in wine fermented by the X16 to 3.275 g/L in wine fermented by C6. The final concentration of lactic acid in wines fermented by mixed Burgundian strains was toward the high end of the range.

3.2.8 Production of sulphur dioxide varied significantly across wine yeast strains and fermentation temperatures

The sulphur dioxide production by *S. cerevisiae* yeast strains was quantified at 16 °C and 20 °C (Figure 9). Some significant (p ≤ 0.05, F = 21.12) differences existed in sulphur dioxide concentrations produced by yeast strains at each temperature. The yeast strains produced significantly (p ≤ 0.05, F = 126.82) greater amount of sulphur dioxide at 16 °C than 20 °C with exception of Blanc and C6.

The sulphur dioxide production ranged from 17.7 mg/L for C6 to 52.91 mg/L for X16 at 16 °C. The sulphur dioxide production at 20 °C ranged from 15.29 mg/L to 30.69 mg/L for mixed Burgundian strains M3 and M4, respectively. The production of sulphur dioxide by all of the yeasts strains was greater at 16 °C than 20°C except for C6.
Figure 9. Sulphur dioxide concentration (mg/L) in Chardonnay wines fermented at 16 °C and 20 °C with six individual industrial, two individual Burgundian and four mixed Burgundian S. cerevisiae strains (n=3). Error bars indicate standard errors.

The C6 produced the lowest amount of sulphur dioxide at 16 °C. The sulphur dioxide production by C2 and the two mixed Burgundian strains M2 and M3 was toward the low end of the range of this study at 16 °C, while the other two mixed Burgundian, M1 and M4, fit into the middle range of this study. This pattern was different at 20 °C; M3 and M4 produced the highest and lowest amount of the sulphur dioxide, respectively. No significant (p ≤ 0.05) difference existed in sulphur dioxide production by M4 and X16 at 20 °C.
Table 9. Concentration of sulphur dioxide (mg/L) of Chardonnay wines fermented at 16 °C and 20 °C with six individual industrial, two individual Burgundian and four mixed Burgundian *S. cerevisiae* strains (*n*=3) using synthetic grape juice. Strain and temperature effects are shown. A, B indicate strain effect for each temperature. C shows the temperature effect.

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>16 °C A</th>
<th>20 °C B</th>
<th>Temperature effect C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Individual Industrial Strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blanc</td>
<td>27.90 de</td>
<td>23.56 fg</td>
<td>Ns</td>
</tr>
<tr>
<td>Elegance</td>
<td>32.65 f</td>
<td>25.52 h</td>
<td>S</td>
</tr>
<tr>
<td>Fusion</td>
<td>30.48 ef</td>
<td>21.7 defgh</td>
<td>S</td>
</tr>
<tr>
<td>CY3079</td>
<td>40.20 ij</td>
<td>25.21 gh</td>
<td>S</td>
</tr>
<tr>
<td>ICV-D254</td>
<td>41.23 i</td>
<td>20.67 cdef</td>
<td>S</td>
</tr>
<tr>
<td>X16</td>
<td>52.91 k</td>
<td>29.86 ij</td>
<td>S</td>
</tr>
<tr>
<td><strong>Individual Burgundian Strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>26.90 cde</td>
<td>20.06 bcdef</td>
<td>S</td>
</tr>
<tr>
<td>C6</td>
<td>17.17 a</td>
<td>19.43 a</td>
<td>Ns</td>
</tr>
<tr>
<td><strong>Mixed Burgundian Strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C2/C6:1/1), M1</td>
<td>38.44 hij</td>
<td>22.11 efgh</td>
<td>S</td>
</tr>
<tr>
<td>(C2/C6:1/2), M2</td>
<td>22.73 bc</td>
<td>16.53 a</td>
<td>S</td>
</tr>
<tr>
<td>(C2/C6:1/3), M3</td>
<td>21.29 ab</td>
<td>15.29 a</td>
<td>S</td>
</tr>
<tr>
<td>(C2/C6:3/2), M4</td>
<td>37.51 ghij</td>
<td>30.69 ij</td>
<td>S</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>17.17-52.91</td>
<td>15.29-30.69</td>
<td></td>
</tr>
</tbody>
</table>

A, B: The mean values of the biological replicates of each strain are indicated (*n*=3) at 16 °C and 20 °C.

At a given temperature yeast strains with different superscripts are significantly (*p* ≤ 0.05) different.

C: s and ns indicate significant and no significant difference between the fermentation temperatures for each yeast strain (*p* ≤ 0.05).

### 3.2.9 Foam production varied significantly across wine yeast strains and fermentation temperatures

The foam production of all *S. cerevisiae* yeast strains was measured during fermentation of Chardonnay must at 16 °C and 20 °C (Figure 10).
Figure 10. Foam formation (mm) by six individual industrial, two individual Burgundian and four mixed Burgundian *S. cerevisiae* strains during fermentation of Chardonnay must at 16 °C and 20 °C with (n=3). Error bars indicate standard errors.

Some significant (p ≤ 0.05, F = 16.09) differences were found in foam formation by yeast strains at either temperature. All yeast strains produced significantly (p ≤ 0.05, F = 385.50) higher foam at 20 °C than 16 °C. Foam formation at 16 °C ranged from 3.5 mm in Chardonnay must fermented by Elegance to 7.33 mm in Chardonnay must inoculated with M3. The range of foam production at 20 °C ranged from 7 mm by Elegance to 11 mm by ICV-D254.

The foam formation at 16 °C by individual Burgundian and mixed Burgundian strains was significantly (p ≤ 0.05) higher than Blanc, Elegance, Fusion and CY3079. This pattern is slightly different at 20 °C; one industrial strain, ICV-D254, produced significantly (p ≤ 0.05) higher foam than all strains. At 20 °C, all Burgundian mixtures produced higher foam than the individual Burgundian strains. The foam formation by Burgundian mixtures was significantly (p ≤ 0.05) higher than the Elegance and Fusion.
Table 10. Foam formation height (mm) in Chardonnay must fermented at 16 °C and 20 °C with six individual industrial, two individual Burgundian and four mixed Burgundian S. cerevisiae strains (n=3). Strain and temperature effects are shown. A,B indicate strain effect for each temperature. C shows the temperature effect.

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>16 °C A</th>
<th>20 °C B</th>
<th>Temperature effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Individual Industrial Strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blanc</td>
<td>4.17ab</td>
<td>9.50gh</td>
<td>s</td>
</tr>
<tr>
<td>Elegance</td>
<td>3.50a</td>
<td>7.00a</td>
<td>s</td>
</tr>
<tr>
<td>Fusion</td>
<td>3.67a</td>
<td>7.83bc</td>
<td>s</td>
</tr>
<tr>
<td>CY3079</td>
<td>4.83bc</td>
<td>9.17efg</td>
<td>s</td>
</tr>
<tr>
<td>ICV-D254</td>
<td>6.33efg</td>
<td>11.00ij</td>
<td>s</td>
</tr>
<tr>
<td>X16</td>
<td>5.00c</td>
<td>9.00defg</td>
<td>s</td>
</tr>
<tr>
<td><strong>Individual Burgundian Strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>6.67fgh</td>
<td>7.67abc</td>
<td>ns</td>
</tr>
<tr>
<td>C6</td>
<td>6.67fgh</td>
<td>7.33ab</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Mixed Burgundian Strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C2/C6:1/1), M1</td>
<td>6.17def</td>
<td>10.00h</td>
<td>s</td>
</tr>
<tr>
<td>(C2/C6:1/2), M2</td>
<td>6.17def</td>
<td>9.33fgh</td>
<td>s</td>
</tr>
<tr>
<td>(C2/C6:1/3), M3</td>
<td>7.33h</td>
<td>8.33cd</td>
<td>ns</td>
</tr>
<tr>
<td>(C2/C6:3/2), M4</td>
<td>7.17gh</td>
<td>10.00h</td>
<td>s</td>
</tr>
</tbody>
</table>

| Range                      | 3.50-7.33 | 7.00-11.00 |

^A,B^ The mean values of the biological replicates of each strain are indicated (n=3) at 16 °C and 20 °C.

At a given temperature yeast strains with different superscripts are significantly (p ≤ 0.05) different.

^C^ s and ns indicate significant and no significant difference between the fermentation temperatures for each yeast strain (p ≤ 0.05).
3.2.10 Growth patterns varied significantly among wine yeast strains and fermentation temperatures

The growth phenotype of all of the *S. cerevisiae* yeast strains was evaluated at 16 °C and 20 °C (Figure11).

![Figure 11](image)

**Figure 11.** Growth phenotypes of six individual industrial and two individual Burgundian *S. cerevisiae* strains (n=3). (A) growth at 16°C and (B) growth at 20 °C.

The growth pattern of all yeast strains at 16 °C was quite similar except for X16. The lag phase during the growth of the X16 strain was shorter than the other strains at 16°C and 20 °C. Individual Burgundian strains had a slightly longer lag phase than the other strains at either temperature.

Significant (p ≤ 0.05, F = 253.85) differences existed in the optical density values of the strains at either temperature. The final optical densities of strains at 20 °C were significantly (p ≤ 0.05, F = 930.53) greater than 16 °C. The optical densities of the individual Burgundian strains were significantly (p ≤ 0.05, F =253.85) lower than industrial strains at either temperature. The optical density of C6 was significantly (p ≤ 0.05) greater than the C2 at both temperatures.
Table 11. Final optical densities (λ= 600) recorded during growth of six individual industrial and two individual Burgundian S. cerevisiae strains (n=3) in filter-sterilized Chardonnay must. Strain and temperature effects are shown. A, B indicate strain effect for each temperature. C shows the temperature effect.

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>16 °C A</th>
<th>20 °C B</th>
<th>Temperature effect C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Individual Industrial Strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blanc</td>
<td>1.570 b</td>
<td>1.660 cd</td>
<td>S</td>
</tr>
<tr>
<td>Elegance</td>
<td>1.594 de</td>
<td>1.666 d</td>
<td>S</td>
</tr>
<tr>
<td>Fusion</td>
<td>1.618 f</td>
<td>1.709 ef</td>
<td>S</td>
</tr>
<tr>
<td>CY3079</td>
<td>1.656 g</td>
<td>1.719 g</td>
<td>S</td>
</tr>
<tr>
<td>ICV-D254</td>
<td>1.601 e</td>
<td>1.734 h</td>
<td>S</td>
</tr>
<tr>
<td>X16</td>
<td>1.703 h</td>
<td>1.717 fg</td>
<td>S</td>
</tr>
<tr>
<td><strong>Individual Burgundian Strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>1.495 a</td>
<td>1.585 a</td>
<td>S</td>
</tr>
<tr>
<td>C6</td>
<td>1.582 c</td>
<td>1.618 b</td>
<td>S</td>
</tr>
<tr>
<td><strong>Rang</strong></td>
<td>1.495-1.703</td>
<td>1.585-1.734</td>
<td></td>
</tr>
</tbody>
</table>

A, B The mean values of the biological replicates of each strain are indicated (n=3) at 16 °C and 20 °C.
At a given temperature yeast strains with different superscripts are significantly (p ≤ 0.05) different.
C's indicates significant difference between the fermentation temperatures for each yeast strain (p ≤ 0.05).

The final optical densities of yeast strains at 16 °C ranged from 1.495 for C2 to 1.703 for X16. No significant (p > 0.05) differences existed between final optical densities of the Elegance and ICV-D254 at 16 °C, while the final optical densities differed significantly (p ≤ 0.05) among the other yeast strains. The final optical density values at 20 °C varied from 1.585 to 1.734 for C2 and ICV-D254, respectively. X16 had the highest optical density value among yeast strains at either temperature. Individual Burgundian strains had the lowest optical densities among yeast strains at either temperature.
3.2.11 Principal component analysis differentiated wine yeast strains according to their enological properties

Principal Component Analysis (PCA) was conducted to observe groupings among the individual industrial, individual Burgundian and mixed Burgundian strains at 16 °C (Figure 12) and 20 °C (Figure 13).

PCA of the enological properties accounted for 67.1 % of the total variability in data, with 43.6% and 23.5 % of the variability explained by principal component (PC) I and PCII, respectively. The longer vectors on the PCA plot represented the most important traits in explaining the variability among yeast strains. The most heavily loaded vector on PCI were ethanol production and ethanol tolerance, indicating that ethanol production and ethanol tolerance were the most important attributes in the explanation of variability among yeast strains. Foam formation was the most heavily variable on PCII (Figure 12). The ethanol production and ethanol tolerance increased by moving to the right on the PCA plot. The foam formation increased by moving up on the PCA plot. In contrast the production of glycerol and sulphur dioxide increased by moving down on the plot (Figure 12).

The PCA plots showed the groupings among yeast strains according to their enological properties. The mixed Burgundian strains clustered together in Figure 12, with high glycerol production and foam formation, consistent with Table 5 and 10, respectively. The individual and mixed Burgundian strains had lower ethanol production, ethanol tolerance, and sulphur dioxide production and were grouped together. In contrast, the individual industrial strains showed no groups (Figure 12) and were located throughout the plot. The industrial strain Fusion was the only strain in the lower left quadrant, showing a very dissimilar enological pattern from all other strains. The
Burgundian strains were mostly positioned just to the left above center of the PCA plot. The industrial strain ICV-D254 was positioned relatively close to the Burgundian strains and was therefore the most similar to them. The individual and mixed Burgundian strains were more similar to one another in enological properties than to other industrial strains.

**Figure 12.** PCA plots of the enological properties of Chardonnay wines fermented at 16°C. Individual industrial strains are indicated with ●, individual Burgundian strains are shown with ▲ and mixed Burgundian strains are illustrated with ▲. Red ellipse shows the cluster of individual Burgundian strains. Green ellipse indicates the cluster of mixed Burgundian strains. Each vector represents an enological trait (Vector coordinates = 3 × sample coordinates).

The PCA plots showed the interrelationship among the enological properties as reflected by the angle between two vectors. The smaller the angle, the higher the correlation; 90° and 180° angles reflected no correlation and inverse correlation, respectively. Therefore, ethanol production and ethanol tolerance traits were highly correlated, but there was no correlation between acetic acid production and ethanol tolerance, as well as glycerol and sulphur dioxide production. Glycerol and acetic acid production were inversely correlated (Figure 12).
The PCA for yeasts at 20 °C is shown in Figure 13. PCA plot of the enological properties accounted for 64.7% of the total variability in the data, with 36.3% and 28.4% of the variability explained by PCI and PCII, respectively.

The most heavily loaded vector on PCI was the glycerol production; it was the most important trait in explanation of variability among the yeast strains. The most heavily loaded vector on PCII was sulphur dioxide production; it was the most important descriptor of the variability on PCII (Figure 13).

The glycerol production increased by moving to the right; moving to the left side indicated an increase in ethanol, acetic acid and foam production. The ethanol tolerance and sulphur dioxide production increased by moving down side on the plot (Figure 13).

The mixed Burgundian strains at 20 °C clustered together just slightly above center on the plot (Figure 13) and showed a similar pattern as observed at 16 °C; one Burgundian mixture (M4) showed a quite different location than the other mixtures; it
had higher ethanol tolerance and produced more sulphur dioxide than the other mixed Burgundian strains; this is consistent with results in Tables 7 and 9, respectively. In contrast, the individual industrial strains were located throughout the plot with very different characteristics (Figure 13). The industrial strain Elegance was as most similar in enological characteristics as the single and mixed Burgundian strains.

Ethanol, acetic acid and foam production were highly correlated at 20 °C, while no correlation existed between acetic acid production and ethanol tolerance (Figure 13); relationship which similar to those observed at 16 °C (Figure 12). The glycerol and sulphur dioxide production were almost inversely correlated (Figure 13), consistent with the observed pattern at 16 °C (Figure 12).

The most important descriptors on PCI and PCII were changed by increasing temperature to 20 °C; the most important descriptors on PCI and PCII at 16 °C were ethanol production and foam formation, respectively (Figure 12).

Interestingly, the single and mixed fermentations with Burgundian strains at both temperatures (Figure 12 and 13) are more similar to one another than to the industrial strains.
3.2.12 Comparison of mean enological traits for Burgundian yeast strains at 16 °C and 20 °C

Figure 14 summarizes the influence of temperature for the enological traits of the Burgundian mixtures.

![Figure 14. Summary of mean enological traits of mixed Burgundian yeast strains (n=4) at two fermentation temperatures.](image)

As evident in Figure 14, the Burgundian strains perform similarly at 16 °C and 20 °C, with the exception of their foam and sulphur dioxide production. Mean values for ethanol, glycerol, acetic acid, ethanol tolerance, sulphur dioxide and foam production are reported in Tables 3, 5, 6, 7, 9 and 10, respectively. As a group, the mixed Burgundian strains have higher foam production at 20 °C than at 16°C. The sulphur dioxide production by mixed Burgundian strains at 16°C was higher than 20 °C.
3.3 Production of volatile compounds by wine yeast strains

The volatile compounds in headspace of Chardonnay wines fermented by the six individual industrial, two individual Burgundian, and four mixed Burgundian strains was quantified at 16 °C and 20 °C. Figure 14 indicates a chromatogram representing the quantifiable peaks. The numbers beside peaks match with the quantifiable compounds and their retention times as shown in Table 12.

Table 12. Quantifiable volatile compounds in Chardonnay wines fermented by six individual industrial, two individual Burgundian and four mixed Burgundian *S. cerevisiae* strains at 16 °C and 20 °C.

<table>
<thead>
<tr>
<th>Peak Number</th>
<th>Volatile Compound</th>
<th>Class</th>
<th>Retention Time (Minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acetaldehyde</td>
<td>Aldehyde</td>
<td>4.257</td>
</tr>
<tr>
<td>2</td>
<td>Ethyl acetate</td>
<td>Acetate ester</td>
<td>5.290</td>
</tr>
<tr>
<td>3</td>
<td>Ethyl butanoate</td>
<td>Ethyl ester</td>
<td>8.771</td>
</tr>
<tr>
<td>4</td>
<td>Propanol</td>
<td>Higher alcohol</td>
<td>8.997</td>
</tr>
<tr>
<td>5</td>
<td>Isobutanol</td>
<td>Higher alcohol</td>
<td>10.788</td>
</tr>
<tr>
<td>6</td>
<td>Isoamyl acetate</td>
<td>Acetate ester</td>
<td>11.148</td>
</tr>
<tr>
<td>7</td>
<td>n-butanol</td>
<td>Higher alcohol</td>
<td>12.302</td>
</tr>
<tr>
<td>8</td>
<td>2-methyl-1-butanol</td>
<td>Higher alcohol</td>
<td>14.035</td>
</tr>
<tr>
<td>9</td>
<td>3-methyl-1-butanol</td>
<td>Higher alcohol</td>
<td>14.131</td>
</tr>
<tr>
<td>10</td>
<td>Ethyl hexanoate</td>
<td>Ethyl ester</td>
<td>14.624</td>
</tr>
<tr>
<td>11</td>
<td>Hexyl acetate</td>
<td>Acetate ester</td>
<td>15.798</td>
</tr>
<tr>
<td>12</td>
<td>1-hexanol</td>
<td>Higher alcohol</td>
<td>18.264</td>
</tr>
<tr>
<td>13</td>
<td>Ethyl octanoate</td>
<td>Ethyl ester</td>
<td>20.727</td>
</tr>
<tr>
<td>14</td>
<td>Acetic acid</td>
<td>Acid</td>
<td>20.847</td>
</tr>
<tr>
<td>15</td>
<td>2,3-butanediol</td>
<td>Higher alcohol</td>
<td>25.152</td>
</tr>
<tr>
<td>16</td>
<td>Ethyl decanoate</td>
<td>Ethyl ester</td>
<td>27.407</td>
</tr>
<tr>
<td>17</td>
<td>Ethyl laurate</td>
<td>Ethyl ester</td>
<td>34.312</td>
</tr>
<tr>
<td>18</td>
<td>Phenylethanol</td>
<td>Higher alcohol</td>
<td>36.077</td>
</tr>
</tbody>
</table>

The eight higher alcohols, five ethyl esters, three acetate esters, one aldehyde and one organic acid out of 18 quantifiable compounds were identified in the headspace of Chardonnay wines (Table 13).
Figure 15. A representative chromatogram obtained by GC-MS headspace analysis of Chardonnay wine. Quantifiable peaks are numbered; their corresponding compound names and retention times are listed in Table 1.
Table 13. Quantifiable volatile compounds in Chardonnay wine fermented by six individual industrial, two individual Burgundian and four mixed Burgundian *S. cerevisiae* strain at 16 °C and 20 °C. The quantifiable compounds were listed by their class.

<table>
<thead>
<tr>
<th>Volatile Compound</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3-butanediol</td>
<td>Higher alcohol (HA-1)</td>
</tr>
<tr>
<td>2-methyl-1-butanol</td>
<td>Higher alcohol (HA-2)</td>
</tr>
<tr>
<td>3-methyl-1-butanol</td>
<td>Higher alcohol (HA-3)</td>
</tr>
<tr>
<td>n-butanol</td>
<td>Higher alcohol (HA-4)</td>
</tr>
<tr>
<td>1-hexanol</td>
<td>Higher alcohol (HA-5)</td>
</tr>
<tr>
<td>Isobutanol</td>
<td>Higher alcohol (HA-6)</td>
</tr>
<tr>
<td>Phenylethanol</td>
<td>Higher alcohol (HA-7)</td>
</tr>
<tr>
<td>Propanol</td>
<td>Higher alcohol (HA-8)</td>
</tr>
<tr>
<td>Ethyl butanoate</td>
<td>Ethyl ester (EE-1)</td>
</tr>
<tr>
<td>Ethyl hexanoate</td>
<td>Ethyl ester (EE-2)</td>
</tr>
<tr>
<td>Ethyl octanoate</td>
<td>Ethyl ester (EE-3)</td>
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<tr>
<td>Ethyl decanoate</td>
<td>Ethyl ester (EE-4)</td>
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<td>Ethyl laurate</td>
<td>Ethyl ester (EE-5)</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>Acetate ester (AE-1)</td>
</tr>
<tr>
<td>Isoamyl acetate</td>
<td>Acetate ester (AE-2)</td>
</tr>
<tr>
<td>Hexyl acetate</td>
<td>Acetate ester (AE-3)</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>Aldehyde (ACET)</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>Acid (AA)</td>
</tr>
</tbody>
</table>

3.3.1 Production of higher alcohols varied among wine yeast strains

The eight compounds out of the 18 quantified volatile compounds were higher alcohols. Higher alcohols were identified as the most dominant volatile class in the Chardonnay wine samples. The eight higher alcohols were quantified in wine fermented by the six individual industrial, two individual Burgundian and four mixed Burgundian *S. cerevisiae* strains at 16 °C and 20 °C. No significant (p > 0.05) differences existed in higher alcohol concentrations produced by wine yeast strains between the two temperatures; therefore, only the strain effect for production of higher alcohols is reported in Table 14. The strain effect was assessed using the average of higher alcohol concentrations at both fermentation temperatures as shown in Table 14(n=6).
Figure 16. Mean concentrations (mg/L) of higher alcohols in Chardonnay wines fermented with six individual industrial yeast strains, two individual Burgundian and four mixed Burgundian yeast strains, averaged across two fermentation temperatures (n=6). Error bars indicate standard errors.
Figure 16 (Continued)

D

Butanol (mg/L)

Yeast Strain

E

Propanol (mg/L)

Yeast Strain

F

1-hexanol (mg/L)

Yeast Strain
Figure 16 (Continued)

G

Isobutanol (mg/L)

Yeast Strain

H

Phenyl ethanol (mg/L)

Yeast Strain
Table 14. Mean concentrations (mg/L) of higher alcohols in Chardonnay wines fermented with six individual industrial yeast strains, two individual Burgundian and four mixed Burgundian yeast strains, averaged across two fermentation temperatures (n=6). Strain effect is shown for each volatile compound.

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>2,3-butanediol</th>
<th>2-methyl-1-butanol</th>
<th>3-methyl-1-butanol</th>
<th>n-butanol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Individual Industrial Strains</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blanc</td>
<td>1.235&lt;sup&gt;jk&lt;/sup&gt;</td>
<td>1.150&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.789&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.049&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Elegance</td>
<td>1.065&lt;sup&gt;ghi&lt;/sup&gt;</td>
<td>1.170&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.167&lt;sup&gt;defg&lt;/sup&gt;</td>
<td>0.046&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fusion</td>
<td>1.223&lt;sup&gt;ijk&lt;/sup&gt;</td>
<td>1.420&lt;sup&gt;def&lt;/sup&gt;</td>
<td>7.056&lt;sup&gt;defg&lt;/sup&gt;</td>
<td>0.056&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>CY3079</td>
<td>1.110&lt;sup&gt;hij&lt;/sup&gt;</td>
<td>1.181&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.723&lt;sup&gt;abcdefg&lt;/sup&gt;</td>
<td>0.033&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ICV-D254</td>
<td>0.864&lt;sup&gt;bcdef&lt;/sup&gt;</td>
<td>1.249&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>6.609&lt;sup&gt;abcdefg&lt;/sup&gt;</td>
<td>0.101&lt;sup&gt;ef&lt;/sup&gt;</td>
</tr>
<tr>
<td>X16</td>
<td>1.326&lt;sup&gt;k&lt;/sup&gt;</td>
<td>1.763&lt;sup&gt;f&lt;/sup&gt;</td>
<td>9.292&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.047&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Individual Burgundian Strains</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>1.063&lt;sup&gt;ghi&lt;/sup&gt;</td>
<td>1.375&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>7.190&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>0.072&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>C6</td>
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<td>1.291&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>7.007&lt;sup&gt;bcdefg&lt;/sup&gt;</td>
<td>0.039&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Mixed Burgundian Strains</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C2/C6:1/1), M1</td>
<td>0.975&lt;sup&gt;efgh&lt;/sup&gt;</td>
<td>1.375&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>6.188&lt;sup&gt;abcdef&lt;/sup&gt;</td>
<td>0.056&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>(C2/C6:1/2), M2</td>
<td>0.920&lt;sup&gt;defg&lt;/sup&gt;</td>
<td>1.349&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>7.203&lt;sup&gt;fg&lt;/sup&gt;</td>
<td>0.056&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>(C2/C6:1/3), M3</td>
<td>0.990&lt;sup&gt;gh&lt;/sup&gt;</td>
<td>1.572&lt;sup&gt;er&lt;/sup&gt;</td>
<td>7.453&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.054&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>(C2/C6:3/2), M4</td>
<td>0.545&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.291&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>6.740&lt;sup&gt;abcddefg&lt;/sup&gt;</td>
<td>0.053&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>0.545-1.326</td>
<td>1.150-1.763</td>
<td>5.789-9.292</td>
<td>0.033-0.101</td>
</tr>
<tr>
<td>Yeast Strain</td>
<td>1-hexanol</td>
<td>Isobutanol</td>
<td>Phenylethanol</td>
<td>Propanol</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-----------</td>
<td>------------</td>
<td>---------------</td>
<td>----------</td>
</tr>
<tr>
<td><strong>Individual Industrial Strains</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blanc</td>
<td>1.918&lt;sup&gt;de&lt;/sup&gt;</td>
<td>6.666&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.906&lt;sup&gt;def&lt;/sup&gt;</td>
<td>5.034&lt;sup&gt;k&lt;/sup&gt;</td>
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<tr>
<td>Elegance</td>
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<td>9.744&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.254&lt;sup&gt;lj&lt;/sup&gt;</td>
<td>2.713&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fusion</td>
<td>2.212&lt;sup&gt;h&lt;/sup&gt;</td>
<td>7.400&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.242&lt;sup&gt;ji&lt;/sup&gt;</td>
<td>2.111&lt;sup&gt;hi&lt;/sup&gt;</td>
</tr>
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<td>CY3079</td>
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<td>12.829&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.070&lt;sup&gt;h&lt;/sup&gt;</td>
<td>1.427&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
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<td>0.879&lt;sup&gt;bcede&lt;/sup&gt;</td>
<td>1.614&lt;sup&gt;abc&lt;/sup&gt;</td>
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<td>1.658&lt;sup&gt;k&lt;/sup&gt;</td>
<td>2.015&lt;sup&gt;fghi&lt;/sup&gt;</td>
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<td><strong>Individual Burgundian Strains</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>1.993&lt;sup&gt;g&lt;/sup&gt;</td>
<td>11.999&lt;sup&gt;cdef&lt;/sup&gt;</td>
<td>1.023&lt;sup&gt;fgi&lt;/sup&gt;</td>
<td>2.136&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
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<td>19.183&lt;sup&gt;k&lt;/sup&gt;</td>
<td>1.056&lt;sup&gt;gh&lt;/sup&gt;</td>
<td>1.941&lt;sup&gt;defgh&lt;/sup&gt;</td>
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<td><strong>Mixed Burgundian Strains</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C2/C6:1/1), M1</td>
<td>1.904&lt;sup&gt;cdef&lt;/sup&gt;</td>
<td>14.892&lt;sup&gt;hi&lt;/sup&gt;</td>
<td>1.070&lt;sup&gt;h&lt;/sup&gt;</td>
<td>2.019&lt;sup&gt;ghi&lt;/sup&gt;</td>
</tr>
<tr>
<td>(C2/C6:1/2), M2</td>
<td>1.809&lt;sup&gt;bcddef&lt;/sup&gt;</td>
<td>14.744&lt;sup&gt;ghi&lt;/sup&gt;</td>
<td>0.933&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>1.786&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
<tr>
<td>(C2/C6:1/3), M3</td>
<td>1.960&lt;sup&gt;fg&lt;/sup&gt;</td>
<td>17.327&lt;sup&gt;j&lt;/sup&gt;</td>
<td>0.916&lt;sup&gt;def&lt;/sup&gt;</td>
<td>1.960&lt;sup&gt;efghi&lt;/sup&gt;</td>
</tr>
<tr>
<td>(C2/C6:3/2), M4</td>
<td>1.716&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>12.781&lt;sup&gt;cdef&lt;/sup&gt;</td>
<td>0.474&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.664&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>1.603-2.212</td>
<td>6.666-19.183</td>
<td>0.474-1.658</td>
<td>1.427-5.034</td>
</tr>
</tbody>
</table>

The mean values of the biological replicates of each yeast strain at two temperatures are shown (n=6). Yeast strain means with different superscripts are significantly (p ≤ 0.05) different.
3.3.1.1 Production of 2,3-butanediol

The higher alcohol 2,3-butanediol was quantified in the headspace of all Chardonnay wines fermented at both fermentation temperatures. The concentration of 2,3-butanediol differed significantly (p ≤ 0.05, F = 10.73) among wine yeast strains (Figure 16A and Table 14). The concentration of 2,3-butanediol in the headspace of the Chardonnay wines ranged from 0.545 to 1.326 mg/L in wines fermented by Burgundian mixture M4 and individual industrial strain X16, respectively.

The low producer strain M4 was significantly (p ≤ 0.05) different from all other wine yeast strains, while no significant (p > 0.05) differences existed between high producer strain X16 and the other two industrial strains Blanc and Fusion. One individual Burgundian strain C2 and one individual industrial strain Elegance produced the same amount of 2,3-butanediol which was 1.063 mg/L. The mixed Burgundian strains M1, M2 and M3 did not differ significantly (p > 0.05) from the individual Burgundian strains, ICV-D254 and Elegance. M1 and M3 produced a higher amount of 2,3-butanediol among Burgundian mixtures which was not significantly (p > 0.05) different with the amount produced by the individual strains ICV-D254, Elegance and CY3079. The mixed Burgundian strains fit into the low end of the range of this study.

3.3.1.2 Production of 2-methyl-1-butanol

The volatile compound 2-methyl-1-butanol was quantified in the headspace of all Chardonnay wines fermented at either fermentation temperature (Figure 16B and Table 14). The concentration of 2-methyl-1-butanol changed from 1.150 mg/L for Blanc to 1.763 mg/L for X16. The wine yeast strains produced significant (p ≤ 0.05, F = 9.00) different concentrations of 2-methyl-1-butanol. Statistical analysis differentiated the low producer strains Blanc, Elegance, CY3070, ICV-D254, C6 and M4 (1.15-1.29 mg/L)
from high producer strains Fusion, M3 and X16 (1.572 and 1.763 mg/L). The individual Burgundian strains C2, M1 and M2 produced moderate amounts of 2-methyl-1-butanol.

3.3.1.3 Production of 3-methyl-1-butanol

The volatile higher alcohol 3-methyl-1-butanol was quantified in the headspace of all Chardonnay wines fermented at both fermentation temperatures (Figure 16C and Table 14). The production of 3-methyl-1-butanol in Chardonnay wine samples differed significantly ($p \leq 0.05$, $F = 4.97$) and ranged between 5.789-9.292 mg/L in wines inoculated with Blanc and X16, respectively. The 3-methyl-1-butanol production by X16 was significantly ($p \leq 0.05$) greater than all other strains. The low end strain Blanc did not significantly ($p > 0.05$) differ with all other low producer strains M1, ICV-D254, CY3079 and M4 (6.188-6.723 mg/L). No significant ($p > 0.05$) differences existed among the moderate producers C6, Fusion, Elegance and C2 (7.007-7.190 mg/L). The M2 and M3 did not differ significantly ($p > 0.05$) from the moderate producer strains. The M3 and M2 produced the second and third highest concentrations of 3-methyl-1-butanol, respectively.

3.3.1.4 Production of n-butanol

The higher alcohol, n-butanol, was quantified in all Chardonnay wines fermented at both fermentation temperatures (Figure 16D and Table 14). Butanol production by wine yeast strains ranged from 0.033 mg/L for CY3079 to 0.101 for ICV-D254. Few significant differences ($p \leq 0.05$, $F = 31.47$) existed in n-butanol production by yeast strains. The high end producer strain ICV-D254 differed significantly ($p \leq 0.05$) from all other yeast strains. No significant ($p > 0.05$) differences existed between low end
producer strain CY3079 and individual Burgundian strain C2. The C6, M1 and M2
strains produced the highest amount of n-butanol after ICV-D254. The n-butanol
production by mixed Burgundian strains was higher than the amount produced by C6 and
lower than the amount produced by C2.

3.3.1.5 Production of 1-hexanol

The 1-hexanol was quantified in the headspace of all Chardonnay wine samples
fermented at both fermentation temperatures is shown in Figure 16E and Table 14. The
wine yeast strains produced significantly (p ≤ 0.05, F = 8.4) different amounts of 1-
hexanol; the concentration ranged from 1.603 to 2.212 mg/L in wines fermented by X16
and Fusion, respectively. No significant (p > 0.05) differences was detected between low-
end strain X16 and one Burgundian mixture M4, while the high-end strain Fusion
differed significantly (p ≤ 0.05) from all other yeast strains. The C2, C6 and M3 strains
produced the highest amount of the 1-hexanol following the high-end strain Fusion and
they did not significantly (p > 0.05) differ with Elegance, CY3079 and ICV-D254.

3.3.1.6 Production of isobutanol

Isobutanol was measured in the headspace of all Chardonnay wine samples at
either fermentation temperature (Figure 16F and Table 14). The production range of
isobutanol by wine yeast strains differed significantly (p ≤ 0.05, F = 58.81) and changed
from 6.666 mg/L in wine fermented by Blanc to 19.183 mg/L in wine fermented by C6.
Blanc, Fusion and Elegance produced the lowest amount of isobutanol, respectively.
Blanc and Fusion strains differed significantly (p ≤ 0.05) from Elegance and all other
strains, while no significant (p > 0.05) differences existed between Blanc and Fusion. The
Elegance strain varied significantly (p ≤ 0.05) from all other yeast strains. The C6 and M3 strains produced the highest amounts of isobutanol. Isobutanol production by C6 and M3 varied significantly (p ≤ 0.05) different from each other and all other strains. The other yeast strains fell into the middle range of the isobutanol production. Isobutanol production by C6 was significantly (p ≤ 0.05) greater than C2. The isobutanol production by all mixed Burgundian strains was significantly (p ≤ 0.05) less than isobutanol production by C6 and higher than C2.

3.3.1.7 Production of phenylethanol

Phenylethanol was quantified in the headspace of all Chardonnay wines fermented at both fermentation temperatures (Figure 16G and Table 14). The yeast strains produced significantly (p ≤ 0.05, F = 36.52) different amounts. The phenylethanol content of the Chardonnay wines ranged from 0.474 mg/L for M4 to 1.658 mg/L for X16. These low- and high-end strains differed significantly (p ≤ 0.05) from all other yeasts strains. The individual and mixed Burgundian strains M1, M2 and M3 strains had significant (p ≤ 0.05) difference compared to the high-end strain X16 and low-end strain M4. No significant (p > 0.05) differences existed among strains C2, C6, M1 and CY3079. Additionally, no significant (p > 0.05) differences was detected among M2, M3, Blanc and ICV-D254. This group of yeasts produced more phenylethanol than the previous group. Elegance and Fusion produced the second and third highest amounts of phenylethanol, respectively. The phenylethanol production by these two strains was significantly (p ≤ 0.05) less than X16 and significantly (p ≤ 0.05) greater than all other yeast strains.
3.3.1.8 Production of propanol

Propanol was detected in the headspace of all Chardonnay wines fermented at either fermentation temperature (Figure 16H and Table 14). Propanol production by yeast strains differed significantly \((p \leq 0.05, F = 204.80)\) and ranged from 1.427 to 5.034 mg/L in wines fermented with CY3079 and Blanc, respectively. Propanol production by Blanc varied significantly \((p \leq 0.05)\) from all other yeasts. The propanol concentration in wines fermented with CY3079 and ICV-D254 did not significantly \((p > 0.05)\) vary. The propanol content of wine fermented with C2 was significantly \((p \leq 0.05)\) greater than propanol concentration in wine fermented with C6. No significant \((p > 0.05)\) difference existed among M1, M3, C2, C6 and X16. Additionally, no significant \((p > 0.05)\) difference was detected among M2, M4 and ICV-D254. This group of strains produced a lower amount of propanol than the previous group. The Elegance strain which produced the second highest amount of propanol was significantly \((p \leq 0.05)\) different from all other yeast strains.
3.3.2 Production of ethyl esters varied among wine yeast strains

The five out of the 18 quantified volatile compounds in the headspace of Chardonnay wines were ethyl esters, making ethyl esters the second most prevalent volatile class in the Chardonnay wine samples. The five ethyl ester compounds were quantified in wine fermented by all *S. cerevisiae* strains at 16 °C and 20 °C. The ethyl ester content of Chardonnay wines fermented at 16 °C and 20 °C did not significantly (p > 0.05) differ. The strain effect was evaluated using the average content of ethyl esters in wines fermented at both fermentation temperatures (n=6) (Table 15).
Figure 17. Mean concentrations (mg/L) of ethyl esters in Chardonnay wines fermented with six individual industrial yeast strains, two individual Burgundian and four mixed Burgundian yeast strains, averaged across two fermentation temperatures (n=6). Error bars indicate standard errors.
Figure 17 (Continued)

D

E
Table 15. Mean concentrations (mg/L) of ethyl esters in Chardonnay wines fermented with six individual industrial yeast strains, two individual Burgundian and four mixed Burgundian yeasts trains, averaged across two fermentation temperatures (n=6). Strain effect is shown for each volatile compound.

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Ethyl butanoate</th>
<th>Ethyl hexanoate</th>
<th>Ethyl octanoate</th>
<th>Ethyl decanoate</th>
<th>Ethyl laurate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Individual Industrial Strains</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blanc</td>
<td>0.200&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.036&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.035&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.028&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Elegance</td>
<td>0.275&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.045&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.048&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.031&lt;sup&gt;fg&lt;/sup&gt;</td>
<td>0.005&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
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<td>0.059&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>0.045&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td>0.039&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.036&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.024&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.004&lt;sup&gt;ad&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Individual Burgundian Strains</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>0.211&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.043&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.040&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.017&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.002&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>C6</td>
<td>0.229&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.044&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.040&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.020&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>0.002&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Mixed Burgundian Strains</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C2/C6:1/1), M1</td>
<td>0.246&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>0.041&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.042&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.023&lt;sup&gt;bcde&lt;/sup&gt;</td>
<td>0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(C2/C6:1/2), M2</td>
<td>0.217&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.040&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.042&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.025&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.002&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>(C2/C6:1/3), M3</td>
<td>0.218&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.040&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.040&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.022&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>0.002&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>(C2/C6:3/2), M4</td>
<td>0.215&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.042&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.041&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.018&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.001&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td><strong>Range</strong></td>
<td>0.200-0.361</td>
<td>0.030-0.059</td>
<td>0.030-0.059</td>
<td>0.017-0.034</td>
<td>0.001-0.01</td>
</tr>
</tbody>
</table>

The mean values of the biological replicates of each yeast strain at two temperatures are shown (n=6). Yeast strain means with different superscripts are significantly (p ≤ 0.05) different.
3.3.2.1 Production of ethyl butanoate

Ethyl butanoate was quantified in all Chardonnay wines fermented by all strains of *S. cerevisiae* strain at 16 °C and 20 °C (Figure 17A and Table 15). The yeast strains produced significantly (p ≤ 0.05, F = 7.46) different amounts of the ethyl butanoate. The ethyl butanoate ranged from 0.200- 0.361 mg/L in wines fermented by Blanc and ICV-D254, respectively. No significant (p > 0.05) differences existed among low-end strain Blanc, individual Burgundian strains and mixed Burgundian strains, showing that ethyl butanoate production by Burgundian strains was toward the low end of the range. The high-end strain ICV-D254 was significantly (p ≤ 0.05) different from all other strains. The CY3079 and Fusion produced the second and third highest concentrations of ethyl butanoate, respectively.

3.3.2.2 Production of ethyl hexanoate

Ethyl hexanoate was quantified in the headspace of all Chardonnay wine samples fermented at both fermentation temperatures (Figure 17B and Table 15). The production of ethyl hexanoate by wine yeast strains ranged from 0.030 mg/L in wine fermented by ICV-D254 to 0.059 mg/L in wine fermented by Fusion. Statistical analysis revealed few significant (p ≤ 0.05, F = 11.82) differences between yeast strains. The low and high producer strains differed significantly (p ≤ 0.05) from each other; additionally, each of them varied significantly (p ≤ 0.05) from all other wine yeast strains. The production of ethyl hexanoate by Burgundian strains fell into the middle of the range and only significantly (p ≤ 0.05) differed with the low-end and high-end strains.
3.3.2.3 Production of ethyl octanoate

Ethyl octanoate was one of the quantified ethyl esters in the headspace of all Chardonnay wines fermented at either fermentation temperature (Figure 17C and Table 15). The concentration of ethyl octanoate in Chardonnay wines significantly (p ≤ 0.05, F = 3.88) differed and ranged from 0.030 mg/L in wine fermented by ICV-D254 to 0.059 mg/L in wine inoculated with Fusion. The ICV-D254 strain significantly (p ≤ 0.05) differed from CY3079, Elegance and Fusion. Elegance and CY3079 produced the highest amounts of ethyl octanoate after Fusion. No significant (p > 0.05) difference existed among Blanc, X16, C2, C6, M1, M2, M3 and M4. The production of ethyl hexanoate within all Burgundian strains was quite similar together and higher than low-end producers and significantly (p ≤ 0.05) less than high-end producer strains.

3.3.2.4 Production of ethyl decanoate

The volatile compound ethyl decanoate was quantified in the headspace of all Chardonnay wines after fermentation at 16 °C and 20 °C (Figure 17D and Table 15). The wine yeast strains produced significantly (p ≤ 0.05, F = 8.96) different amounts of ethyl decanoate ranging from 0.017 mg/L to 0.034 mg/L in wines fermented by C2 and Fusion, respectively. Individual industrial strains, Blanc and Fusion, were the second and third highest producer strains, respectively. No significant (p > 0.05) difference existed among Blanc, Elegance and Fusion. The other group of strains including C2, M4, C6, M3 and ICV-D254 produced non-significantly (p > 0.05) different concentrations of ethyl decanoate and were classified as lower producer strains (0.17-0.22 mg/L). Another group of strains produced non-significant (p > 0.05) moderate levels of ethyl decanoate including M1, X16, M2 and CY3079 (0.23-0.25 mg/L).
3.3.2.5 Production of ethyl laurate

Ethyl laurate was quantified in the headspace of all Chardonnay wines fermented at both fermentation temperatures (Figure 17E and Table 15). The ethyl laurate produced by wine yeast strains ranged from 0.001mg/L in wines fermented by Blanc, M1 and M4 to 0.010 mg/L in wine fermented with Fusion. Statistical analysis revealed few significant (p ≤ 0.05, F = 53.73) differences between yeast strains. The Fusion strain produced significant (p ≤ 0.05) different amount of ethyl laurate from all other strains. No significant (p > 0.05) difference existed among low producer strains including Blanc, M1, M4, ICV-D254, C2, C6, M1 and M3 (0.001-0.002 mg/L). The Elegance and X16 strains were the second and third highest producing strains.
3.3.3 Production of acetate esters varied among wine yeast strains

Acetate esters were the third most prevalent volatile class compounds in the headspace of Chardonnay wine samples fermented at 16 °C and 20 °C. The production of acetate esters by wine yeast strains did not significantly (p > 0.05) differ across the two fermentation temperatures. Therefore, only the strain effect on production of acetate esters was analyzed and indicated in Table 16. The strain effect was analyzed using the average of acetate esters concentrations in wines fermented at both fermentation temperatures, as shown in Table 16 (n=6).
Figure 18. Mean concentrations (mg/L) of acetate esters in Chardonnay wines fermented with six individual industrial yeast strains, two individual Burgundian and four mixed Burgundian yeast strains, averaged across two fermentation temperatures (n=6). Error bars indicate standard errors.
Table 16. Mean concentration (mg/L) of acetate esters in Chardonnay wines fermented with six individual industrial strains, two individual Burgundian and four mixed Burgundian yeast strains, averaged across two fermentation temperatures (n=6). Strain effect is shown for each volatile compound.

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Ethyl acetate</th>
<th>Hexyl acetate</th>
<th>Isoamyl acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Individual Industrial Strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blanc</td>
<td>7.761&lt;sup&gt;ij&lt;/sup&gt;</td>
<td>0.024&lt;sup&gt;bcde&lt;/sup&gt;</td>
<td>0.093&lt;sup&gt;defgh&lt;/sup&gt;</td>
</tr>
<tr>
<td>Elegance</td>
<td>6.954&lt;sup&gt;hi&lt;/sup&gt;</td>
<td>0.027&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>0.109&lt;sup&gt;hij&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fusion</td>
<td>5.703&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.019&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.059&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CY3079</td>
<td>6.635&lt;sup&gt;cddefgh&lt;/sup&gt;</td>
<td>0.030&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.118&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td>ICV-D254</td>
<td>6.245&lt;sup&gt;abcdefgh&lt;/sup&gt;</td>
<td>0.024&lt;sup&gt;bcde&lt;/sup&gt;</td>
<td>0.107&lt;sup&gt;ghij&lt;/sup&gt;</td>
</tr>
<tr>
<td>X16</td>
<td>8.448&lt;sup&gt;j&lt;/sup&gt;</td>
<td>0.035&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.220&lt;sup&gt;k&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Individual Burgundian Strains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>6.617&lt;sup&gt;bcdefgh&lt;/sup&gt;</td>
<td>0.024&lt;sup&gt;bcde&lt;/sup&gt;</td>
<td>0.090&lt;sup&gt;cddefgh&lt;/sup&gt;</td>
</tr>
<tr>
<td>C6</td>
<td>6.663&lt;sup&gt;defgh&lt;/sup&gt;</td>
<td>0.024&lt;sup&gt;bcde&lt;/sup&gt;</td>
<td>0.116&lt;sup&gt;ij&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Mixed Burgundian Strains</strong></td>
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<td></td>
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</tr>
<tr>
<td>(C2/C6:1/1), M1</td>
<td>6.554&lt;sup&gt;abcdefgh&lt;/sup&gt;</td>
<td>0.025&lt;sup&gt;cdde&lt;/sup&gt;</td>
<td>0.100&lt;sup&gt;efghij&lt;/sup&gt;</td>
</tr>
<tr>
<td>(C2/C6:1/2), M2</td>
<td>6.756&lt;sup&gt;efgh&lt;/sup&gt;</td>
<td>0.027&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>0.109&lt;sup&gt;hij&lt;/sup&gt;</td>
</tr>
<tr>
<td>(C2/C6:1/3), M3</td>
<td>6.917&lt;sup&gt;ghi&lt;/sup&gt;</td>
<td>0.024&lt;sup&gt;bcde&lt;/sup&gt;</td>
<td>0.104&lt;sup&gt;ghi&lt;/sup&gt;</td>
</tr>
<tr>
<td>(C2/C6:3/2), M4</td>
<td>6.809&lt;sup&gt;fg&lt;/sup&gt;</td>
<td>0.026&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.085&lt;sup&gt;bcdef&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>5.703-8.448</td>
<td>0.019-0.030</td>
<td>0.059-0.118</td>
</tr>
</tbody>
</table>

The mean values of the biological replicates of each yeast strain at two temperatures are shown (n=6). Yeast strain means with different superscripts are significantly (p ≤ 0.05) different.
3.3.3.1 Production of ethyl acetate

Ethyl acetate was quantified in the headspace of all Chardonnay wines fermented at both fermentation temperatures (Figure 18A and Table 16) and ranged from 5.703 mg/L in wine fermented by Fusion to 8.448 mg/L in wine fermented by X16. The yeasts strains produced significantly (p ≤ 0.05, F = 5.33) different amounts of the ethyl acetate. No significant (p > 0.05) differences existed between the two highest producer strains X16 and Blanc.

3.3.3.2 Production of hexyl acetate

Hexyl acetate was quantified in the headspace of all Chardonnay wines fermented at both fermentation temperatures (Figure 18B and Table 16). The hexyl acetate concentration in Chardonnay wines ranged from 0.019 mg/L to 0.035 mg/L in wines fermented by Fusion and X16, respectively. The Fusion strain produced significantly (p ≤ 0.05) lower amounts of hexyl acetate than all other wine yeast strains. The CY3079, Elegance and M2 strains fit into the high end of the range of the hexyl acetate production and did not vary significantly (p > 0.05) from each other. The remaining strains, including Blanc, ICV-D254, C2, M3, M1, M4 and C6 (0.024-0.026) did not produce significantly (p > 0.05) different amounts of hexyl acetate from each other.

3.3.3.3 Production of isoamyl acetate

Isoamyl acetate is an acetate ester which was found in the headspace of all Chardonnay wines fermented at either fermentation temperature (Figure 17C and Table 16). The yeast strains produced significantly (p ≤ 0.05, F = 10.92) different concentrations of isoamyl acetate. The range of isoamyl acetate by wine yeasts strains varied from 0.059 to 0.220 mg/L in wines
fermented by Fusion and X16 strains, respectively. Each of these low- and high-end strains produced significantly ($p \leq 0.05$) different amounts of isoamyl acetate from each other and all other wine yeast strains. The C2 strain produced a significantly ($p \leq 0.05$) greater amount of isoamyl acetate than C6. The low producing strains without significant ($p > 0.05$) difference were M4, C2, Blanc and M1 (0.085-0.10 mg/L). The remaining strains produced moderate levels of isoamyl acetate.
3.3.4 Production of acetaldehyde varied among wine yeast strains

Acetaldehyde was quantified in the headspace of all Chardonnay wine samples fermented at 16 °C and 20 °C (Figure 19 and Table 17). No significant (p > 0.05) difference was observed between acetaldehyde concentration in wines fermented at the two fermentation temperatures. Therefore, only the strain effect on production of acetaldehyde was analyzed and indicated in Table 17. The strain effect was analyzed using the average of acetaldehyde concentrations in wines fermented at both fermentation temperatures (n=6).

![Graph showing mean acetaldehyde concentrations (mg/L) for different yeast strains.](image)

**Figure 19.** Mean concentrations (mg/L) of acetaldehyde in Chardonnay wines fermented with six individual industrial yeast strains, two individual Burgundian and four mixed Burgundian yeast strains, averaged across two fermentation temperatures (n=6). Error bars indicate standard errors.

The yeast strains produced significantly (p ≤ 0.05, F = 8.59) different concentrations of acetaldehyde. The acetaldehyde concentration ranged from 0.732 mg/L in wine fermented by M4 to 1.422 mg/L in wine fermented by M3. No significant (p > 0.05) difference existed among M4 and the other low producer strains including ICV-D254, CY3079, Elegance and C6 (0.732-
The high-end strain M3 did not produce significantly (p > 0.05) different amounts of acetaldehyde from the M2 and Blanc strains. The remaining strains fell into the middle range of acetaldehyde production.

Table 17. Mean concentrations (mg/L) of acetaldehyde in Chardonnay wines fermented with six individual industrial yeast strains, two individual Burgundian and four mixed Burgundian yeast strains, averaged across two fermentation temperatures (n=6). Strain effect is shown for acetaldehyde production.

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Acetaldehyde</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Individual Industrial Strains</strong></td>
<td></td>
</tr>
<tr>
<td>Blanc</td>
<td>1.235ghi</td>
</tr>
<tr>
<td>Elegance</td>
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<tr>
<td>Fusion</td>
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<tr>
<td>CY3079</td>
<td>0.914abc</td>
</tr>
<tr>
<td>ICV-D254</td>
<td>0.829abc</td>
</tr>
<tr>
<td>X16</td>
<td>1.150fgh</td>
</tr>
<tr>
<td><strong>Individual Burgundian Strains</strong></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>0.967bcde</td>
</tr>
<tr>
<td>C6</td>
<td>1.031cdefg</td>
</tr>
<tr>
<td><strong>Mixed Burgundian Strains</strong></td>
<td></td>
</tr>
<tr>
<td>(C2/C6:1/1), M1</td>
<td>1.115defg</td>
</tr>
<tr>
<td>(C2/C6:1/2), M2</td>
<td>1.345hi</td>
</tr>
<tr>
<td>(C2/C6:1/3), M3</td>
<td>1.422i</td>
</tr>
<tr>
<td>(C2/C6:3/2), M4</td>
<td>0.732a</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>0.732-1.422</td>
</tr>
</tbody>
</table>

The mean values of the biological replicates of each yeast strain at two temperatures are shown (n=6). Yeast strain means with different superscripts are significantly (p ≤ 0.05) different.
3.3.5 Production of acetic acid varied among wine yeast strains

Acetic acid was quantified in the headspace of Chardonnay wine samples fermented at 16 °C and 20 °C. No significant (p > 0.05) differences existed between acetic acid concentrations in wines fermented across the two fermentation temperatures. Therefore, only the strain effect on production of acetic acid was assessed and is indicated in Table 18. The strain effect was analyzed using the average of acetic acid content in wines fermented at both fermentation temperatures (n=6).

![Graph showing acetic acid concentrations in Chardonnay wines](image)

**Figure 20.** Mean concentrations (mg/L) of acetic acid in Chardonnay wines fermented with six individual industrial yeast strains, two individual Burgundian and four mixed Burgundian yeast strains, averaged across two fermentation temperatures (n=6). Error bars indicate standard errors.

The acetic acid was quantified in the headspace of all Chardonnay wines fermented at both fermentation temperatures (Figure 20 and Table 18). The production of acetic acid by wine yeast strains differed significantly (p ≤ 0.05, F = 4.19) among yeast strains and ranged from 0.250-0.375 mg/L in wines fermented by the Blanc and CY3079 strains, respectively. The low
producing strains including Blanc, C2 and C6, did not produce significantly (p > 0.05) different amounts of acetic acid. The acetic acid production by Elegance, Fusion, X16, M3 and CY3079 strains was toward the high end of the range (0.358-0.372 mg/L) and not significantly (p > 0.05) different from one another. The remaining strains, including M2, M4, ICV-D254 and M1 produced moderate levels of acetic acid with no significant (p > 0.05) difference.

Table 18. Mean concentrations (mg/L) of acetic acid in Chardonnay wines fermented with six individual industrial yeast strains, two individual Burgundian and four mixed Burgundian yeast strains, averaged across two fermentation temperatures (n=6). Strain effect is shown for volatile compound.

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Acetic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Individual Industrial Strains</strong></td>
<td></td>
</tr>
<tr>
<td>Blanc</td>
<td>0.250&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Elegance</td>
<td>0.358&lt;sup&gt;def&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fusion</td>
<td>0.361&lt;sup&gt;def&lt;/sup&gt;</td>
</tr>
<tr>
<td>CY3079</td>
<td>0.375&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>ICV-D254</td>
<td>0.322&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
<tr>
<td>X16</td>
<td>0.365&lt;sup&gt;ef&lt;/sup&gt;</td>
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<tr>
<td>C2</td>
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</tr>
<tr>
<td>C6</td>
<td>0.303&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Mixed Burgundian Strains</strong></td>
<td></td>
</tr>
<tr>
<td>(C2/C6:1/1), M1</td>
<td>0.323&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>(C2/C6:1/2), M2</td>
<td>0.312&lt;sup&gt;bcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>(C2/C6:1/3), M3</td>
<td>0.372&lt;sup&gt;ef&lt;/sup&gt;</td>
</tr>
<tr>
<td>(C2/C6:3/2), M4</td>
<td>0.317&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>0.250-0.375</td>
</tr>
</tbody>
</table>

The mean values of the biological replicates of each yeast strain at two temperatures are shown (n=6). Yeast strain means with different superscripts are significantly (p ≤ 0.05) different.
3.4 Principal component analysis differentiated wine yeast strains according to their volatile profiles

Principal Component Analysis (PCA) was conducted for higher alcohols (Figure 21), ethyl esters (Figure 22) and acetate esters (Figure 23), and finally for all volatile compounds (Figure 24). PCA plots represented the averages of volatile compounds at both fermentation temperatures among the three groups of yeast strains (individual industrial, individual Burgundian and mixed Burgundian) since volatile compounds did not significantly (p > 0.05) differ between the two fermentation temperatures. PCA analysis revealed the groups in the yeast strains and indicated the relationships among the volatile compounds.

3.4.1 Principal component analysis differentiated wine yeast strains according to their higher alcohol profiles

PCA of the higher alcohols in the Chardonnay wines accounted for 65.6 % of the total variability in the data, with 37% and 28.6 % of the variance explained by the PCI and PCII, respectively (Figure 21). The individual industrial strains were scattered throughout the plot, reflecting different patterns of higher alcohol production. Three yeast strains (Fusion, Elegance and CY3079) were positioned slightly above CY3079 and C2, reflecting high concentrations of HA-1, HA-5, HA-7 and HA-8. The X16 and Blanc strains had very dissimilar patterns of higher alcohol production compared to all other strains as shown in Figure 21.

The mixed Burgundian strains composed a distinct group, with the exception of mixed Burgundian M4, which was most heavily loaded on negative PC II (Figure 21).
The mixed Burgundian strains and C6 had relatively high concentrations of 2-methyl-1-butanol (HA-2), n-butanol (HA-4) and isobutanol (HA-6) and lower concentrations of 2,3-butanediol (HA-1), n-butanol (HA-4) and propanol (HA-8) (Figure 21), consistent with Table 14.

The mixed Burgundian strains produced moderate amounts of the higher alcohols compared to X16 and Blanc as shown in Table 14. The mixed Burgundian strains were more similar to one another than other industrial strains with the exception of ICV-D254.
3.4.2 Principal component analysis differentiated wine yeast strains according to their ethyl ester profiles

PCA plot of ethyl esters production in the Chardonnay wines described 88.3% of the total variability in the data, with 65% and 23.3 % explaining by PCI and PCII, respectively (Figure 22).

![PCA plot of ethyl esters production in Chardonnay wines](image)

**Figure 22.** Plot obtained from mean values of the ethyl esters averaged across two fermentation temperatures in Chardonnay wine. Individual industrial strains are indicated with ○, individual Burgundian strains are shown with ■ and mixed Burgundian strains are illustrated with ▲. The red ellipse shows the cluster of individual Burgundian strains. The green ellipse indicates the cluster of mixed Burgundian strains. Each vector represents a higher alcohol compound. The ethyl ester compounds are ethyl butanoate (EE-1), ethyl hexanoate (EE-2), ethyl octanoate (EE-3), ethyl decanoate (EE-4) and ethyl laurate (EE-5) (Vector coordinates= 3× sample coordinates).

The individual industrial strains showed different patterns of ethyl ester production. The Fusion and ICV-D254 strains had very dissimilar patterns of ethyl ester production compared to all other strains as shown in Figure 22. The individual and mixed Burgundian strains clustered tightly together. The position of industrial strain Blanc was very close to the mixed Burgundian strains, representing a similar ethyl ester profile to the mixed Burgundian strains. The other three industrial strains including X16, CY3079...
and Elegance had similar ethyl ester profiles and were positioned slightly above the PCA center (Figure 22).

The individual and mixed Burgundian strains produced more ethyl hexanoate (EE-2) and ethyl octanoate (EE-3) (Figure 22). The Burgundian strains produced less ethyl butanoate (EE-1), while most of the industrial strains produced more ethyl butanoate, as shown in Table 15. The individual industrial strains showed different patterns of ethyl ester production (Figure 22).

The individual and mixed Burgundian strains were more similar to one another than to the industrial strains except for Blanc. The pattern of ethyl esters production by Blanc was very similar to the Burgundian strains, as shown in Table 15.

3.4.3 Principal component analysis differentiated wine yeast strains according to their acetate ester profiles

PCA plot of acetate esters production in the Chardonnay wines accounted for 97.2 % of the total variability in the data, with 85.8% and 10.4 % of the variance explained by PCI and PCII, respectively (Figure 23).

The industrial strains X16 and Fusion showed very dissimilar acetate ester profiles to all other yeast strains. The three other industrial strains including ICV-D254, Elegance and CY3079 were positioned around the center of the PCA plot and close to the Burgundian strains.

The individual and mixed Burgundian strains clustered tightly together in the center of PCA plots and showed a distinct pattern of acetate ester production from the individual industrial strains which fell outside of this cluster. The mixed Burgundian M2
and industrial strain Elegance overlapped together and showed very similar patterns of acetate ester production.

![Figure 23](image)

**Figure 23.** Plot obtained from mean values of the acetate esters averaged across two fermentation temperatures in Chardonnay wine. Individual industrial strains are indicated with blue, individual Burgundian strains are shown with red and mixed Burgundian strains are illustrated with green. The red ellipse shows the cluster of individual Burgundian strains. The green ellipse indicates the cluster of mixed Burgundian strains. Each vector represents a higher alcohol compound. The ethyl ester compounds ethyl acetate (AE-1), hexyl acetate (AE-2) and isoamyl acetate (AE-3) (Vector coordinates= 3× sample coordinates).

The individual industrial strains showed less-scattered acetate ester patterns compared to PCA plots of higher alcohols and even ethyl esters. The individual and mixed Burgundian strains fit into the central part of PCA plot, showing moderate production of acetate esters, while X16 produced much more ethyl acetate (AE-1) and isoamyl acetate (AE-3). The Fusion strain produced lesser amounts of acetate esters compared to the Burgundian strains, consistent with the results in Table 16.
3.4.4 Principal component analysis differentiated wine yeast strains based on the production of volatile compounds

PCA plot of all volatile compounds in the Chardonnay wines accounted for 56.1 % of the total variability in the data, with 29.4 % and 26.7 % of the variance explained by PCI and PCII, respectively (Figure 24).

The individual industrial strains were scattered throughout the PCA plots, representing the various patterns of volatile compounds. The X16 and Fusion had very dissimilar volatile profiles to all other yeast strains (Figure 24).

The individual and mixed Burgundian strains composed a cluster in the PCA plot, although one of the Burgundian mixtures, M4, was relatively apart from the other Burgundian strains in the cluster due to a higher loading factor on PC II, consistent with higher alcohol patterns. The Burgundian strains showed a distinct pattern of volatile compounds from most of the individual industrial strains outside of the Burgundian cluster.

The industrial strain ICV-D254 showed a very similar pattern to M4. The industrial strain Blanc was close to individual Burgundian strains and M1. Interestingly, the mixed Burgundian strain M1 (one to one ratio of individual Burgundian strains) was positioned between the two individual Burgundian strains in the PCA plot, which may possibly have happened due to the metabolic effects of individual Burgundian strains.
Figure 24. Plot obtained from mean values of the all volatile compounds averaged across two fermentation temperatures in Chardonnay wine. Individual industrial strains are indicated with ●, individual Burgundian strains are shown with ▲ and mixed Burgundian strains are illustrated with △. The red ellipse shows the cluster of individual Burgundian strains. The green ellipse indicates the cluster of mixed Burgundian strains. Each vector represents a volatile compound (Vector coordinates = 3× sample coordinates).
3.5 Cluster analysis distinguished yeasts strains according to their volatile profiles

Cluster analysis was used as an extra test to group the PCA results. Similar clusters are linked together in the dendogram diagram.

**Figure 25.** Dendogram of cluster analysis on mean volatile compounds for yeast groups [individual industrial (n=36), individual Burgundian (n=12), mixed Burgundian strains (n=24)], as determined by GC-MS and averaged across both fermentation temperatures.

The cluster analysis was conducted on the yeast groups of individual industrial, individual Burgundian and mixed Burgundian strains (Figure 25) and revealed that individual Burgundian and mixed Burgundian groups clustered together at a low distance showing volatile production by individual and mixed Burgundian strains are most similar together, while the relative distance of individual industrial strains from Burgundian strains was much greater than the relative distance between individual and mixed...
Burgundian strains. These primary clusters confirmed that individual and mixed Burgundian groups were more similar to one another than to the industrial group.
3.6 Comparison of odour and estimated aroma sensory profiles of Chardonnay wines

Odour and estimated aroma sensory profiles of the volatile compounds in Chardonnay wines were created. The odour profiles represented the summary of the volatile compound concentrations (mg/L) (Tables 14-18); whereas the estimated aroma sensory profile for the same volatile compounds represented the summary of the Odour Active Values (OAVs). The OAVs were on a log scale; those above zero were above the threshold and those below zero were below the threshold. The volatile compounds were either perceptible or non-perceptible according to their OAVs.

The 18 volatile compounds were quantified by GC-MS in the headspace of Chardonnay wines fermented with individual industrial, individual Burgundian and mixed Burgundian strains. The name of the volatile compounds and their aroma descriptors are listed in Table 19. Additionally, the water threshold of the volatile compounds (Campo et al., 2005; Cullerré et al., 2004) and OAVs for mean of individual Burgundian and mean of mixed Burgundian strains are indicated in Table 19.

According to the OAVs of individual and mixed Burgundian strains in Table 19, the OAVs of all higher alcohols were below their sensory thresholds except for 2-methyl-1-butanol. In contrast, the OAVs of the three ethyl esters, ethyl butanoate, ethyl hexanoate and ethyl octanoate, were above their sensory thresholds. The OAV of isoamyl acetate was above its perception threshold. The OAV of acetaldehyde was more than its threshold, while the OAV of the acetic acid was lower than its sensory threshold.
The odour threshold and Odour Active Value (OAV) of individual volatile compounds calculated by dividing the mean odour concentration for the yeast groups by the odourant threshold (mg/L). The odourant concentrations (mg/L) were determined by GC-MS and averaged between two fermentation temperatures. [individual Burgundian (n=12), mixed Burgundian strains (n=24)].

Table 19. The odour threshold and Odour Active Value (OAV) of individual volatile compounds calculated by dividing the mean odour concentration for the yeast groups by the odourant threshold (mg/L). The odourant concentrations (mg/L) were determined by GC-MS and averaged between two fermentation temperatures. [individual Burgundian (n=12), mixed Burgundian strains (n=24)].

<table>
<thead>
<tr>
<th>Compound</th>
<th>Aroma Descriptor</th>
<th>Odour threshold (mg/L)</th>
<th>Individual Burgundian</th>
<th>Mixed Burgundian</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Higher alcohols</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3-butanediol</td>
<td>Creamy&lt;sup&gt;1&lt;/sup&gt;</td>
<td>150&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.007</td>
<td>0.006</td>
</tr>
<tr>
<td>2-methyl-1-butanol</td>
<td>Sweet fruit&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.42&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3.169</td>
<td>3.322</td>
</tr>
<tr>
<td>3-methyl-1-butanol</td>
<td>Berry&lt;sup&gt;3&lt;/sup&gt;</td>
<td>30&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.237</td>
<td>0.230</td>
</tr>
<tr>
<td>n-butanol</td>
<td>Nail polish&lt;sup&gt;3&lt;/sup&gt;</td>
<td>150&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.0004</td>
<td>0.0004</td>
</tr>
<tr>
<td>1-hexanol</td>
<td>Grass&lt;sup&gt;1&lt;/sup&gt;</td>
<td>8&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.247</td>
<td>0.231</td>
</tr>
<tr>
<td>Isobutanol</td>
<td>Fusel&lt;sup&gt;1&lt;/sup&gt;</td>
<td>40&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.390</td>
<td>0.373</td>
</tr>
<tr>
<td>Phenylethanol</td>
<td>Rose&lt;sup&gt;1&lt;/sup&gt;</td>
<td>14&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.074</td>
<td>0.061</td>
</tr>
<tr>
<td>Propanol</td>
<td>Candy&lt;sup&gt;3&lt;/sup&gt;</td>
<td>306&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.007</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>Ethyl esters</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Ethyl butanoate</td>
<td>Strawberry&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.02&lt;sup&gt;2&lt;/sup&gt;</td>
<td>11.001</td>
<td>11.200</td>
</tr>
<tr>
<td>Ethyl hexanoate</td>
<td>Green apple&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.14&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3.130</td>
<td>2.914</td>
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<tr>
<td>Ethyl octanoate</td>
<td>Pear&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.005&lt;sup&gt;2&lt;/sup&gt;</td>
<td>8.005</td>
<td>8.215</td>
</tr>
<tr>
<td>Ethyl decanoate</td>
<td>Dried fruit&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.2&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.092</td>
<td>0.110</td>
</tr>
<tr>
<td>Ethyl laurate</td>
<td>Waxy&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.5&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.004</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>Acetate esters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>Balsamic&lt;sup&gt;2&lt;/sup&gt;</td>
<td>12&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.553</td>
<td>0.563</td>
</tr>
<tr>
<td>Hexyl acetate</td>
<td>Apple&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.016</td>
<td>0.017</td>
</tr>
<tr>
<td>Isoamyl acetate</td>
<td>Banana&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.03&lt;sup&gt;1&lt;/sup&gt;</td>
<td>3.432</td>
<td>3.312</td>
</tr>
<tr>
<td><strong>Aldehyde</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>Overripe apple&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.5&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.998</td>
<td>2.307</td>
</tr>
<tr>
<td><strong>Organic acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>Vinegar&lt;sup&gt;3&lt;/sup&gt;</td>
<td>600&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

<sup>1</sup>Odour Active Values (OAVs) obtained by dividing the mean concentration of the volatiles by the published water threshold value, for the yeast groups (individual and mixed Burgundian Strains). The OAVs of the individual industrial strains are reported in Table 32, Appendix A.

<sup>2</sup>Obtained from Campo et al., 2005
<sup>3</sup>Obtained from Francis and Newton, 2005
<sup>4</sup>Obtained from Cullere et al., 2004
<sup>4</sup>Obtained from Schieberle and Hofmann, 1997
Figures 26, 27, and 28 show aroma and estimated sensory profiles. The odourant concentrations (mg/L) for mean of individual Burgundian and mean of mixed Burgundian strains were plotted versus two different individual industrial strains at each aroma radar diagram (Figures 26 A, 27 A and 28 A) and compared with their estimated aroma sensory profile on another radar diagram (Figures 26 B, 27 B, and 28 B).

The individual and mixed Burgundian strains showed minor differences in their odour profiles (Figures 26 A, 27 A, and 28 A); the mixed Burgundian strains produced slightly more acetaldehyde and slightly less 2,3-butanediol than individual Burgundian strains. The odour profile of Fusion was different from Burgundian strains. Fusion produced slightly higher concentrations of higher alcohols such as 2,3-butanediol, 1-hexanol, and phenyl ethanol than Burgundian strains. Furthermore, Fusion produced considerably more concentrations of ethyl esters such as ethyl butanoate, ethyl hexanoate and ethyl laurate than Burgundian strains. Burgundian strains produced higher concentrations of acetate esters, specifically isoamyl acetate, than Fusion. The acetic acid production by Fusion was slightly higher than Burgundian strains (Figure 26 A). The odour profile of the Blanc strain showed some differences with Burgundian strains and X16; Blanc produced higher concentrations of propanol and 2,3-butanediol, ethyl decanoate, and acetaldehyde than Burgundian strains. The acetic acid production by Burgundian strains was slightly higher than that of Blanc (Figure 26 A).

The estimated aroma sensory profile of the individual and mixed Burgundian strains overlapped and showed very similar patterns. The industrial strains Blanc and X16 showed similar estimated sensory profiles, although Blanc had less waxy aroma and more candy aroma than X16 (Figure 26 B).
Figure 26. (A) Odour profile of the volatile compounds in Chardonnay wines. Mean odourant concentrations (mg/L) for individual Burgundian (n=12), mixed Burgundian strains (n=24) and odourant concentrations (mg/L) of individual industrial strains Blanc (n=6) and Fusion (n=6) averaged across both fermentation temperatures. (B) Estimated aroma sensory profile of the volatile compounds. OAV of individual volatile compounds calculated as the log of the ratio of odourant concentration to odourant threshold.
The Burgundian strains produced more berry, fusel oil, candy, and balsamic aromas than industrial strains; all of these aromas were below the human perception threshold. The production of nail polish and vinegar aromas by Burgundian strains was lower than industrial strains. The industrial strains produced more ethyl ester aromas such as strawberry, green apple, pear and waxy aromas. The Burgundian strains produced fruity aromas such as sweet fruit, strawberry, green apple, pear and banana at concentrations above their sensory threshold (Figure 26 B).

The X16 strain produced higher alcohols, ethyl butanoate, acetate esters and acetic acid than Burgundian strains. The Blanc strain produced more ethyl butanoate but less ethyl hexanoate than Burgundian strains (Figure 27 A). The industrial strains X16 and ICV-D254 showed very similar estimated sensory profiles. Theses industrial strains produced less berry, fusel oil, and candy aromas but more nail polish, strawberry, green apple, pear, waxy, banana and vinegar aromas than Burgundian strains (Figure 27 B).

Figure 28 compared the Burgundian strains with the other two individual industrial strains Elegance and CY3079. The industrial strains showed similar estimated sensory profiles to the other industrial strains as they produced less berry, fusel oil, candy, and balsamic aromas but more nail polish, ethyl ester fruity and waxy aromas and vinegars compared to the Burgundian strains (Figure 28).

The estimated sensory profiles of the individual and mixed Burgundian strains showed that production of fruity aromas such as sweet fruit, strawberry, green apple, pear and banana were above their sensory threshold, while the production of nail polish, waxy, balsamic and vinegar were below their human perception threshold.
Figure 27. (A) Odour profile of the volatile compounds in Chardonnay wines. Mean odourant concentrations (mg/L) for individual Burgundian (n=12), mixed Burgundian strains (n=24) and odourant concentrations (mg/L) of individual industrial strains X16 (n=6) and ICV-D254 (n=6) averaged across both fermentation temperatures. (B) Estimated aroma sensory profile of the volatile compounds (Log values of OAVs). OAV of individual volatile compounds calculated as the log of the ratio of odourant concentration to odourant threshold.
Figure 28. (A) Odour profile of the volatile compounds in Chardonnay wines. Mean odourant concentrations (mg/L) for individual Burgundian (n=12), mixed Burgundian strains (n=24) and odourant concentrations (mg/L) of individual industrial strains Elegance (n=6) and CY3079 (n=6) averaged across both fermentation temperatures. (B) Estimated aroma sensory profile of the volatile compounds (Log values of OAVs). OAV of individual volatile compounds calculated as the log of the ratio of odourant concentration to odourant threshold.
4 DISCUSSION

4.1 Genetic and phenotypic characterization of wine yeast strains

4.1.1 Genetic fingerprinting

The wine yeast strains were fingerprinted after isolation from the enological environment. The δ sequence typing genetically differentiated the six individual industrial strains and two novel Burgundian strains. This method revealed that Burgundian strains were different from each other and different from the control industrial strains. It is known that strains with the same molecular patterns have identical enological properties (Rainieri and Pretorius, 2004). The Burgundian strains are thus expected to have different enological properties.

4.1.2 Killer phenotype of Burgundian yeast strains

The two individual Burgundian strains are killer positive. The individual industrial strains Blanc, Elegance and ICV-D254 are killer positive, while the other individual industrial strains Fusion, CY3079 and X16 are sensitive strains. The growth of natural sensitive yeast strains is inhibited when fermentation is inoculated with a killer strain since killer inoculum strains become the predominate strain (van Vuuren and Jacobs, 1992). Killer sensitive yeast inocula may be inhibited by naturally occurring killer yeasts, causing stuck fermentations. The individual Burgundian strains have the potential to become predominant in single fermentations due to their killer positive phenotype. Furthermore, the Burgundian strains do not prevent the growth of the other Burgundian strain used in this study in mixed starter cultures, allowing them to grow simultaneously.
4.2 Enological characteristics of wine yeast strains

The wine yeast strains were enologically characterized after genetic characterization. Several desirable characteristics have been identified for wine yeast strains including fermentation kinetics, ethanol production, the efficient conversion of grape must sugar to ethanol, high glycerol production, low acetic acid production, high ethanol tolerance, compatibility with malolactic bacteria, low production of sulphur dioxide, and low foam formation (Pretorius, 2000). These traits were examined and compared to six industrial Chardonnay yeast strains to assess the enological equivalency of two novel Burgundian wine yeast strains and their mixed cultures.

Some of the enological characteristics were slightly different among all yeast strains, such as the fermentation kinetics at 16 °C and 20 °C. The two individual industrial strains Elegance and X16 had a higher rate of ethanol production at both fermentation temperatures. The Burgundian strains showed the similar growth kinetics patterns to the other industrial strains, which allowed them to ferment grape must vigorously and produce wines with high ethanol concentrations even at the lower temperature (16 °C).

All *S. cerevisiae* wine yeast strains produced significantly (p ≤ 0.05) higher concentrations of ethanol at 16 °C than at 20 °C, consistent with previous study by Torija et al. (2003); they investigated the effect of temperature (10, 20, 25, 30 and 35 °C) on ethanol production by *S. cerevisiae* wine yeast strains and reported that alcohol yield was higher at low temperatures due to greater viability of cells at low temperatures. The fermentation temperature has a main effect on ethanol tolerance of *S. cerevisiae* strains. The sensitivity of *S. cerevisiae* strains to ethanol increases as temperature
increases, particularly at temperatures above 20 °C (Ribereau-Gayon and Peynaud, 1960). Fermentation capacity of *S. cerevisiae* strains in this study was negatively affected at higher temperature (20 °C) and the yield of ethanol production was decreased. This may have simply occurred due to the volatilization of some of the ethanol produced at the higher temperature.

Fusion and CY3079 produced the lowest and highest amounts of ethanol, respectively, at both fermentation temperatures. The individual Burgundian and mixed Burgundian strains fell in the middle of the range of ethanol production in this study. Ethanol production is considered an important criterion of wine yeast strains. However, the positive effects of wine yeast strains on the composition and sensorial characteristics of wine are also essential. Excessive ethanol production can create the perception of hotness and mask the aroma and flavor of wine (Guth and Sies, 2002). Furthermore, the market’s demand for wines with reduced alcohol content has recently increased due to increased health awareness, more restricted drinking and driving laws, and increased taxes on high ethanol wines. Wine makers are encouraged to produce wines with moderate alcohol content, especially in warm climates where grape sugar content is high (Day et al., 2002). Strains which produce moderate levels of ethanol, such as the Burgundian strains, could be a better choice for wine makers producing wines with moderate alcohol content to meet the market’s demand.

No significant (*p* ≤ 0.05) difference existed in the ethanol production by individual and mixed Burgundian strains at either fermentation temperature; except for M4 which produced significantly (*p* ≤ 0.05) less ethanol than the individual Burgundian strain C2 at 20 °C. The individual Burgundian strain C2 was significantly (*p* ≤ 0.05) more ethanol tolerant than the other individual Burgundian strain C6 at both fermentation
temperatures. The mixed Burgundian strains were moderately ethanol tolerant at both fermentation temperatures. Although statistically significant differences in ethanol tolerance existed among the yeast strains, the Burgundian strains showed moderate and high ranges of ethanol tolerance, making them enologically equivalent to the individual industrial strains.

Conversion factor (sugar to ethanol) of Burgundian strains did not significantly (p ≤ 0.05) differ from industrial strains. The individual Burgundian and mixed Burgundian strains indicated the equivalent fermentation kinetics, ethanol production, ethanol tolerance, and sugar to ethanol conversion factors in comparison with the individual industrial strains. The Burgundian strains were able to ferment grape must vigorously and produce ethanol.

Glycerol is the most important product of alcoholic fermentation after ethanol and carbon dioxide. The glycerol production in this study ranged from 4.45-6.3 g/L at 16 °C and 4.58-6.11 g/L at 20 °C. The individual and mixed Burgundian strains produced 5.35-5.55 g/L of glycerol at 16 °C and 5.3-5.54 g/L at 20 °C, which was significantly (p ≤ 0.05) higher than the glycerol concentration in Chardonnay wines fermented by industrial strains ICV-D254 and CY3079. Glycerol is a non-volatile compound with no aromatic effect. Yet, glycerol improves the quality of wine by providing sweetness and mouth feel (Eustace and Thornton, 1987). The production of glycerol by S. cerevisiae ranges from 2 to 11 g/L in different wines; however, the usual range of glycerol production is between 4 and 9 g/L (Grazia et al., 1995). The sweet taste of glycerol is perceived in white table wine at a concentration of 5.2 g/L; while the perceptible threshold for viscosity of glycerol is at 25.8 g/L (Scanes et al., 1998). The moderate glycerol content of Chardonnay wines fermented by the individual and mixed Burgundian strains were above
the sweetness threshold of glycerol. Oak aging will increase the perception threshold for glycerol and make the sweet taste unperceivable. Wine composition may change the glycerol thresholds; for example, wine acidity and ethanol concentration increase the sensory threshold of glycerol (Hinreiner et al., 1955).

Glycerol production by wine yeast strains did not significantly (p > 0.05) differ across the two fermentation temperatures except for Blanc and M1. Glycerol production by yeast strains increased by 0.5% when the temperature increased from 16 °C to 20 °C except for Fusion, M1 and M4. Remize et al. (1999) assessed the effects of yeast strains and environmental factors on glycerol production in wine. The production of glycerol by 19 industrial wine strains was between 6.4-8.9 g/L and varied significantly among strains. Temperature had a slight impact on glycerol production. The production of glycerol increased by less than 15% when the fermentation temperature was raised from 18 °C to 28 °C. Agitation had a minor effect on the glycerol production (0-13%). The initial concentration of nitrogen in grape must had a slight effect on glycerol production. According to Remize et al. (1999), environmental factors also had a slight effect on glycerol production, while different wine yeast strains produced significantly different amounts of glycerol, consistent with the current study. Therefore, production of wines with high glycerol content requires the selection of wine yeast strains that produce high glycerol concentrations. The Burgundian strains produced moderate amounts of glycerol; some industrial strains such as CY3079 and ICV-D254 produced less glycerol than Burgundian strains.

Acetic acid usually constitutes about 90% of the volatile acids in wine. The range of acetic acid production by Burgundian strains at 16 °C was 0.095-0.181 g/L and at
20 °C 0.077-0.178 g/L. The acetic acid content of a normal dry table wine ranges from 0.2 to 0.4 g/L (Ribereau-Gayon, 1961). The U.S legal limit of acetic acid in white wines is 1.1 g/L. The sensorial perception threshold of acetic acid is more than 0.7 g/L, higher than the normal production level of acetic acid by \textit{S. cerevisiae} (Zoecklein et al., 1995). Acetic acid production by all of the wine yeast strains available in this study, including the Burgundian strains, was much lower than the legal limit and also below the sensory detection threshold. The Burgundian strains are therefore equivalent to individual industrial strains in terms of acetic acid production. High acetic acid content in wines is usually caused by stressed yeast cells and by acetic acid bacteria that originate from bad fermentation practices.

Production of a well-balanced wine is important in wine making. Malolactic fermentation is conducted to decrease the acidity of wine through conversion of malic acid to lactic acid (Beelman and Gallander, 1979). Inoculated lactic acid bacteria may grow slowly in wines which results in stuck malolactic fermentation and spoilage of wines when wine yeast strains are incompatible with lactic acid bacteria (Davis et al., 1985). The malic acid was degraded up to 90% in the Burgundian strains fermentations, which was comparable with industrial strains fermentations. Therefore, compatibility of the Burgundian strains with malolactic bacteria was equivalent to the industrial strains. Interestingly, the final malic acid content in the wines fermented by the mixed Burgundian strains was less than in wine fermented by C6 and higher than wine fermented by C2; this could be due to unknown metabolic interactions in mixed cultures.

The industrial wine yeast strains used in this study produced sulphur dioxide in the range of 27.9 to 59.91mg/L at 16 °C and 21.7 to 29.86 mg/L at 20 °C. The range of sulphur dioxide production at 16 °C was above the usual range (10-30 mg/L) of sulphur
dioxide production by the *S. cerevisiae* (Regadon et al., 1997). The range of sulphur dioxide production by Burgundian strains ranged between 17.17-38.44 mg/L at 16 °C and between 15.29-30.69 mg/L at 20 °C, showing low to moderate production of sulphur dioxide compared to the industrial strains.

Sulphur dioxide production by yeast strains may be influenced by the use of a synthetic juice with a poor nutrient profile (Spiropoulis et al. 2000), we had to use synthetic juice to ensure the absence of initial sulphites. Wine yeasts can tolerate around 250 mg/L total sulphur dioxide (Regadon et al., 1997). This attribute might come into consideration because of the sensitivity of the lactic acid bacterial *O. oeni* to sulphur dioxide. Most of the *O. oeni* strains can tolerate 15 mg/L free sulphur dioxide and 60-100 mg/L of total sulphur dioxide. Therefore, high concentrations of sulphur dioxide produced during alcoholic fermentation may cause stuck or sluggish malolactic fermentation (Eschenbruch, 1974), which may negatively influence the final balance of the wines. The individual and mixed Burgundian strains seem to be a better choice than the industrial strains in terms of sulphur dioxide production. It is unlikely that Burgundian strains will negatively affect malolactic fermentation due to sulphur dioxide production. Sulphur dioxide production does not principally contribute to the selection of wine yeast strains, unless low production of sulphur dioxide is required for malolactic fermentation. Another possible benefit of low sulphur dioxide producing yeasts is when a low sulphur dioxide strain is needed to respond to the market’s demand for organic wine.

Foam formation is one of the undesirable technological properties of wine yeasts. Foaming occurs in the initial phases of wine fermentation. Approximately 5% of the capacity of fermentation vessels is assigned to accommodate foam formation. The real volume of the fermenter is reduced. Furthermore, foam contains a significant amount of
yeast cells which may accumulate above the grape juice on the walls and roof of a closed tank. The accumulated yeasts will no longer participate in the fermentation and may form a black, unpleasant-smelling layer on the surface (Thornton, 1978). Therefore, low formation of foam is a valuable quality for wine yeasts due to limited space available in fermentation tanks in wineries during the vintage.

Although it is known that grape must profiles greatly affect foam formation; foam formation of the wine yeast strains is an important consideration for wineries (Esch and Rassell, 1975). Foam production by Burgundian strains was higher but primarily fell within the range of foam production by the individual industrial strains. However, further tests using different grape musts would be required to confirm that foam formation by the Burgundian strains is not an industrial issue.

The one observed trend is optimization of enological properties in mixed Burgundian strains. When one of the individual Burgundian strains produced a higher amount of one enological property than the other, the mixing of these two produced an optimized level usually dependent on the ratio of the mixture. The trend was observed for enological properties such as ethanol tolerance, acetic acid and sulphur dioxide production; this could have happened due to unknown metabolic interactions in mixed cultures.

In conclusion, the novel Burgundian strains and their mixtures were enologically competent and equivalent to individual industrial strains for the production of Chardonnay wine.
4.3 Enological patterns of wine yeast strains

The PCA grouped wine yeast strains according to their enological traits. The individual and mixed Burgundian strains clustered together and formed distinct groups from the industrial strains; they collectively carry most of the important desirable characteristics. The individual and mixed Burgundian strains are enologically more similar to one another than to the industrial strains. As an example, the industrial strain X16 produced a high concentration of sulphur dioxide, which is not desirable. Furthermore, the industrial strains ICV-D254 produced foam either comparable to or even greater than Burgundian strains depending on the temperature.

The enological patterns of mixed Burgundian strains were similar to the individual Burgundian strains; the M4 mixture was the only Burgundian mixture that showed markedly different enological pattern from individual and other mixed Burgundian strains at 20 °C. The M4 strain was significantly (p ≤ 0.05) more ethanol tolerant than the individual Burgundian strains and produced significantly more (p ≤ 0.05) sulphur dioxide than individual Burgundian strains. This different pattern may be due to the combined effects of temperature and the metabolic interaction of the individual strains in the mixed culture.
4.4 Volatile profiles of wines produced with different yeast strains

Use of mixed *S. cerevisiae* strains is one of the recent technologies for fermentations to produce more complex wine (Lambrechts & Pretorius, 2000; Fleet, 2003). Wine makers are currently looking for mixtures of indigenous yeast species and strains to enhance wine flavour (Grossmann et al., 1996). Few studies have thus far directly investigated the effect of the mixed *S. cerevisiae* strains on wine fermentation, specifically on Chardonnay wine. More studies are required to understand the impact of indigenous and mixed starter cultures on wine composition. The current study investigated the impact of indigenous Burgundian *S. cerevisiae* strains and their mixtures on the volatile profile of Chardonnay wine.

Eighteen volatile compounds were identified and quantified in the chemical profile of Chardonnay wines. Differences existed in concentrations of the volatile compounds in the headspace of Chardonnay wines fermented with individual industrial, individual Burgundian and mixed Burgundian strains were different, while no different volatile compounds were detected in the chemical profile of the Chardonnay wines fermented by the Burgundian strains. Howell et al. (2005) investigated the effect of mixed *S. cerevisiae* strains on the chemical profile and aromatic properties of Chardonnay wine; three industrial strains which were inoculated at the same rate. The chemical profiles of the wines fermented by mixed *S. cerevisiae* strains were different from the chemical profiles of the wines fermented by individual *S. cerevisiae* strains. Blending the single fermented wines did not create the same chemical profile as the wine fermented by mixed culture fermentation. The metabolic interactions of the wine yeast strains were regarded as the cause of the different chemical composition and aromatic properties in the wines fermented by mixed cultures.
Another study by Swiegers et al. (2007) from the Australian Wine Research Institute indicated that mixed *S. cerevisiae* strain fermentations produced lower thiol concentrations and enhanced sensory properties in Sauvignon Blanc; wines produced with the individual yeasts strains did not show these properties.

In this study, the Chardonnay wines fermented by individual Burgundian and mixed Burgundian strains had similar volatile compounds, but in different quantities. This effect was markedly noticeable in the production of higher alcohols. One of the individual Burgundian strains produced a lower amount of higher alcohols such as n-butanol and isobutanol than the other individual Burgundian yeast strain, while the Burgundian mixtures produced moderate levels of that higher alcohol. These differences could affect the sensory quality of Chardonnay wines.

The fermentation temperature did not significantly (*p > 0.05*) affect the production of volatile compounds in this study, while previous studies (Ough and Aremine, 1967; Killian and Ough 1979) showed that production of higher alcohols and esters were affected by fermentation temperature. They did, however, investigate the effect of low, middle, and high fermentation temperatures (10 °C, 21 °C and 30 °C) on the production of volatile compounds and found that the production of higher alcohols, such as isoamyl alcohol, was increased at 21 °C, while isobutanol production remained relatively the same under different fermentation temperatures. The production of 2,3-butanediol increased as fermentation temperature increased. The maximum production of volatile esters occurred at 21 °C. In the current study, the production of 2, 3-butandiol was slightly higher at 20 °C than at 16 °C, while the isobutanol production remained relatively unchanged over the temperature increase. The ethyl ester production at 20 °C was slightly higher than 16 °C, consistent with previous studies.
The fermentation temperature can affect the production of volatile compounds indirectly. The rate of carbon dioxide production increases as fermentation temperature increases. The rapid evolution of carbon dioxide removes wine volatile compounds (Kunkee and Bisson, 1993). No significant (p > 0.05) difference in volatile production by wine yeast strains at 16 °C and 20 °C could be related to the slight difference in fermentation temperature.

The PCA grouped wine yeast strains according to their volatile compounds produced. The individual and mixed Burgundian strains clustered together in distinct groups from the industrial strains. The volatile profiles of individual and mixed Burgundian strains were more similar to one another than to the industrial strains. The current study summarizes the effect of volatile compounds by calculating the odour active values (OAVs) to create the estimated sensory profiles of the wines fermented with Burgundian strains against industrial strains. The higher alcohols quantitatively constitute the largest group of volatile compounds in wine (Amerine et al., 1980). Eight higher alcohols were quantified in the headspace of Chardonnay wines fermented with individual industrial, individual and mixed Burgundian strains and constituted the largest group of volatile compounds, consistent with previous studies.

The production of higher alcohols by individual and mixed Burgundian strains was more similar to one another than to that produced by the industrial strains. The mixed Burgundian strains produced moderate levels of higher alcohols compared to the industrial strains. The higher alcohols mostly contribute to the fruity aroma and flavour of the wine at low levels. Higher alcohols at concentrations more than 300 mg/L are destructive of wine flavour and aroma (Swiegers et al., 2005).
The OAVs of all higher alcohol compounds produced by Burgundian strains were below human thresholds with the exception of 2-methyl-1-butanol, which is responsible for sweet fruit aroma (Table 19). The OAVs for n-butanol, 1-hexanol and isobutanol, which are responsible for unpleasant nail polish, grass, and fusel oil, were far below their sensory thresholds. The mixed Burgundian strains tended to produce very low amounts of 2,3-butanediol, which is a slightly bitter creamy compound. Although 2,3-butanediol is usually produced in large concentrations, its contribution to the sensory profile of the wine is not well established due to its high perception threshold (150 mg/L) (Dubois, 1994). The indigenous Burgundian strains produced less higher alcohols than industrial strains. In a study by Romano et al. (1992), 14 S. cerevisiae strains were isolated from the Salnés region in Spain and screened for production of volatile compounds such as higher alcohols. This study revealed that production of higher alcohols by the indigenous yeast strains in single inoculated fermentations was lower than normal levels in Salnés spontaneous fermentations. This could have been attributed to the use of indigenous yeast strains as pure culture, as spontaneous fermentations are conducted by original microflora which are a mixture of different strains and species (Longo et al., 1992).

Two patterns were observed in the production of higher alcohols by mixed Burgundian strains compared with individual strains. The mixed Burgundian strains mostly produced similar amounts of some higher alcohols such as 2,3-butanediol, 2-methyl-1-butanol, 3-methyl-1-butanol to individual Burgundian strains, while two individual Burgundian strains produced slightly different amount of higher alcohols from each other. The mixed Burgundian strains produced moderate levels of higher alcohols such as n-butanol and isobutanol compared to individual Burgundian strains, while one of
the individual Burgundian strains produced a higher amount of n-butanol and isobutanol than the other Burgundian strain. C2 produced significantly (p ≤ 0.05) greater amount of n-butanol than C6, while the mixed Burgundian strains produced moderate amounts of n-butanol. The C6 produced a significantly (p ≤ 0.05) greater amount of isobutanol than C2, while the Burgundian mixtures produced moderate levels of isobutanol. These different patterns in the volatile profile of the Chardonnay wines fermented by mixed Burgundian strains compared with pure Burgundian and industrial cultures were attributed to potential metabolic interactions in mixed cultures. The individual Burgundian strains and particularly the mixed Burgundian strains could therefore positively contribute to the sensory profile of wine in terms of moderate levels of pleasant higher alcohols compared with industrial strains.

Esters are volatile compounds which significantly contribute to the fruity flavour and aroma of wine (Herraiz and Ough, 1993). Five ethyl esters were quantified in the headspace of the Chardonnay wines produced in this study. The individual Burgundian strains produced similar amounts of ethyl esters. The mixed Burgundian strains produced similar or slightly higher amounts of ethyl esters compared to individual Burgundian strains. The sensory descriptors and human thresholds of the ethyl esters are shown in Table 19. The OAVs of ethyl butanoate, ethyl hexanoate and ethyl octanoate produced by Burgundian strains were above the sensory threshold. Ethyl butanoate, ethyl hexanoate and ethyl octanoate are responsible for strawberry, green apple and pear aromas. The OAVs of ethyl laurate produced by Burgundian strains was below the human threshold. The ethyl laurate is responsible for a less pleasant waxy aroma. Some industrial strains produced higher concentrations of ethyl laurate. The Burgundian strains produced ethyl
esters with pleasant fruity aromas above their perception threshold, which could positively contribute to the overall aroma of wine.

Ethyl acetate, hexyl acetate, and isoamyl acetate were quantified in the headspace of Chardonnay wines. The hexyl acetate and isoamyl acetate contribute to the fruity aroma of wine. The hexyl acetate produces a balsamic aroma. The OAVs of isoamyl acetate produced by Burgundian strains were above its threshold. Isoamyl acetate produces a pleasant banana aroma in wine. The OAVs of ethyl acetate produced by Burgundian strains were below the human threshold. The ethyl acetate produces balsamic aroma.

The mixed Burgundian strains produced acetate esters either within or slightly higher than the range of individual Burgundian strains. The Burgundian strains could thus positively contribute to the fruity aroma of the wine.

Although the OAVs of individual and mixed Burgundian strains for pleasant esters were above their sensory thresholds, the indigenous Burgundian strains produced smaller amounts of the esters compared to the industrial strains. As previously mentioned (Longo et al., 1992), the production of esters by indigenous strains used as pure culture was below the usual levels in Salnés spontaneous fermentations in which different yeast strains and species were present. The production of ethyl decanoate by mixed Burgundian strains was higher than individual strains in the present study, consistent with Longo et al. (1992).

Acetaldehyde was the only aldehyde analyzed in the chemical profile of the Chardonnay wines in this study. Acetaldehyde usually represents more than 90% of the total aldehydes (Nykanen, 1986). The sensory contribution of aldehydes in wine is important due to their low perceived sensory threshold (Suomalainen and Lehtonen,
The sensory threshold of acetaldehyde is 0.5 mg/L, at which a sour green apple aroma can be perceived (Lambrechts and Pretorious, 2000). The OAVs of acetaldehyde produced by Burgundian strains were above its sensory threshold. The Burgundian strains produced similar amounts of acetaldehyde compared to the industrial strains.

The acetaldehyde content of wine is affected by factors such as grape must, aging, and the sulphur dioxide content of the grape must (Romano et al., 1994).

A study by Salgado (1987) showed the indigenous *S. cerevisiae* strains isolated from the Salnés region in Spain produced more acetaldehyde than found in commercial Salnés wines. The use of grape must with high concentrations of sulphur dioxide (150 mg/L) compared with the grape must used in Salnés wineries with typical levels of sulphur dioxide ranging from 30-60 mg/L may explain the reason for the higher acetaldehyde production (Salgado, 1987).

Acetic acid was the only quantified organic acid in the headspace of Chardonnay wine in this study. The OAVs of acetic acid produced by Burgundian strains were zero. The range of acetic acid production by Burgundian strains was lower than all other industrial strains. The Burgundian strains should positively contribute to the overall aroma of wine by not producing acetic acid.

In summary, the estimated sensory profiles of individual and mixed Burgundian strains were similar to each other but different from those of the industrial strains. The Burgundian strains collectively carry pleasant aromas with OAVs above the sensory threshold. These novel yeast strains produced lower concentrations of n-butanol (nail polish), isobutanol (fusel), and acetic acid (vinegar), when compared to industrial wine yeast strains. Furthermore, the Burgundian strains showed similar OAVs for phenylethanol, which is responsible for rose notes in wines. The industrial strains showed
higher OAVs of ethyl esters, however, the OAVs of the Burgundian strains for fruity ethyl esters were above the threshold. The Burgundian strains could positively contribute to the overall aroma of wine according to their estimated sensory profiles.

It is important to note that the human nose has a different sensitivity to volatile compounds than mechanical detectors such as GC’s which identifies individual compounds by separating them. Furthermore, human beings perceive flavour by subconsciously integrating their sense response with the different substances present (Keith and Powers, 2006). Therefore, volatile compounds with OAVs below the sensory threshold might also be considered in sensory effects.

The OAVs of 2-methyl-1-butanol, ethyl butanoate, ethyl hexanoate, ethyl octanoate, isoamyl acetate and acetaldehyde produced by Burgundian strains were above their sensory threshold. The rest of compounds had OAVs below the sensory thresholds (Table 19).

Few studies address the effect of the subthresholds and their contribution to the overall perceived aroma. Chemical compounds with minor individual impact might combine with other compounds to have a considerable impact. The subthreshold concentrations may have positive or synergistic effects on the perceived overall aroma of a mixture of compounds present at super threshold and subthresholds (Bult et al., 2001). The compounds with OAVs below their sensory threshold might combine with other compounds and contribute to the overall flavour of Chardonnay wines in this study.

In conclusion, the individual and mixed Burgundian strains seem to influence the aroma and flavour of a wine in a way which is different from existing yeast strains resulting in wines with different chemical composition.
5 CONCLUSIONS

The novel Burgundian strains C2 and C6 had unique genetic fingerprints compared to the commercially available wine yeast strains, validating that they were different strains of *S. cerevisiae* which could be used individually or in combination. The C2 and C6 strains are both killer positive, allowing them to dominate during fermentation and grow simultaneously.

The individual Burgundian and mixed Burgundian yeast strains completed alcoholic fermentation successfully and produced 13.94-14.02 % (v/v) ethanol at 16 °C and 13.06-13.23 % (v/v) at 20 °C. The Burgundian strains showed intermediate enological properties (ethanol tolerance, glycerol, acetic acid, and sulphur dioxide production) compared to industrial strains, with the exception of foam production. The Burgundian strains were enologically equivalent to the industrial strains, making them appropriate for commercial wine fermentation.

PCA analysis for the enological properties of the yeast strains revealed that individual and mixed Burgundian yeast strains were more similar to one another than to the industrial yeast strains. GC-MS headspace analysis of 18 volatile compounds (higher alcohols, ethyl esters, acetate esters, aldehyde, and organic acid) revealed no significant (p > 0.05) difference between volatile compounds produced by the same yeast at the two fermentation temperatures, while significant (p ≤ 0.05) differences occurred among the wine yeast strains.

PCA analysis of the 18 volatile compounds (higher alcohols, ethyl esters, acetate esters, aldehyde, and organic acid) indicated that individual and mixed Burgundian strains were more similar to one another than to the industrial strains. In general, the concentration of the higher alcohols, ethyl esters, and acetate esters from the individual
and mixed Burgundian strains were different but within the range typically observed for commercial yeast strains.

Mixed Burgundian yeast strains produced higher amounts of higher alcohols such as 2-methyl-1-butanol resulting in wine with fruity aromas and lower amounts of higher alcohols like 2,3-butanediol (Creamy), propanol (Candy) and phenylethanol (Rose). These yeast strains also produced more ethyl hexanoate and ethyl octanoate, which contribute to fruity notes. The Burgundian strains produced low to moderate levels of acetate esters, while most of the industrial strains tended to produce either very low or very high levels of acetate esters. The individual and mixed Burgundian strains produced wines that had distinct patterns of volatile compounds, which were unique from industrial strains; however the relative impact of these volatiles could not be assessed without interpreting their OAVs.

The estimated sensory profiles of individual and mixed Burgundian strains were similar but different from estimated aroma sensory profiles of wines produced by the industrial strains. In general, wines produced with the Burgundian strains had OAV of pleasant aromas, which were above the sensory threshold. Therefore, it is speculated that the Burgundian strains would positively contribute to the overall wine aroma.

Individual and mixed Burgundian yeast strains offer wine makers the opportunity to influence the bouquet a wine in a way which is not currently available using single yeast strains. However, further research is necessary to confirm that the volatile profiles are consistent with those obtained in commercial size fermentations. In addition, the use of threshold values obtained in wine rather than water would provide a more satisfactorily estimated OAVs. Unfortunately, such values are not currently available in
the literature. Therefore, the effect of the yeasts should be confirmed using the sensory analysis.
6 FURTHER STUDIES

The relative sensory impact of volatile compounds in this study was determined using the estimated sensory profiles which are new in wine research. The estimated sensory profiles showed that the Chardonnay wines fermented by Burgundian strains had different aromatic profiles from Chardonnay wines fermented with commercially available industrial strains. We matched the analytical data obtained by GC with their sensory contributions. Quantitative gas chromatography-olfactometry (GC/O) is another method which can be used to link volatile compounds to aromatic characteristics.

The GC identifies the compounds by separating them, while human beings perceive flavour by subconsciously integrating their sense response with the different substances present. Additionally, the nose is more sensitive to volatile compounds than mechanical detectors. Furthermore, some organoleptic interactions may happen among wine compounds; one compound may mask or decrease sensitivity to another compound (Keith and Powers, 2006). It is thus highly recommended to hold sensory tests to examine the effect of indigenous Burgundian strains and their mixtures on wine flavour.

Furthermore, the aging process has a considerable effect on the development of aromatic compounds in wine; headspace volatiles are not static but dynamic. The data prepared in this research is a snap-shot in time only. Chardonnay wines fermented with the individual Burgundian and mixed Burgundian strains could show more sensory differences from each other and industrial strains after the aging process. It is recommended to have analytical tests in conjunction with the sensory tests after aging the Chardonnay wines fermented with Burgundian and industrial strains.

The laboratory model of wine fermentation is different from industrial wine making. Wine making in small scale in the laboratory does not include factors such as
surface area, potential exposure to oxygen, bulk fermentations following bulk aging. The chemical and sensory profiles of wines fermented with Burgundian strains at an industry scale might be different due to the large scale fermentations, aging process and other factors involved.
REFERENCES


### APPENDIX A: Raw data of enological properties and volatile compounds

Table 20. Ethanol concentration (% v/v) of Chardonnay wines fermented at 16 °C (A) and 20 °C (B) with *S. cerevisiae* strains.

#### (A)

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std*</th>
<th>Std error**</th>
<th>RSD (%)***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blanc</td>
<td>14.11</td>
<td>14.15</td>
<td>14.13</td>
<td>14.13</td>
<td>0.02</td>
<td>0.01</td>
<td>0.13</td>
</tr>
<tr>
<td>Elegance</td>
<td>14.22</td>
<td>14.21</td>
<td>14.22</td>
<td>14.21</td>
<td>0.01</td>
<td>0.00</td>
<td>0.06</td>
</tr>
<tr>
<td>Fusion</td>
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<td>13.81</td>
<td>13.88</td>
<td>13.86</td>
<td>0.04</td>
<td>0.02</td>
<td>0.31</td>
</tr>
<tr>
<td>CY3079</td>
<td>14.27</td>
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<td>14.27</td>
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<td>0.01</td>
<td>0.07</td>
</tr>
<tr>
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<td>14.04</td>
<td>0.46</td>
<td>0.27</td>
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<td>14.07</td>
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<td>14.15</td>
<td>0.07</td>
<td>0.04</td>
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<tr>
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<td>0.02</td>
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<tr>
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<tr>
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<td>0.95</td>
</tr>
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#### (B)

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<th>Yeast Strain</th>
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<th>Replicate 3</th>
<th>Average</th>
<th>Std*</th>
<th>Std error**</th>
<th>RSD (%)***</th>
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<td>13.34</td>
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<td>13.32</td>
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<td>0.02</td>
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<td>0.02</td>
<td>0.01</td>
<td>0.12</td>
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<td>13.23</td>
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<tr>
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<td>13.12</td>
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<td>0.02</td>
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<tr>
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<tr>
<td>M3</td>
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<td>13.00</td>
<td>13.06</td>
<td>0.11</td>
<td>0.06</td>
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*Standard Deviation  **Standard error  ***Relative Standard Error
Table 21. Conversion factors (ethanol produced/sugar consumed) by *S. cerevisiae* strains at 16 °C (A) and 20 °C (B).

(A)  

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std</th>
<th>Std error</th>
<th>RSD (%)</th>
</tr>
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<tr>
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<td>0.463</td>
<td>0.007</td>
<td>0.004</td>
<td>1.496</td>
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<td>Elegance</td>
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<td>0.465</td>
<td>0.008</td>
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<tr>
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<td>0.459</td>
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</tr>
<tr>
<td>X16</td>
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<td>0.457</td>
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<td>0.465</td>
<td>0.001</td>
<td>0.001</td>
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<td>0.001</td>
<td>0.323</td>
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</table>

(B)  

<table>
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<tr>
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<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std</th>
<th>Std error</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blanc</td>
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<td>0.428</td>
<td>0.434</td>
<td>0.431</td>
<td>0.003</td>
<td>0.002</td>
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<td>0.438</td>
<td>0.437</td>
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<td>0.001</td>
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<td>0.423</td>
<td>0.427</td>
<td>0.425</td>
<td>0.002</td>
<td>0.001</td>
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</tr>
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<td>0.436</td>
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</tr>
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<td>0.431</td>
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<td>0.431</td>
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</table>

*Standard Deviation  **Standard error  ***Relative Standard Error
Table 22. Glycerol concentration (% v/v) of Chardonnay wines fermented at 16 °C (A) and 20 °C (B) with *S. cerevisiae* strains.

(A)  

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std</th>
<th>Std error</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blanc</td>
<td>5.07</td>
<td>4.97</td>
<td>4.95</td>
<td>4.99</td>
<td>0.06</td>
<td>0.04</td>
<td>1.26</td>
</tr>
<tr>
<td>Elegance</td>
<td>5.37</td>
<td>5.46</td>
<td>5.46</td>
<td>5.43</td>
<td>0.05</td>
<td>0.03</td>
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<tr>
<td>Fusion</td>
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<td>6.26</td>
<td>6.30</td>
<td>6.30</td>
<td>0.04</td>
<td>0.02</td>
<td>0.68</td>
</tr>
<tr>
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<td>4.77</td>
<td>4.83</td>
<td>4.76</td>
<td>4.78</td>
<td>0.04</td>
<td>0.02</td>
<td>0.75</td>
</tr>
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<td>4.45</td>
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<td>4.70</td>
</tr>
<tr>
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<td>5.72</td>
<td>5.66</td>
<td>5.67</td>
<td>0.05</td>
<td>0.03</td>
<td>0.90</td>
</tr>
<tr>
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<td>5.48</td>
<td>0.13</td>
<td>0.07</td>
<td>2.33</td>
</tr>
<tr>
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<td>5.54</td>
<td>5.49</td>
<td>0.06</td>
<td>0.04</td>
<td>1.16</td>
</tr>
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<td>5.60</td>
<td>5.55</td>
<td>5.55</td>
<td>0.05</td>
<td>0.03</td>
<td>0.94</td>
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<tr>
<td>M2</td>
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<td>5.43</td>
<td>0.10</td>
<td>0.06</td>
<td>1.83</td>
</tr>
<tr>
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<td>5.36</td>
<td>5.35</td>
<td>0.03</td>
<td>0.02</td>
<td>0.62</td>
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<tr>
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<td>5.47</td>
<td>5.53</td>
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<td>0.03</td>
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</table>

(B)  

<table>
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<th>Replicate 3</th>
<th>Average</th>
<th>Std</th>
<th>Std error</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blanc</td>
<td>5.46</td>
<td>5.75</td>
<td>5.90</td>
<td>5.71</td>
<td>0.23</td>
<td>0.13</td>
<td>3.95</td>
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<tr>
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<td>5.46</td>
<td>5.46</td>
<td>5.43</td>
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<td>0.03</td>
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<tr>
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<td>6.32</td>
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<td>0.26</td>
<td>0.15</td>
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<td>0.05</td>
<td>1.76</td>
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<td>0.11</td>
<td>0.06</td>
<td>2.02</td>
</tr>
<tr>
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<td>5.13</td>
<td>5.32</td>
<td>5.30</td>
<td>0.16</td>
<td>0.09</td>
<td>2.99</td>
</tr>
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<td>5.33</td>
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<td>5.30</td>
<td>0.05</td>
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<td>0.05</td>
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<td>5.70</td>
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*Standard Deviation** Standard error*** Relative Standard Error
Table 23. Acetic acid concentration (% v/v) of Chardonnay wines fermented at 16 °C (A) and 20 °C (B) with *S. cerevisiae* strains.

(A)

<table>
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<tr>
<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std</th>
<th>Std error</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
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<td>0.10</td>
<td>0.095</td>
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<td>0.01</td>
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</tr>
<tr>
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<td>0.09</td>
<td>0.103</td>
<td>0.02</td>
<td>0.01</td>
<td>14.78</td>
</tr>
<tr>
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<td>0.153</td>
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<td>0.01</td>
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</tr>
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<td>0.01</td>
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<td>0.01</td>
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(B)

<table>
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<th>Replicate 3</th>
<th>Average</th>
<th>Std</th>
<th>Std error</th>
<th>RSD (%)</th>
</tr>
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<tbody>
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<td>0.077</td>
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<td>0.01</td>
<td>9.05</td>
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<td>0.01</td>
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</tr>
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<td>0.178</td>
<td>0.02</td>
<td>0.01</td>
<td>9.89</td>
</tr>
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<td>0.01</td>
<td>11.76</td>
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<td>0.097</td>
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<td>10.15</td>
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</table>

*Standard Deviation  **Standard error  ***Relative Standard Error
Table 24. Ethanol tolerance of *S. cerevisiae* strains at 16 °C (A) and 20 °C (B).

<table>
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<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std*</th>
<th>Std error**</th>
<th>RSD (%)***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blanc</td>
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<td>17.08</td>
<td>17.35</td>
<td>17.26</td>
<td>0.16</td>
<td>0.09</td>
<td>0.90</td>
</tr>
<tr>
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<td>18.33</td>
<td>18.37</td>
<td>18.32</td>
<td>0.06</td>
<td>0.03</td>
<td>0.32</td>
</tr>
<tr>
<td>Fusion</td>
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<td>15.12</td>
<td>15.01</td>
<td>15.19</td>
<td>0.22</td>
<td>0.13</td>
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</tr>
<tr>
<td>CY3079</td>
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<td>17.11</td>
<td>17.47</td>
<td>17.37</td>
<td>0.23</td>
<td>0.13</td>
<td>1.30</td>
</tr>
<tr>
<td>ICV-D254</td>
<td>16.50</td>
<td>16.75</td>
<td>16.56</td>
<td>16.60</td>
<td>0.13</td>
<td>0.07</td>
<td>0.77</td>
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<tr>
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<td>18.52</td>
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<td>18.48</td>
<td>0.06</td>
<td>0.04</td>
<td>0.35</td>
</tr>
<tr>
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<td>17.23</td>
<td>17.75</td>
<td>17.37</td>
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<td>0.19</td>
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<tr>
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<td>16.52</td>
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<td>0.64</td>
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<tr>
<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std*</th>
<th>Std error**</th>
<th>RSD (%)***</th>
</tr>
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<tbody>
<tr>
<td>Blanc</td>
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<td>17.52</td>
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<td>17.26</td>
<td>16.85</td>
<td>0.47</td>
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<td>2.78</td>
</tr>
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<td>16.59</td>
<td>16.82</td>
<td>16.69</td>
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<td>15.30</td>
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*Standard Deviation  **Standard error  ***Relative Standard Error
Table 25. Malolactic compatibility of *S. cerevisiae* strains at 20 °C. Initial (A) and final (B) concentrations of malic acid (g/L); initial (C) and final (D) concentrations (g/L) of lactic acid.

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std*</th>
<th>Std error**</th>
<th>RSD (%)***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blanc</td>
<td>5.077</td>
<td>5.000</td>
<td>5.016</td>
<td>5.031</td>
<td>0.04</td>
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<td>0.81</td>
</tr>
<tr>
<td>Elegance</td>
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<tr>
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<td>4.623</td>
<td>4.677</td>
<td>4.650</td>
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<td>0.02</td>
<td>0.58</td>
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<td>0.89</td>
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<td>4.923</td>
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<td>4.994</td>
<td>0.09</td>
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<th>Std error**</th>
<th>RSD (%)***</th>
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<tbody>
<tr>
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<td>0.443</td>
<td>0.472</td>
<td>0.450</td>
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<tr>
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<td>0.469</td>
<td>0.463</td>
<td>0.464</td>
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* Standard Deviation  ** Standard error  *** Relative Standard Error
### Table 25 (Continued)

#### (C)

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<th>Std*</th>
<th>Std error**</th>
<th>RSD (%)***</th>
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<td>0.031</td>
<td>0.031</td>
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### (D)

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<th>Replicate 3</th>
<th>Average</th>
<th>Std*</th>
<th>Std error**</th>
<th>RSD (%)***</th>
</tr>
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<tbody>
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</tr>
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<td>1.81</td>
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*Standard Deviation  **Standard error  ***Relative Standard Error
Table 26. Sulphur dioxide concentration (mg/L) of Chardonnay wines fermented at 16 °C (A) and 20 °C (B) with *S. cerevisiae* strains.

(A)  
<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std</th>
<th>Std error</th>
<th>RSD (%)</th>
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</thead>
<tbody>
<tr>
<td>Blanc</td>
<td>25.73</td>
<td>30.38</td>
<td>27.59</td>
<td>27.90</td>
<td>2.34</td>
<td>1.35</td>
<td>8.39</td>
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<td>32.65</td>
<td>2.64</td>
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<td>30.48</td>
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<td>1.81</td>
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<tr>
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<td>2.95</td>
<td>1.70</td>
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(B)  
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<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std</th>
<th>Std error</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blanc</td>
<td>23.56</td>
<td>22.63</td>
<td>24.49</td>
<td>23.56</td>
<td>0.93</td>
<td>0.54</td>
<td>3.95</td>
</tr>
<tr>
<td>Elegance</td>
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<td>24.76</td>
<td>23.92</td>
<td>25.52</td>
<td>2.09</td>
<td>1.20</td>
<td>8.18</td>
</tr>
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<td>19.53</td>
<td>21.70</td>
<td>23.87</td>
<td>21.70</td>
<td>2.17</td>
<td>1.25</td>
<td>10.00</td>
</tr>
<tr>
<td>CY3079</td>
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<td>26.35</td>
<td>24.18</td>
<td>25.21</td>
<td>1.09</td>
<td>0.63</td>
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</tr>
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<td>20.15</td>
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<td>20.67</td>
<td>1.76</td>
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</tr>
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<td>20.06</td>
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<td>0.45</td>
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</tr>
<tr>
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</tr>
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<td>19.08</td>
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<td>22.11</td>
<td>2.94</td>
<td>1.69</td>
<td>13.28</td>
</tr>
<tr>
<td>M2</td>
<td>18.60</td>
<td>13.95</td>
<td>17.05</td>
<td>16.53</td>
<td>2.37</td>
<td>1.37</td>
<td>14.32</td>
</tr>
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<td>M3</td>
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<td>14.75</td>
<td>14.09</td>
<td>15.29</td>
<td>1.54</td>
<td>1.70</td>
<td>10.05</td>
</tr>
<tr>
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<td>27.78</td>
<td>33.59</td>
<td>30.69</td>
<td>2.91</td>
<td>30.69</td>
<td>9.47</td>
</tr>
</tbody>
</table>

*Standard Deviation  **Standard error  ***Relative Standard Error
Table 27. Foam formation height (mm) in Chardonnay wines fermented at 16 °C (A) and 20 °C (B) with *S. cerevisiae* strains.

### (A)

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std</th>
<th>Std error**</th>
<th>RSD (%)***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blanc</td>
<td>4.00</td>
<td>4.00</td>
<td>4.50</td>
<td>4.17</td>
<td>0.29</td>
<td>0.17</td>
<td>6.93</td>
</tr>
<tr>
<td>Elegance</td>
<td>3.00</td>
<td>4.00</td>
<td>3.50</td>
<td>3.50</td>
<td>0.50</td>
<td>0.29</td>
<td>14.29</td>
</tr>
<tr>
<td>Fusion</td>
<td>3.50</td>
<td>3.60</td>
<td>3.91</td>
<td>3.67</td>
<td>0.21</td>
<td>0.12</td>
<td>5.82</td>
</tr>
<tr>
<td>CY3079</td>
<td>4.50</td>
<td>5.00</td>
<td>5.00</td>
<td>4.83</td>
<td>0.29</td>
<td>0.17</td>
<td>5.97</td>
</tr>
<tr>
<td>ICV-D254</td>
<td>6.00</td>
<td>6.00</td>
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<td>0.33</td>
<td>9.12</td>
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<td>5.00</td>
<td>5.50</td>
<td>5.00</td>
<td>0.50</td>
<td>0.29</td>
<td>10.00</td>
</tr>
<tr>
<td>C2</td>
<td>7.00</td>
<td>7.00</td>
<td>6.00</td>
<td>6.67</td>
<td>0.58</td>
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</tr>
<tr>
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<td>7.00</td>
<td>6.00</td>
<td>6.67</td>
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<td>0.33</td>
<td>8.66</td>
</tr>
<tr>
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<td>6.00</td>
<td>6.17</td>
<td>0.76</td>
<td>0.44</td>
<td>12.39</td>
</tr>
<tr>
<td>M2</td>
<td>7.00</td>
<td>5.50</td>
<td>6.00</td>
<td>6.17</td>
<td>0.76</td>
<td>0.44</td>
<td>12.39</td>
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<tr>
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<td>8.00</td>
<td>6.22</td>
<td>7.78</td>
<td>7.33</td>
<td>0.97</td>
<td>0.56</td>
<td>13.23</td>
</tr>
<tr>
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<td>6.00</td>
<td>7.17</td>
<td>1.04</td>
<td>0.60</td>
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### (B)

<table>
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<tr>
<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std</th>
<th>Std error**</th>
<th>RSD (%)***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blanc</td>
<td>9.00</td>
<td>9.50</td>
<td>10.00</td>
<td>9.50</td>
<td>0.50</td>
<td>0.29</td>
<td>5.26</td>
</tr>
<tr>
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<td>7.00</td>
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<td>7.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Fusion</td>
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<td>8.00</td>
<td>7.50</td>
<td>7.83</td>
<td>0.29</td>
<td>0.17</td>
<td>3.69</td>
</tr>
<tr>
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<td>9.00</td>
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<td>9.17</td>
<td>0.29</td>
<td>0.17</td>
<td>3.15</td>
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<td>0.00</td>
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<td>9.00</td>
<td>1.00</td>
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<td>11.11</td>
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<tr>
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<td>7.50</td>
<td>7.00</td>
<td>7.67</td>
<td>0.76</td>
<td>0.44</td>
<td>9.96</td>
</tr>
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<td>7.00</td>
<td>8.00</td>
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<td>0.58</td>
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<td>7.87</td>
</tr>
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<td>10.00</td>
<td>1.00</td>
<td>0.58</td>
<td>10.00</td>
</tr>
<tr>
<td>M2</td>
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<td>9.33</td>
<td>0.58</td>
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<tr>
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<td>8.00</td>
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<td>0.58</td>
<td>0.33</td>
<td>6.93</td>
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<tr>
<td>M4</td>
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<td>10.00</td>
<td>10.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

*Standard Deviation*  
**Standard error**  
***Relative Standard Error***
Table 28. Final optical densities of (λ=600) recorded during growth of *S. cerevisiae* strains at 16 °C (A) and 20 °C (B).

(A)

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std'</th>
<th>Std error''</th>
<th>RSD (%) ***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blanc</td>
<td>1.567</td>
<td>1.569</td>
<td>1.575</td>
<td>1.570</td>
<td>0.00</td>
<td>0.00</td>
<td>0.27</td>
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<td>1.591</td>
<td>1.603</td>
<td>1.594</td>
<td>0.01</td>
<td>0.00</td>
<td>0.50</td>
</tr>
<tr>
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<td>1.616</td>
<td>1.619</td>
<td>1.62</td>
<td>1.618</td>
<td>0.00</td>
<td>0.00</td>
<td>0.13</td>
</tr>
<tr>
<td>CY3079</td>
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<td>1.655</td>
<td>1.665</td>
<td>1.656</td>
<td>0.01</td>
<td>0.01</td>
<td>0.54</td>
</tr>
<tr>
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<td>1.601</td>
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<td>0.00</td>
<td>0.19</td>
</tr>
<tr>
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<td>1.705</td>
<td>1.703</td>
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<td>1.582</td>
<td>0.01</td>
<td>0.01</td>
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</table>

(B)

<table>
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<tr>
<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std'</th>
<th>Std error''</th>
<th>RSD (%) ***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blanc</td>
<td>1.653</td>
<td>1.663</td>
<td>1.665</td>
<td>1.660</td>
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<td>1.713</td>
<td>1.709</td>
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<td>0.00</td>
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<td>0.01</td>
<td>0.77</td>
</tr>
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</tr>
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<td>1.596</td>
<td>1.585</td>
<td>0.02</td>
<td>0.01</td>
<td>1.04</td>
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<td>1.620</td>
<td>1.621</td>
<td>1.618</td>
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</table>

*Standard Deviation  **Standard error  ***Relative Standard Error
Table 29. Concentrations (mg/L) of volatile compounds produced by *S. cerevisiae* strains in Chardonnay wine fermented at 16°C and 20 °C. Temperature effect is shown.

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>2,3-butanediol</th>
<th>2-methyl-1-butanol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16 °C</td>
<td>20 °C</td>
</tr>
<tr>
<td>Blanc</td>
<td>1.206</td>
<td>1.264</td>
</tr>
<tr>
<td>Elegance</td>
<td>1.065</td>
<td>1.065</td>
</tr>
<tr>
<td>Fusion</td>
<td>1.185</td>
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</tr>
<tr>
<td>CY3079</td>
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</tr>
<tr>
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<td>0.843</td>
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<tr>
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</tr>
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</tr>
<tr>
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<tr>
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<td>0.952</td>
</tr>
<tr>
<td>M2</td>
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<td>0.976</td>
</tr>
<tr>
<td>M3</td>
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<tr>
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<td>0.545</td>
<td>0.544</td>
</tr>
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</table>

<table>
<thead>
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<th>Yeast Strain</th>
<th>3-methyl-1-butanol</th>
<th>n-butanol</th>
</tr>
</thead>
<tbody>
<tr>
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<td>20 °C</td>
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<tr>
<td>Blanc</td>
<td>5.923</td>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
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<tr>
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<tr>
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</table>

Temperature effect: ns indicates no significant difference between the fermentation temperatures for each yeast strain at p ≤ 0.05.
Table 29 (Continued)

<table>
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<th>Yeast Strain</th>
<th>1-hexanol</th>
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<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>20 °C</td>
<td>Average</td>
<td>Temp.e</td>
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<td>11.699</td>
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<table>
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<th></th>
<th>Propanol</th>
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<tr>
<td></td>
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<td>20 °C</td>
<td>Average</td>
<td>Temp.e</td>
<td>16 °C</td>
</tr>
<tr>
<td>Blanc</td>
<td>0.904</td>
<td>0.909</td>
<td>0.906</td>
<td>ns</td>
<td>5.148</td>
</tr>
<tr>
<td>Elegance</td>
<td>1.254</td>
<td>1.254</td>
<td>1.254</td>
<td>ns</td>
<td>2.713</td>
</tr>
<tr>
<td>Fusion</td>
<td>1.203</td>
<td>1.281</td>
<td>1.242</td>
<td>ns</td>
<td>2.043</td>
</tr>
<tr>
<td>CY3079</td>
<td>1.025</td>
<td>1.115</td>
<td>1.070</td>
<td>ns</td>
<td>1.380</td>
</tr>
<tr>
<td>ICV-D254</td>
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<td>0.879</td>
<td>ns</td>
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</tr>
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<td>ns</td>
<td>1.876</td>
</tr>
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<td>1.015</td>
<td>1.031</td>
<td>1.023</td>
<td>ns</td>
<td>2.161</td>
</tr>
<tr>
<td>C6</td>
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<td>1.047</td>
<td>1.056</td>
<td>ns</td>
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</tr>
<tr>
<td>M1</td>
<td>1.066</td>
<td>1.074</td>
<td>1.070</td>
<td>ns</td>
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</tr>
<tr>
<td>M2</td>
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<td>0.952</td>
<td>0.933</td>
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</tr>
<tr>
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<td>0.882</td>
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</tr>
<tr>
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<td>0.474</td>
<td>0.474</td>
<td>ns</td>
<td>1.674</td>
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<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Ethyl butanoate</th>
<th></th>
<th>Ethyl hexanoate</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16 °C</td>
<td>20 °C</td>
<td>Average</td>
<td>Temp.e</td>
<td>16 °C</td>
</tr>
<tr>
<td>Blanc</td>
<td>0.195</td>
<td>0.206</td>
<td>0.200</td>
<td>ns</td>
<td>0.035</td>
</tr>
<tr>
<td>Elegance</td>
<td>0.275</td>
<td>0.275</td>
<td>0.275</td>
<td>ns</td>
<td>0.045</td>
</tr>
<tr>
<td>Fusion</td>
<td>0.285</td>
<td>0.314</td>
<td>0.299</td>
<td>ns</td>
<td>0.057</td>
</tr>
<tr>
<td>CY3079</td>
<td>0.290</td>
<td>0.308</td>
<td>0.299</td>
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<td>0.041</td>
</tr>
<tr>
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<td>0.364</td>
<td>0.358</td>
<td>0.361</td>
<td>ns</td>
<td>0.030</td>
</tr>
<tr>
<td>X16</td>
<td>0.260</td>
<td>0.275</td>
<td>0.268</td>
<td>ns</td>
<td>0.038</td>
</tr>
<tr>
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<td>0.214</td>
<td>0.209</td>
<td>0.211</td>
<td>ns</td>
<td>0.044</td>
</tr>
<tr>
<td>C6</td>
<td>0.230</td>
<td>0.227</td>
<td>0.229</td>
<td>ns</td>
<td>0.046</td>
</tr>
<tr>
<td>M1</td>
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<td>0.209</td>
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<td>0.041</td>
</tr>
<tr>
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<td>0.209</td>
<td>0.225</td>
<td>0.217</td>
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<tr>
<td>M3</td>
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<td>0.211</td>
<td>0.218</td>
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</tr>
<tr>
<td>M4</td>
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<td>0.215</td>
<td>0.215</td>
<td>ns</td>
<td>0.043</td>
</tr>
</tbody>
</table>

**Temperature effect:** ns indicates no significant difference between the fermentation temperatures for each yeast strain at p ≤ 0.05.
<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Ethyl octanoate</th>
<th>Ethyl decanoate</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>16 °C</td>
<td>20 °C</td>
</tr>
<tr>
<td>Blanc</td>
<td>0.035</td>
<td>0.035</td>
</tr>
<tr>
<td>Elegance</td>
<td>0.048</td>
<td>0.048</td>
</tr>
<tr>
<td>Fusion</td>
<td>0.058</td>
<td>0.061</td>
</tr>
<tr>
<td>CY3079</td>
<td>0.044</td>
<td>0.046</td>
</tr>
<tr>
<td>ICV-D254</td>
<td>0.030</td>
<td>0.031</td>
</tr>
<tr>
<td>X16</td>
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<td>0.036</td>
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<tr>
<td>C2</td>
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<td>0.039</td>
</tr>
<tr>
<td>C6</td>
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<td>0.039</td>
</tr>
<tr>
<td>M1</td>
<td>0.042</td>
<td>0.042</td>
</tr>
<tr>
<td>M2</td>
<td>0.039</td>
<td>0.045</td>
</tr>
<tr>
<td>M3</td>
<td>0.038</td>
<td>0.041</td>
</tr>
<tr>
<td>M4</td>
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<td>0.037</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Ethyl laurate</th>
<th>Ethyl acetate</th>
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<td></td>
<td>16 °C</td>
<td>20 °C</td>
</tr>
<tr>
<td>Blanc</td>
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<td>0.001</td>
</tr>
<tr>
<td>Elegance</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>Fusion</td>
<td>0.009</td>
<td>0.010</td>
</tr>
<tr>
<td>CY3079</td>
<td>0.003</td>
<td>0.003</td>
</tr>
<tr>
<td>ICV-D254</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>X16</td>
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<td>0.004</td>
</tr>
<tr>
<td>C2</td>
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<td>0.002</td>
</tr>
<tr>
<td>C6</td>
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<td>0.002</td>
</tr>
<tr>
<td>M1</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>M2</td>
<td>0.002</td>
<td>0.003</td>
</tr>
<tr>
<td>M3</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>M4</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Hexyl acetate</th>
<th>Isoamyl acetate</th>
</tr>
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<tr>
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<td>16 °C</td>
<td>20 °C</td>
</tr>
<tr>
<td>Blanc</td>
<td>0.024</td>
<td>0.024</td>
</tr>
<tr>
<td>Elegance</td>
<td>0.027</td>
<td>0.027</td>
</tr>
<tr>
<td>Fusion</td>
<td>0.019</td>
<td>0.020</td>
</tr>
<tr>
<td>CY3079</td>
<td>0.029</td>
<td>0.031</td>
</tr>
<tr>
<td>ICV-D254</td>
<td>0.025</td>
<td>0.023</td>
</tr>
<tr>
<td>X16</td>
<td>0.035</td>
<td>0.035</td>
</tr>
<tr>
<td>C2</td>
<td>0.024</td>
<td>0.024</td>
</tr>
<tr>
<td>C6</td>
<td>0.024</td>
<td>0.023</td>
</tr>
<tr>
<td>M1</td>
<td>0.025</td>
<td>0.025</td>
</tr>
<tr>
<td>M2</td>
<td>0.025</td>
<td>0.029</td>
</tr>
<tr>
<td>M3</td>
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<td>0.023</td>
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<tr>
<td>M4</td>
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Temperature effect: ns indicates no significant difference between the fermentation temperatures for each yeast strain at p≤ 0.05.
Table 29 (Continued)

<table>
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<tr>
<th>Yeast Strain</th>
<th>Acetaldehyde</th>
<th></th>
<th>Acetic acid</th>
<th></th>
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<tr>
<td></td>
<td>16 °C</td>
<td>20 °C</td>
<td>Average</td>
<td>Temp.e</td>
</tr>
<tr>
<td>Blanc</td>
<td>1.184</td>
<td>1.286</td>
<td>1.235</td>
<td>ns</td>
</tr>
<tr>
<td>Elegance</td>
<td>0.945</td>
<td>0.924</td>
<td>0.934</td>
<td>ns</td>
</tr>
<tr>
<td>Fusion</td>
<td>0.988</td>
<td>1.279</td>
<td>1.133</td>
<td>ns</td>
</tr>
<tr>
<td>CY3079</td>
<td>0.836</td>
<td>0.992</td>
<td>0.914</td>
<td>ns</td>
</tr>
<tr>
<td>ICV-D254</td>
<td>0.745</td>
<td>0.913</td>
<td>0.829</td>
<td>ns</td>
</tr>
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<td>1.032</td>
<td>1.031</td>
<td>ns</td>
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<td>1.119</td>
<td>1.115</td>
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<tr>
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<td>0.677</td>
<td>0.732</td>
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</table>

Temperature effect: ns indicates no significant difference between the fermentation temperatures for each yeast strain at p≤ 0.05.
Table 30. Concentrations (mg/L) of all volatile compounds quantified by GC-MS. Chardonnay wines (n=3) were fermented at 16 °C by industrial yeast strains Blanc, Elegance, Fusion, CY3079, ICV-D254 and X16; individual Burgundian strains C2, C6 and mixed Burgundian strains M1, M2, M3 and M.

### 2,3-butanediol

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std*</th>
<th>Std error**</th>
<th>RSD (%)***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blanc</td>
<td>1.137</td>
<td>1.132</td>
<td>1.349</td>
<td>1.206</td>
<td>0.12</td>
<td>0.07</td>
<td>10.27</td>
</tr>
<tr>
<td>Elegance</td>
<td>1.067</td>
<td>1.105</td>
<td>1.021</td>
<td>1.065</td>
<td>0.04</td>
<td>0.02</td>
<td>3.94</td>
</tr>
<tr>
<td>Fusion</td>
<td>1.080</td>
<td>1.103</td>
<td>1.372</td>
<td>1.185</td>
<td>0.16</td>
<td>0.09</td>
<td>13.72</td>
</tr>
<tr>
<td>CY3079</td>
<td>1.185</td>
<td>1.158</td>
<td>0.982</td>
<td>1.109</td>
<td>0.11</td>
<td>0.06</td>
<td>9.94</td>
</tr>
<tr>
<td>ICV-D254</td>
<td>0.891</td>
<td>0.819</td>
<td>0.820</td>
<td>0.843</td>
<td>0.04</td>
<td>0.02</td>
<td>4.87</td>
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<td>1.256</td>
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<td>0.04</td>
<td>4.93</td>
</tr>
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<td>1.041</td>
<td>1.151</td>
<td>1.077</td>
<td>0.06</td>
<td>0.04</td>
<td>6.01</td>
</tr>
<tr>
<td>C6</td>
<td>0.969</td>
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<td>0.07</td>
<td>0.04</td>
<td>6.96</td>
</tr>
<tr>
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<td>0.07</td>
<td>11.51</td>
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<td>0.06</td>
<td>11.58</td>
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<tr>
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<td>13.11</td>
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</table>

### 2,3-methyl-1-butanol

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std*</th>
<th>Std error**</th>
<th>RSD (%)***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blanc</td>
<td>1.288</td>
<td>1.170</td>
<td>1.060</td>
<td>1.173</td>
<td>0.11</td>
<td>0.07</td>
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</tr>
<tr>
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<td>1.198</td>
<td>1.170</td>
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<td>0.01</td>
<td>2.09</td>
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<tr>
<td>Fusion</td>
<td>1.381</td>
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<td>1.376</td>
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<td>0.02</td>
<td>2.78</td>
</tr>
<tr>
<td>CY3079</td>
<td>1.035</td>
<td>1.209</td>
<td>1.180</td>
<td>1.141</td>
<td>0.09</td>
<td>0.05</td>
<td>8.17</td>
</tr>
<tr>
<td>ICV-D254</td>
<td>1.181</td>
<td>1.187</td>
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<td>1.204</td>
<td>0.03</td>
<td>0.02</td>
<td>2.79</td>
</tr>
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<td>8.36</td>
</tr>
<tr>
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<td>1.304</td>
<td>0.09</td>
<td>0.05</td>
<td>6.73</td>
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</table>

*Standard Deviation  **Standard error  ***Relative Standard Error
### Table 30 (Continued)

#### 3, methyl-1-butanol

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std</th>
<th>Std error</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blanc</td>
<td>6.640</td>
<td>5.771</td>
<td>5.359</td>
<td>5.923</td>
<td>0.65</td>
<td>0.38</td>
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<tr>
<td>Elegance</td>
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<td>7.321</td>
<td>7.167</td>
<td>0.14</td>
<td>0.08</td>
<td>1.94</td>
</tr>
<tr>
<td>Fusion</td>
<td>6.936</td>
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<td>0.07</td>
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<tr>
<td>CY3079</td>
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<td>6.728</td>
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#### n-butanol

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<th>Std</th>
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<th>RSD (%)</th>
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*Standard Deviation  **Standard error  ***Relative Standard Error
Table 30 (Continued)

1-hexanol

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<th>Std</th>
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<th>RSD (%)</th>
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Isobutanol

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*Standard Deviation **Standard error ***Relative Standard Error
Table 30 (Continued)

Propanol

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Phenylethanol

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*Standard Deviation **Standard error ***Relative Standard Error

161
Table 30 (Continued)

### Ethyl butanoate

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### Ethyl hexanoate

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<th>Std</th>
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<th>RSD (%)</th>
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*Standard Deviation  **Standard error  ***Relative Standard Error
Table 30 (Continued)

**Ethyl octanoate**

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<th>Replicate 3</th>
<th>Average</th>
<th>Std</th>
<th>Std error</th>
<th>RSD (%)</th>
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**Ethyl decanoate**

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<th>Std</th>
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*Standard Deviation** | **Standard error** | **Relative Standard Error**
(Table 30) Continued

### Ethyl Laurate

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### Ethyl Acetate

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*Standard Deviation **Standard error ***Relative Standard Error
(Table 30) Continued

### Hexyl acetate

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<th>Replicate 2</th>
<th>Replicate 3</th>
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<th>Std</th>
<th>Std error</th>
<th>RSD (%)</th>
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<tbody>
<tr>
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<tr>
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<td>0.001</td>
<td>9.557</td>
</tr>
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</table>

### Isoamyl acetate

<table>
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<tr>
<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std*</th>
<th>Std error**</th>
<th>RSD (%)***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blanc</td>
<td>0.093</td>
<td>0.093</td>
<td>0.086</td>
<td>0.090</td>
<td>0.004</td>
<td>0.002</td>
<td>4.586</td>
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<td>0.110</td>
<td>0.096</td>
<td>0.109</td>
<td>0.013</td>
<td>0.008</td>
<td>11.953</td>
</tr>
<tr>
<td>Fusion</td>
<td>0.060</td>
<td>0.053</td>
<td>0.058</td>
<td>0.057</td>
<td>0.004</td>
<td>0.002</td>
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</tr>
<tr>
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<td>0.126</td>
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<td>0.107</td>
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<td>0.010</td>
<td>0.006</td>
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</tr>
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<td>0.113</td>
<td>0.088</td>
<td>0.116</td>
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<td>0.015</td>
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<td>0.091</td>
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*Standard Deviation  **Standard error  ***Relative Standard Error
(Table 30) Continued

**Acetaldehyde**

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std</th>
<th>Std error</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blanc</td>
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<td>1.168</td>
<td>1.094</td>
<td>1.184</td>
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<td>0.945</td>
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<td>10.33</td>
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<td>5.88</td>
</tr>
<tr>
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<td>0.09</td>
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</tr>
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<td>15.36</td>
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<td>15.12</td>
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<td>1.071</td>
<td>1.030</td>
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<td>1.011</td>
<td>1.112</td>
<td>0.09</td>
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<td>8.41</td>
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<tr>
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<td>1.094</td>
<td>1.158</td>
<td>1.228</td>
<td>0.18</td>
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<td>14.67</td>
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<tr>
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<td>1.459</td>
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<td>1.497</td>
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<td>0.07</td>
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**Acetic acid**

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std</th>
<th>Std error</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blanc</td>
<td>0.269</td>
<td>0.288</td>
<td>0.228</td>
<td>0.262</td>
<td>0.03</td>
<td>0.02</td>
<td>11.703</td>
</tr>
<tr>
<td>Elegance</td>
<td>0.380</td>
<td>0.319</td>
<td>0.380</td>
<td>0.360</td>
<td>0.04</td>
<td>0.02</td>
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<td>0.341</td>
<td>0.404</td>
<td>0.355</td>
<td>0.04</td>
<td>0.03</td>
<td>12.427</td>
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<td>0.423</td>
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<td>0.373</td>
<td>0.05</td>
<td>0.03</td>
<td>13.894</td>
</tr>
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<td>0.320</td>
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<td>0.01</td>
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<td>0.378</td>
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<td>0.02</td>
<td>9.075</td>
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<td>0.02</td>
<td>0.01</td>
<td>7.634</td>
</tr>
<tr>
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<td>0.342</td>
<td>0.368</td>
<td>0.330</td>
<td>0.04</td>
<td>0.03</td>
<td>13.536</td>
</tr>
<tr>
<td>M1</td>
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<td>0.299</td>
<td>0.328</td>
<td>0.03</td>
<td>0.02</td>
<td>9.345</td>
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<td>0.277</td>
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<td>0.318</td>
<td>0.04</td>
<td>0.02</td>
<td>11.280</td>
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<td>0.325</td>
<td>0.05</td>
<td>0.03</td>
<td>15.322</td>
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</table>

*Standard Deviation  **Standard error  ***Relative Standard Error
Table 31. Concentrations (mg/L) of all volatile compounds quantified by GC-MS. Chardonnay wines (n=3) were fermented at 20 °C by industrial yeast strains Blanc, Elegance, Fusion, CY3079, ICV-D254 and X16; individual Burgundian strains C2, C6 and mixed Burgundian strains M1, M2, M3 and M4.

**2,3-butanediol**

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std*</th>
<th>Std error**</th>
<th>RSD (%)***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blanc</td>
<td>1.212</td>
<td>1.367</td>
<td>1.213</td>
<td>1.264</td>
<td>0.09</td>
<td>0.05</td>
<td>7.06</td>
</tr>
<tr>
<td>Elegance</td>
<td>1.067</td>
<td>1.105</td>
<td>1.021</td>
<td>1.065</td>
<td>0.04</td>
<td>0.02</td>
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</tr>
<tr>
<td>Fusion</td>
<td>1.197</td>
<td>1.162</td>
<td>1.425</td>
<td>1.261</td>
<td>0.14</td>
<td>0.08</td>
<td>11.33</td>
</tr>
<tr>
<td>CY3079</td>
<td>1.197</td>
<td>1.158</td>
<td>0.982</td>
<td>1.112</td>
<td>0.11</td>
<td>0.07</td>
<td>10.28</td>
</tr>
<tr>
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<td>0.891</td>
<td>0.887</td>
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<td>0.00</td>
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<td>9.61</td>
</tr>
<tr>
<td>C2</td>
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</tr>
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<td>0.544</td>
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<td>0.03</td>
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**2-methyl-1-butanol**

<table>
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<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std*</th>
<th>Std error**</th>
<th>RSD (%)***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blanc</td>
<td>1.150</td>
<td>1.170</td>
<td>1.060</td>
<td>1.127</td>
<td>0.06</td>
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<td>5.17</td>
</tr>
<tr>
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<td>1.159</td>
<td>1.198</td>
<td>1.170</td>
<td>0.02</td>
<td>0.01</td>
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</tr>
<tr>
<td>Fusion</td>
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<td>1.464</td>
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<td>0.04</td>
<td>5.08</td>
</tr>
<tr>
<td>CY3079</td>
<td>1.273</td>
<td>1.209</td>
<td>1.180</td>
<td>1.221</td>
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<td>0.03</td>
<td>3.91</td>
</tr>
<tr>
<td>ICV-D254</td>
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<td>1.413</td>
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</tr>
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</table>

*Standard Deviation **Standard error ***Relative Standard Error
Table 31 (Continued)

3-methyl-1-butanol

<table>
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<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std</th>
<th>Std error</th>
<th>RSD (%)***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blanc</td>
<td>5.833</td>
<td>5.771</td>
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<td>5.655</td>
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<td>7.257</td>
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<td>3.88</td>
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<td>6.950</td>
<td>0.36</td>
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<tr>
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<td>0.74</td>
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<tr>
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<tr>
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<td>0.35</td>
<td>8.35</td>
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n-butanol

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<th>Replicate 3</th>
<th>Average</th>
<th>Std</th>
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*Standard Deviation
**Standard error
***Relative Standard Error
### Table 31 (Continued)

**1-hexanol**

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<tr>
<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std*</th>
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<th>RSD (%)***</th>
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**Isobutanol**

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<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std*</th>
<th>Std error**</th>
<th>RSD (%)***</th>
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<tbody>
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*Standard Deviation **Standard error ***Relative Standard Error
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<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
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<th>Replicate 3</th>
<th>Average</th>
<th>Std</th>
<th>Std error</th>
<th>RSD (%)</th>
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*Standard Deviation **Standard error ***Relative Standard Error
Table 31 (Continued)

**Ethyl butanoate**

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<th>Yeast Strain</th>
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<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std</th>
<th>Std error</th>
<th>RSD (%)</th>
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**Ethyl hexanoate**

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<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std*</th>
<th>Std error**</th>
<th>RSD (%)***</th>
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<td>Blanc</td>
<td>0.038</td>
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<td>0.036</td>
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*Standard Deviation  **Standard error  ***Relative Standard Error
Table 31(Continued)

**Ethyl octanoate**

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<tr>
<th>Yeast Strain</th>
<th>Replicate 1</th>
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<th>Replicate 3</th>
<th>Average</th>
<th>Std</th>
<th>Std error</th>
<th>RSD (%)</th>
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**Ethyl decanoate**

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<th>Std</th>
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*Standard Deviation **Standard error ***Relative Standard Error
Table 31 (Continued)

**Ethyl laurate**

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<th>Std</th>
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<th>RSD (%)</th>
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**Ethyl acetate**

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<th>Replicate 3</th>
<th>Average</th>
<th>Std*</th>
<th>Std error**</th>
<th>RSD (%)***</th>
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</tr>
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<td>0.12</td>
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*Standard Deviation **Standard error ***Relative Standard Error
Table 31 (Continued)

**Hexyl acetate**

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<th>Replicate 3</th>
<th>Average</th>
<th>Std</th>
<th>Std error</th>
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<tbody>
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**Isoamyl acetate**

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<th>Replicate 3</th>
<th>Average</th>
<th>Std*</th>
<th>Std error**</th>
<th>RSD (%)***</th>
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<td>0.061</td>
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<td>0.002</td>
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</tr>
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<td>0.113</td>
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*Standard Deviation  **Standard error  ***Relative Standard Error
Table 31 (Continued)

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std</th>
<th>Std error*</th>
<th>RSD (%)***</th>
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<tbody>
<tr>
<td>Blanc</td>
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<td>1.168</td>
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<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std*</th>
<th>Std error**</th>
<th>RSD (%)***</th>
</tr>
</thead>
<tbody>
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<td>Blanc</td>
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<td>0.228</td>
<td>0.238</td>
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*Standard Deviation **Standard error ***Relative Standard Error