Evaluating ethanol yields of wine yeast strains under various fermentative conditions

by

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Declaration

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Summary

The market for high quality lower alcohol wines is growing globally. Several factors are responsible for this trend, with socio-economic and health concerns being considered as being the most relevant. It is therefore no surprise that in the past three decades many systems have been developed to reduce wine ethanol levels, each with its own strengths and weaknesses. However, current systems are not always cost effective and frequently result in unwanted side-effects. Microbiological methods primarily based on redirecting carbon flux in existing, or novel *Saccharomyces* and non-*Saccharomyces* yeast strains, might have the potential to eliminate or reduce such shortcomings. However, little base-line information regarding differences in ethanol yields of existing wine yeast strains, and on the impact of fermentation conditions on such yields is currently available.

In this study the ethanol yield of 15 wine yeast strains was investigated in synthetic wine must under varied wine fermentative conditions including changes in yeast assimilable nitrogen, sugar concentration, pH and fermenting temperatures to identify strains that produce lower ethanol yields and conditions that would favour such an outcome. Most strains and conditions resulted in very similar ethanol yields, however in some cases interesting differences were observed. Some of the strains showed significant differences between high and low nitrogen containing must. Results from synthetic must were confirmed in grape must (Sauvignon Blanc, Chardonnay, Shiraz and Cabernet Sauvignon), but no consistent response could be observed. Interestingly the Shiraz fermentations always showed a higher ethanol yield for all strains investigated. This may be due to a parameter (or combination thereof) which was not included as an experimental factor in our study. Glycerol yield was also studied in the grape must experiments and was found to be more significantly condition dependent than ethanol yield. Temperature and glycerol seemed to be directly proportional confirming the results of previous studies. While temperature did increase glycerol production, it was concluded that the redirection of carbon towards glycerol was not substantial enough to have measurable effect on the final ethanol concentration. The most notable differences which were observed were very specific to a particular yeast strain and condition pairing, thus no generally applicable treatment to achieve lower ethanol yields could be established.

Opsomming

Deesdae is daar ‘n groeiende mark vir lae alkohol wyne van hoë gehalte. Verskeie faktore is verantwoordelik vir hierdie verskynsel, met sosio-ekonomiese en gesondheidskwessies as die hoof rolspelers. Vir hierdie rede is daar gedurende die laaste drie dekades baie stelsels ontwikkel om wyn etanol vlakke te verlaag, elkeen met voor- en nadele. Meeste van die huidige stelsels is nie koste effektief nie en lei gewoonlik tot ongewenste newe effekte. Mikrobiologiese metodes wat gebaseer is op koolstof vloei veranderinge in wyn gisrasse mag die potensiaal bied om hierdie tekortkominge te verminder of te oorbrug. ‘n Alternatief is om nuwe *Saccharomyces* en nie-Saccharomyces gisrasse te identifiseer wat laer etanol opbrengste lewer. In hierdie studie is die etanol opbrengste van 15 wyn gisrasse ondersoek in ‘n sintetiese mos in verskeie toestande, bv. veranderde stikstof vlakke, suiker vlakke, pH en temperatuur, om die rasse te identifiseer wat laer etanol opbrengste lewer (asook die toestande wat laer etanol opbrengste bevorder). Meeste rasse en toestande het soortgelyke etanol opbrengste getoon, alhoewel daar in sekere gevalle interessante verskille was rakende sekere rasse wat verskillende resultate lewer in mos met verskillende stikstof vlakke. Die resultate van die sintetiese mos eksperimente was bevestig in druiwe mos van vier kultivars (Sauvignon Blanc, Chardonnay, Shiraz en Cabernet Sauvignon), maar geen algemene tendens kon afgelei word nie. Wat interessant was is die feit dat die Shiraz fermentasies altyd hoër etanol opbrengste gelewer het vir al vier gisrasse wat gebruik is vir hierdie eksperimente. Dit mag wees weens ‘n eksperimentele faktor wat nie bestudeer was in die raamwerk van hierdie projek nie. Die opbrengs van gliserol was ook bepaal in die verskeie eksperimente en daar was gevind dat gliserol opbrengs baie meer kondisie-afhanklik is in vergelyking met etanol. Temperatuur en gliserol het ‘n direkte verbandskap met mekaar getoon, wat die bevindinge van vorige studies bevestig. Alhoewel verhogings in temperatuur wel gliserol produksie vermeerder het, was die effek nie genoeg om ‘n meetbare impak op die finale etanol konsentrasie te hê nie. Verskillende giste in verskeie verskillende fermentasie toestande het soortgelyke etanol opbrengste gelewer. Die mees merkbare verskille wat bevind is was spesifiek tot individuele gisras en kondisie kombinasies, maar geen algemene afleiding kon gemaak word rakende behandelings wat etanol opbrengste kan verlaag nie.

This thesis is dedicated to

My Family

Biographical sketch

Olaf Morgenroth was born in Cape Town on 14 July 1988. He matriculated with exemption from Bellville Technical High School in 2006. Olaf obtained his Bachelor of Science in Molecular Biology and Biotechnology from the University of Stellenbosch in 2009, majoring in Microbiology. He then obtained his HonsBSc in Wine Biotechnology from the Institute of Wine Biotechnology in 2011, University of Stellenbosch. In 2012 he enrolled for an MSc in Wine Biotechnology at the Institute of Wine Biotechnology, University of Stellenbosch.

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Preface

This thesis is presented as a compilation of four chapters. Each chapter is introduced separately and is written according to the style of the American Journal of Oenology & Viticulture to which Chapter three was submitted for publication.

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Chapter 2	**Literature review** Current methods for reducing the ethanol content of wine
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Contents

Chapter 1

Introduction and project aims

Chapter 1

Introduction and project aims

1.1 Introduction

Over the past few years the demand for wines with lower levels of ethanol has increased significantly, in particular due to socioeconomic and health-related factors (Howley and Young 1992, Pickering 2000). Furthermore many countries tax wine based on the percentage of alcohol creating a strong commercial incentive to decrease ethanol levels (Heux et al. 2006).

However, there has also been a growing market for fruitier and full bodied red wines, which requires grapes to be left on the vine for longer periods to increase phenolic ripeness. This practice results in a higher sugar concentration in the must and ultimately a higher final ethanol concentration in the wine. A high alcohol level may negatively affect the balance of the wine and also alters the volatility of other important aroma compounds (Guth and Sies 2002). From a health perspective, ethanol poses concerns in terms of calorie intake and an increased risk for alcohol related illnesses and accidents. Fortunately the many health benefits associated with red wines (anti-oxidant and cardiovascular protection) are retained in low-ethanol wines (Greenrod et al. 2005, Lecour et al. 2006).

Low-ethanol wines have been available for over three decades (Pickering 2000), and many different methods have been developed to reduce the ethanol content of wines. These methods include post-fermentation ethanol removal based on methodologies such as spinning cone columns (SCC) and reverse osmosis. However, the economic viability of these methods is questionable due to the cost of heating and filters (Pickering 2000). Several pre-fermentation methods also exist, such as earlier harvesting times, which are not always easy to implement as picking berries before full maturity can result in off flavors and/or a higher acid content (Pickering 2000). Enzyme treatment is another pre-fermentation option, whereby enzymes are added to convert the sugars in the grape must to other compounds that the yeast cannot metabolize (Heresztyn 1987, Villettaz 1987). Not only is this an expensive option but many products and/or by-products of these enzymatic reactions could have a negative effect on the wine quality.

Another option that has been the focus of much research activity over the past 15 to 20 years relates to yeast strain development through breeding or genetic engineering. The principle behind these approaches is the engineering of yeast strains through heterologous or altered gene expression to modify carbon fluxes in the cell. One of the key target carbon sinks in these approaches has been glycerol, as several research groups have attempted to re-direct carbon towards this sink in order to decrease flux to ethanol. (Nevoigt and Stahl 1996, Michnick et al. 1997, Remize et al. 1999, Lopes et al. 2000, Cambon et al. 2006, Ehsani et al. 2009). These approaches have seen some success in terms of decreasing the ethanol concentration in wine, but off flavours such as acetic acid and butanediol are often produced. Even if these off flavors were reduced to acceptable levels, current legislation does not permit these strains to be used in the South African wine industry since they are genetically modified (GM). The need thus exists for an alternative approach to modulate ethanol levels in wine that does not rely on GM technology and should be inexpensive and avoid the use of costly enzyme addition

Glycerol production in yeast has been shown to be strain dependent and can also be environmentally manipulated (Rankine and Bridson 1971, Torija et al. 2003, Yalcin and Ozbas 2008). It is often inversely correlated to ethanol production as carbon is redirected towards glycerol and away from ethanol. Therefore if glycerol production can be manipulated through environmental factors it is likely that ethanol production could also be similarly manipulated. Different wine yeast strains also display significantly different phenotypes with regard to metabolic fluxes and responses to environmental changes or stress. This project therefore investigated which factors (yeast strain, pH, temperature, yeast assimiable nitrogen, initial sugar concentration and cultivar) would influence the production of ethanol. The experimental factors selected for this study can mostly be easily controlled by the winemaker, making implementation practical, cost-effective and user-friendly. Moreover, this approach avoids the use of genetically modified organisms and can thus be used in industry. To our knowledge, this is the first systematic approach to investigate the link between specific fermentation parameters, yeast strains and ethanol yields.

1.2 Project aims

The first phase of this study was to determine whether commercial yeast strains that are popular in the South African wine industry vary intrinsically with regards to their ethanol yields. The second phase investigated whether the composition of a wine must and fermentation conditions can have an effect on the ethanol yields, using both monofactorial and multifactorial experimental lay-outs. Results from these two objectives were subsequently evaluated in grape must in phase three of the study. Finally aroma compound production was investigated under selected conditions, to determine whether changes to key parameters had an effect on the production of important flavor-active compounds.

This project is therefore divided into four objectives:

1. Influence of yeast strain on ethanol yield
2. Influence of must composition and fermentation conditions on ethanol yield
3. Grape derived must experiments
4. Effect of key parameters on volatile aroma compound production

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Chapter 2

Literature review

Current methods for reducing the ethanol content of wine

Chapter 2

Current methods for reducing the ethanol content of wine

2.1 Introduction

Global trends in viticulture have favoured increased phenolic ripeness of grapes to respond to market demand for fuller and fruitier wines. This is in part achieved by leaving the grapes on the vine for longer periods of time (Heux et al. 2006). However, this practice also results in increased sugar concentration in the berry and ultimately in a high sugar must and thus higher alcohol concentrations in the wine. Not only may high alcohol concentrations negatively affect wine (Guth and Sies 2002) but consumers also tend to prefer lower alcohol wines for health reasons or due to social concerns. The many health benefits associated with red wine consumption (anti-oxidant and cardiovascular protection) are fortunately retained in low alcohol wines (Greenrod et al. 2005, Lecour et al. 2006). Wine is also taxed on alcohol content, hence a commercial interest exists for decreasing the alcohol content of high alcohol wines (Godden 2000).

Currently there are several systems that can decrease alcohol levels in wine, ranging from physical post-fermentation to biological approaches. Discussed first is an over-view of non-GM (genetically modified) strategies which have been pursued in the production of low alcohol wines. A general overview of fermentation and central carbon metabolism, i.e. the biochemical pathways responsible for ethanol production and yield in yeast is then given followed by GM methods to reduce ethanol in wine.

2.2 Current non-GM methods to reduce ethanol content of wine

2.2.1 Physical removal of ethanol

2.2.1.1 Distillation

Due to the fact that the boiling point of ethanol (78°C) is lower than that of water, distillation is a simple option to reduce the ethanol content of wine. However, when this method was first developed to completely dealcoholize wine (<0.5 % v/v ethanol) a total volume loss of 50-70% was expected (Pickering 2000). Furthermore, since this method requires heating many of the aromatic compounds are modified or evaporate along with the ethanol. Due to these shortcomings it is not surprising that many modifications of this system exist (Boucher 1983, Boucher 1988, Schobinger et al. 1986, Gómez-Plaza et al. 1999). Since wine is extremely heat sensitive, decreasing the distilling temperature is advantageous in terms of producing a similar quality yet lower ethanol wine. Using a low pressure system (50 to 60 mm Hg) the temperature at which ethanol will evaporate can be lowered to 25°C (Gómez-Plaza et al. 1999). Furthermore by using different temperatures the volatile aroma compounds can be evaporated off, then condensed and subsequently added back to the wine (Gómez-Plaza et al. 1999). However, this method only has limited success as many volatiles nevertheless escape (Gómez-Plaza et al. 1999).

One increasingly popular system is the spinning cone column (SCC) first introduced in the 1930's (Wright and Pyle 1996). The column consists of a series of alternating rotating and stationary cones. The spinning cones are attached to a centrally spinning shaft. In most SCC's the spinning cones also have fins, creating a low pressure at the bottom of the shaft. The liquid is pumped into the feed, where it falls onto a rotating cone and is forced upwards due to a centrifugal force, creating a very thin film of liquid (Wright and Pyle 1996). Once the liquid reaches the end of a spinning cone it falls onto a stationary cone. The liquid then runs down the stationary cone and onto another spinning cone where the process repeats itself. Essentially a SCC increases the contact surface area between the liquid and gas. A graphic representation is represented in Figure 1. Currently it is the most widely used system for ethanol reduction. However, its economic viability is questionable as these systems are generally expensive to acquire and utilise (García-Martín et al. 2010).

Furthermore, to our knowledge, no scientifically validated sensory analysis has been published on SCC treated wine. However, quantitative analytical analysis has been performed on SCC treated wine, the results of which indicate that most compounds with proposed health benefits (for example phenolic compounds and compounds with antioxidant activity) are retained (Belisario-Sánchezn et al. 2009). SCC treated wines have also been shown retain most of their aroma compounds (Belisario-Sánchezn et al. 2009).

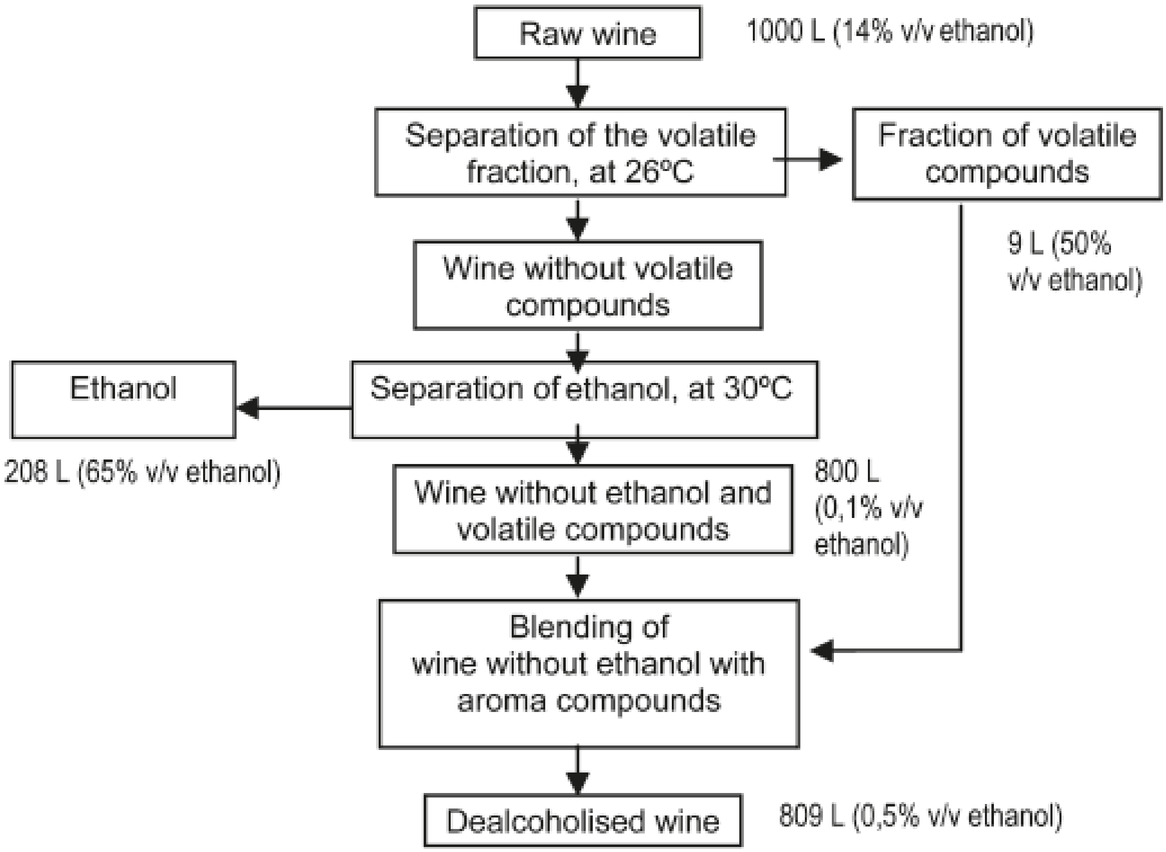

Figure 1. Graphical representation for the production of low ethanol wine using a spinning cone column (Pickering 2000).

2.2.1.2 Filtering

Filtering is another option for winemakers: Reverse osmosis uses either an ethanol permeable or an ethanol retention membrane. Ethanol permeable membrane filters selectively filter out aroma compounds while letting the ethanol and water through. The water and ethanol mixture can then be distilled to remove the ethanol after which the aroma compounds can be added back into this reduced ethanol and water mixture (Bui et al. 1986). Conversely the ethanol retention membrane is permeable to water and aroma compounds while ethanol is retained.

In a recent paper several types of membrane filtration processes such as nanofiltration and reverse osmosis were evaluated. Sensory analysis was then done to determine which membrane would be superior in terms of retaining the relevant aroma compounds. It was concluded that certain nanofiltation systems using a pervaporation process (separation of liquids by partial vaporization through a porous or non-porous membrane) results in the best dealcoholized wines (Catarino and Medes 2011).

An alternative method using filtration to reduce ethanol is to remove some of the initial sugars present in the wine must using nanofiltration (García-Martín et al. 2010) which will ultimately result in a lower ethanol wine. This method also has the potential to reduce aroma loss and has shown promise in being able to reduce the ethanol content of wines by 2% (v/v). However, some aroma loss has been reported (García-Martín et al. 2010). If this is to be a viable option for the wine industry further research will have to be done to perfect this relatively inexpensive system.

2.2.2 Decreasing initial fermentable sugar concentration

Since the final ethanol concentration is directly dependent on the initial sugar concentration, another approach to reduce final ethanol levels is to reduce the amount of fermentable sugars present in the grape must.

2.2.2.1 Earlier harvesting

The sugar concentration in the berry increases the longer it is left on the vine, harvesting the grapes at an earlier stage would be an option. However this method is not applicable to certain styles of wine, as harvesting earlier than recommended can result in unripe aromas and a higher acid content (Pickering 2000).

2.2.2.2 Addition of enzymes

A more practical approach is to degrade or metabolize some of the fermentable sugars by enzymatic or microbial methods. Glucose oxidase (GOX) is one such enzyme: It is an aerobic dehydrogenase which catalyzes the oxidation of glucose to gluconolactone (Pickering et al. 1998). Gluconolactone is subsequently converted non-enzymatically to gluconic acid, generating hydrogen peroxide as a by-product (Pickering et al. 1998). Gluconic acid cannot be metabolized by *Saccharomyces cerevisiae*, therefore the glucose is essentially removed from the must (Heresztyn 1987, Villettaz 1987). Since most grape musts are characterised by an approximate 1:1 ratio of glucose to fructose, the ethanol concentration can theoretically be halved by this method. It has been reported that manipulation of environmental conditions can optimize the system and result in conversion of up to 87% of the glucose to gluconic acid (Pickering et al. 1998). Although the taste and appearance of glucose oxidase treated wine is modified the aroma and mouth feel is unaffected (Pickering et al. 1999).

On the other hand the addition of enzymes is very costly, thus the economic viability of this approach is also questionable. Furthermore, due to the fact that glucose is converted to gluconic acid, there is a notable increase in titrateable acidity (Pickering et al. 1998). Regardless of these shortcomings, Malherbe et al. addressed the cost issue by over-expressing the glucose oxidase gene (*GOX1*) from *Aspergillus niger* in *S. cerevisiae*. The approach was successful in decreasing ethanol by 1.8 to 2.0 % (v/v), however the fermentations required additional oxygenation (Malherbe et al. 2003). The effect of the by-product hydrogen peroxide on wine quality was also not discussed. However, it was postulated that hydrogen peroxide might have an antimicrobial effect in terms of preserving the wine, this has yet to be confirmed. The use of glucose oxidase thus presents many issues that will have to be overcome in order for it to become a viable option for the wine industry.

2.2.2.3 Other methods

By freezing the grape must to form a “slush”, the must can be separated into a low and high sugar containing fraction. The volatile aroma compounds can then be extracted using a custom built extractor and added to the low sugar containing must which is subsequently fermented (Lang and Casimir 1990). In this way some of the initial sugars are removed while the aroma compounds remain in the final wine. However, since its patent approval in 1990, not much research has been done on this approach. This lack of research might be due to the fact that it is mechanistically similar to the reverse osmosis method, which does not require large amounts of energy to cool the must.

2.3 Current GM methods to reduce ethanol content of wine

2.3.1 Background

2.3.1.1 Aerobic carbon metabolism

During aerobic respiration in yeast biochemical energy in the form adenosine triphospate (ATP) is produced from nutrients (sugars like glucose and fructose). These reactions are classified as catabolic reactions, as large molecules are broken down into smaller ones, releasing energy as high-energy bonds break (Willey et al. 2011). This energy is captured and converted to ATP. ATP has been described as the “energy currency” of the cell as these high energy molecules drive anabolic reactions (energy requiring), such as biosynthetic reactions (Willey et al. 2011).

Respiration has been extensively studied and the process is well understood (Fiechter and Seghezzi 1992, Gnaiger et al. 1995, Pronk et al. 1996). Figure 2 shows a simplified diagram depicting the process. ATP is the main product of respiration. During the initial stages of glycolysis (known as the energy investment stage), two ATP molecules are required. This results in two 3-carbon phosphorylated molecules, namely glyceraldehyde-3-phosphate and dihydroxyacetone phosphate (Willey et al. 2011). The next stage (the energy harvesting stage) produces four molecules of ATP via substrate level phosphorylation and four molecules of NADH (Willey et al. 2011). The end result is a net gain of two ATP molecules for every molecule of glucose metabolized. Two molecules of pyruvate remain which are transported into the mitochondria and subsequently fed into the Krebs cycle. In the Krebs cycle two molecules of pyruvate yield six molecules of NADH, two molecules of $FADH_2$ and two molecules of ATP (Willey et al. 2011).

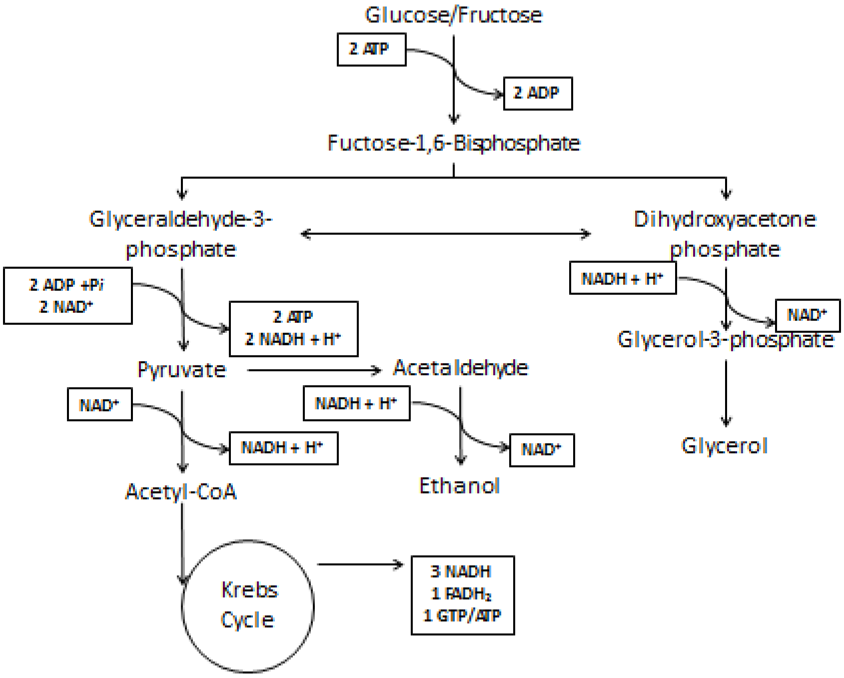

Figure 2. Simplified representation of glycolysis, with regard to the redox balance.

Generating ATP from glucose/fructose results in the accumulation of reducing molecules (NADH and $FADH_2$). For glycolysis to continue the NADH and $FADH_2$ need to be re-oxidized to NAD^+ and FAD^+, respectively. This is where the electron transport chain comes into play. Electrons from NADH and $FADH_2$ are passed onto a trans-membrane protein (Complex I), which “pumps” protons into the inter-membrane space (Willey et al. 2011). Electrons are then successively donated to complexes II, III and IV, at which point the electrons are donated to O_2 as the final electron acceptor resulting in H_2O formation. Each time the electrons are donated from one complex to another, energy is released (known as Gibbs free energy) (Willey et al. 2011). Essentially this release of energy is used to pump the protons into the inter-membrane space resulting in an electrochemical gradient across the inner membrane which powers the ATP Synthase complex (Willey et al. 2011). This protein complex essentially uses the movement of protons back into the mitochondrial matrix to produce ATP by the process of oxidative phosphorylation.

2.3.1.2 Fermentation

When oxygen is not available or when sugar is present at high levels (Crabtree effect), the yeast undergoes a shift towards fermentative metabolism, degrading glucose to ethanol and CO_2 without respiration or mitochondrial involvement. As stated previously, glycolysis generates reducing equivalents such as NADH. Without the electron transport chain to convert NADH back to NAD^+, glycolysis would cease to function due to a lack of NAD^+.

Alcoholic fermentation is an alternative pathway which yeast has acquired to convert NADH to NAD^+ when oxygen is no longer available (Figure 2). Pyruvate from glycolysis is enzymatically converted to acetaldehyde by pyruvate decarboxylase. Acetaldehyde is subsequently converted to ethanol via alcohol dehydrogenase (NADH being the cofactor used in this reaction) and in the process re-oxidizes NADH to NAD^+. In this manner the yeast cells are able to stay metabolically active as glycolysis continues to function, producing a net of two ATP's per mole of glucose/fructose under anaerobic conditions.

2.3.1.3 Glycerol production

Glycerol is a sugar alcohol that is formed as a by-product during fermentation. Its main function is to combat osmotic stress and maintain the NAD^+/NADH redox balance during fermentative growth (Scanes et al. 1998). Many intermediates of glycolysis (including pyruvate) are required for the biosynthesis of compounds required for cell growth. Consequently these intermediate compounds are no longer available for the production of ethanol and thus re-oxidation of NADH. This would upset the redox neutral process of sugar to ethanol conversion, in the absence of an alternative means of NADH oxidation. This is achieved by the enzymatic conversion of dihydroxyacetone phosphate by glycerol-3-phosphate dehydrogenase (*GPD1* & *GPD2*) to glycerol-3-phosphate (NADH being the cofactor used in this reaction) (Scanes et al. 1998). Glycerol-3-phosphate is subsequently converted to glycerol by glycerol-3-phosphatase (*GPP1* & *GPP2*) and exported out of the cell (Scanes et al. 1998). The key "rate limiting" enzymes responsible for glycerol formation are *GPD1* and *GPP2*. The expression of these genes in *S. cerevisiae* is partially controlled by HOG (High Osmolarity Glycerol) and MAP (Mitogen-activated pathway) which is also linked to stress response pathways (Albertyn et al. 1994, Norbeck et al. 1996, Scanes et al. 1998).

Due to the fact that ethanol and glycerol production both convert NADH back to NAD^+ their production in yeast is often inversely correlated. Therefore, theoretically ethanol can be decreased by increasing glycerol production. This can be done by genetic manipulation or by environmental manipulation. In the past temperature, pH, nitrogen and sulphur dioxide treatments have been shown to alter glycerol production (Scanes et al. 1998). For this reason it was hypothesised that manipulation of these and other parameters during alcoholic fermentation may yield similar results in terms of either increases or decreases in ethanol yield.

2.3.2 Metabolic flux engineering

A more targeted approach for ethanol reduction in wine is to redirect the carbon flux derived from glycolytic activity to an alternative end product instead of ethanol. Glycerol is one such possible end product as it is non-volatile and does not contribute to the aroma of wine. Moreover, it has been proposed that it can contribute to the smoothness and mouth-feel of wine (Eustace and Thornton 1987). Directing carbon towards an end product such as glycerol involves the deletion and/or overexpression of certain glycolytic genes. The next section will deal with different genetic modification strategies and highlight some of their strengths as well as their shortcomings.

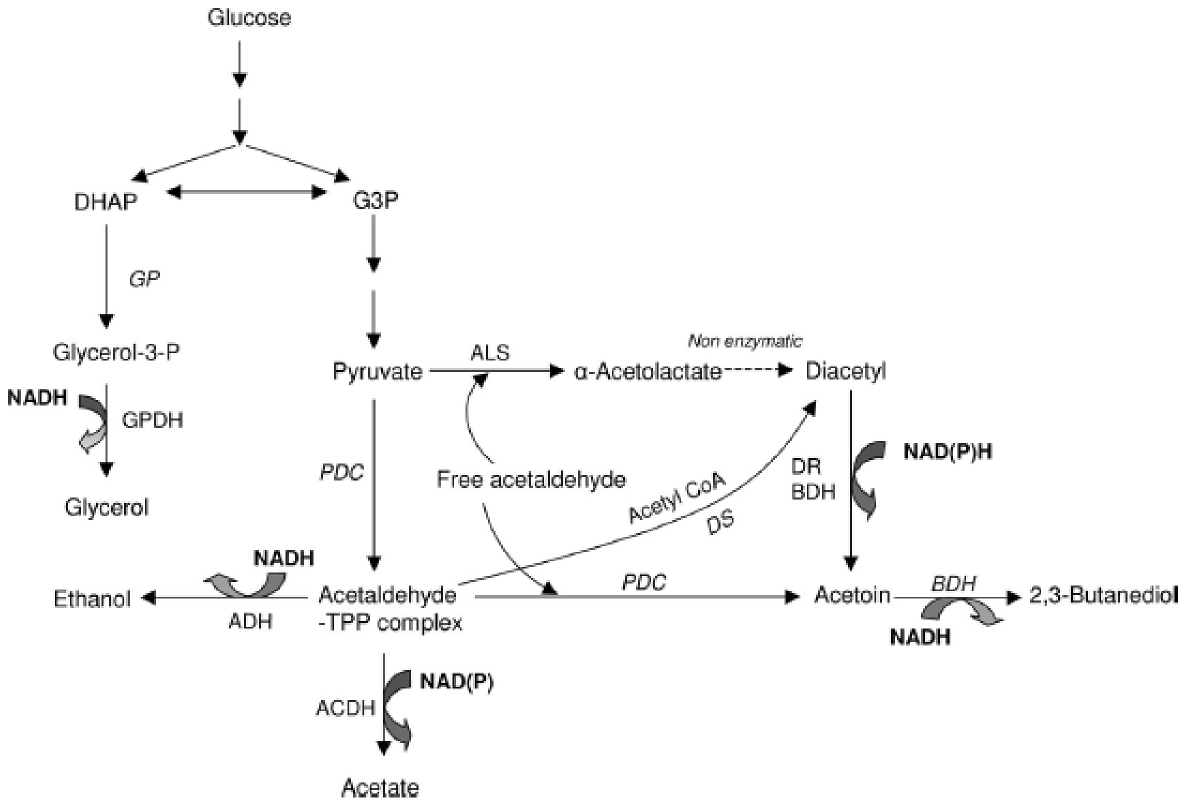

Figure 3. Schematic representation of metabolic pathways implicated in designing low ethanol yielding yeast strains (Ehsani et al. 2009).

2.3.2.1 Glycerol-3-phosphate dehydrogenase

One genetic approach is too over express *GPD1* and *GPD2*, which encode the two glycerol dehydrogenases in *S. cerevisiae (*Nevoigt and Stahl 1996, Michnick et al. 1997, Remize et al. 1999, Lopes et al. 2000, Cambon et al. 2006, Ehsani et al. 2009*).* The isozymes Gpd1p and Gpd2p are responsible for the conversion of dihydroxyacetone phosphate (DHAP) to glycerol-3-phosphate (G3P). This enzymatic reaction has been shown to be the rate limiting step in glycerol formation (Nevoigt and Stahl, 1996). G3P is then subsequently converted to glycerol (Figure 3). In previous studies it was shown that over-expression of *GPD1* increased glycerol concentrations by 548 %, while decreasing ethanol concentrations by 53 % (Nevoigt and Stahl 1996). However, it was also found that the acetic acid concentration increased by 193%. Since acetic acid confers a vinegar taint to wine at high concentrations this issue will have to be resolved to make this a viable option for decreasing ethanol concentrations. When *GPD2* was over-expressed in synthetic grape must it was reported that glycerol levels increased by 109% while the ethanol concentration decreased by only 5.0 %, and acetic acid concentrations increased by 75% (Lopes et al. 2000). Due to the fact that *GPD1* over-expression results in a lower ethanol yield it is more widely used in studies aimed at decreasing ethanol formation (Michnick et al. 1997, Remize et al. 1999, Cambon et al. 2006, Ehsani et al. 2009).

Glycerol-3-phosphate dehydrogenase, the enzyme encoded for by *GPD1*, oxidizes NADH to NAD^+. Consequently *GPD1* over-expression leads to an increase in NAD^+ levels, resulting in an imbalance in the steady state ratio of NAD^+/NADH (Michnick et al. 1997). When this occurs the surplus NAD^+ is reduced back to NADH via other enzymes including aldehyde dehydrogenase resulting in an increased production of acetic acid, which accounts for the link between increased glycerol levels and increased concentrations of this acid (Nevoigt and Stahl 1996, Lopes et al. *2000*).

2.3.2.2 Aldehyde dehydrogenase

One potential option to deal with elevated levels of acetic acid is to delete the stress response gene *AAF1*, which has been shown to regulate the mRNA levels of *ALD6* and *ALD4* (Walkey et al. 2012). Both these genes encode for an aldehyde dehydrogenase (ACDH) which is responsible for the conversion of acetaldehyde to acetic acid. This approach has been shown to reduce acetic acid levels in Chardonnay by up to 39.2% in commercial strain Enoferm M2 (Luo et al. 2013). However, whether deletion of *AAF1* in *GPD1* over-expressing strains will have an effect on acetic acid production has yet to be determined.

Another option to deal with the elevated acetic acid concentrations linked to glycerol overproduction is to directly delete the gene responsible for the majority of acetate production namely *ALD6* (Eglinton et al. 2002). When copies of the *ALD6* gene were disrupted in the laboratory strain V5 the acetate concentration decreased by 60% compared to the wild type strain (Remize et al. 2000). Interestingly when *ALD6* was deleted in the V5 strain over-expressing *GPD1 (V5 GPD1* Δald6*)* acetate concentrations similar to the wild type was observed while glycerol increased by a further 16% compared to V5 only over-expressing *GPD1* (Cambon et al. 2006). This clearly indicated that *ALD6* deletion effectively decreases acetic acid formation in strains where there is a large carbon shift towards glycerol. This decrease in acetic acid production was also confirmed in industrial strains (Cambon et al. 2006).

Unfortunately strains over-expressing *GPD1* and lacking the *ALD6* gene produced elevated concentrations of acetoin, which has a negative sensorial impact on wine (Romano and Suzzi 1996, Cambon et al. 2006).

2.3.2.3 2,3-Butanediol dehydrogenase

Acetoin in wine confers an unpleasant buttery aroma. Under normal circumstances acetoin concentrations in wine range from undetectable to 80 mg/L, while the odour detection threshold is around 150 mg/L (Romano and Suzzi 1996, Romano et al. 2003). Thus the impact on wine quality and aroma is negligible. However, Cambon et al. (2006) reported acetion levels between 5.8 to 9.5 g/L for the *GPD1* overexpression and *ALD6* deletion strategy.

The reduced form of acetoin is 2,3-butanediol, which is regarded to have more neutral sensory properties (Sponholz et al. 1993). *BDH1* codes for the enzyme 2,3-Butanediol dehydrogenase which reduces acetion to 2,3-butanediol (Ehsani et al. *2009*). The gene *BDH1 was* over-expressed in the *V5 GPD1* ∆ald6 strain to determine if acetoin concentrations can be reduced below its sensory threshold (150 mg/L). Ehsani et al*.* were indeed able to slightly decrease acetoin levels, however new strategies will need to be explored to further decrease concentrations. Results with these strains were also not confirmed in a wine yeast genetic background (Ehsani et al. *2009*).

2.3.2.4 Alcohol dehydrogenase

Alcohol dehydrogenase is responsible for the conversion of acetaldehyde to ethanol (Figure 3), in the process regenerating NAD^+ and maintaining the redox balance so that glycolysis can continue operating in the cell. The four isozymes are endcoded by *ADH1, ADH2, ADH3* and *ADH4* (Lutstorf and Megnet 1968). Strains lacking the major isoform of alcohol dehydrogenase (*ADH1*) have a lower ethanol yield than the wild type (Ciriacy 1975, Johansson and Sjöström 1984). Strains lacking all four genes (adh^0) have an even further enhanced glycerol production and lower ethanol yield (Drewke et al. 1990). adh^0 strains produce 25% of the maximum theoretical ethanol yield. However, these strains also produced elevated levels of acetaldehyde and acetic acid, and would thus not be a viable option for wine production.

2.3.2.5 Trehalose

Trehalose is a stress and reserve carbohydrate which could potentially also be used as a carbon sink. Trehalose-6-phosphate synthase (*TPS1*) converts UDP-glucose and glucose-6-phosphate to α,α-trehalose-6-phosphate, which is subsequently converted to trehalose and phosphate by trehalose-6-phosphate phosphatase (*TPS2*) (François and Parrou 2001). Trehalose-6-phosphate phosphatase is also a known hexokinase inhibitor. Therefore *TPS1* not only restricts some carbon entering glycolysis, but also lowers the fermentative efficiency (Rossouw et al. 2013). *TPS1* was expressed using a stationary phase specific promotor *GIP1*, due to the fact that when *TPS1* is over-expressed using a strong promotor fermentative performance is negatively affected (Rossouw et al. 2013). By using this approach ethanol yields were decreased by between 0.5% and 1%, with no increase in acetic acid.

2.3.2.6 Levans

Another option is to direct carbon to non-native storage polymers, such as levan type fructans. Alternative carbon sinks can be introduced into the yeast by expression of relevant genes from other organisms. This was recently achieved in a laboratory yeast strain by over-expressing m1ft from *Leuconostoc mesenteroides* in an invertase negative yeast strain (Franken et al. 2013). The mutant strain was able to accumulate the levan only under aerobic conditions. No change in ethanol was seen under fermentative conditions. However, the fine-tuning of this system or the introduction of alternative genes/pathways for novel polymer production in yeast still holds potential as a strategy for ethanol reduction.

2.3.2.7 Glycerol transporter genes

Fps1p is a member of the MIP (Major Intrinsic Protein) family of proteins, the main function of which is to regulate the intracellular concentration of glycerol by facilitating its efflux (Luyten et al. 1995, Tamás et al. 1999). *FPS1* expression is regulated by the osmolarity of the surrounding environment. The deletion of the *FPS1* gene results in a lower glycerol yield while the ethanol yield increases since glycerol cannot leave the cell (Zhang et al. 2007). When *FPS1* was over-expressed glycerol production was enhanced in strains already over-expressing *GPD1* (Tamás et al. 1999). *FPS1* over-expression has yet to be carried out in wine yeast, and its effect on ethanol yields is also unknown.

2.3.2.8 Multi-gene approach

Another less direct approach is to genetically modify several genes in a wine yeast strain by a combination of gene over-expression, deletion and promotor replacement strategies. By increasing the expression of genes that diverted carbon away from ethanol, and deleting or down regulating genes involved in ethanol formation, it was hypothesized that a low ethanol yielding strain could be generated. In a recent study 41 genetic modifications were performed in the industrial yeast strain AWRI1631, 15 of which had a significant impact in terms of decreasing ethanol formation (Varela et al. 2012). However, only 2 of these 15 strains were chosen, AWRI2531 and AWRI2532, to ferment in grape must. Both of these strains over-expressed *GPD1*. The first (AWRI2531) expressed two copies of the gene, while the second (AWRI2532) expressed three. In both strains the *ALD6* gene was also deleted (Varela et al. 2012). Essentially Varela et al. repeated the experimental design of Cambon et al. (2006) but for the fact that the strains fermented in grape must. In line with expectations and previous findings the wine showed unacceptably high levels of acetoin.

Cordier et al. (2007) investigated glycerol production using a combinatorial genetic approach. The authors investigated genes involved in glycerol production (*GPD1*), glycerol transport (*FPS1*), glycolytic branch point conversion (*TP1*), acetic acid production (*ALD3*) and ethanol production (*ADH1*). Using this approach a yeast strain was constructed that redirected almost half of its sugar towards glycerol (0.46 g.g glucose^{-1}) (Cordier et al. 2007).

The aim of this study was not to decrease ethanol in wine, but rather to increase glycerol for industrial applications. However, it does represent a potential step in the right direction in terms of creating a wine yeast strain capable of decreased ethanol yields.

2.3.3 Non-*Saccharomyces* yeasts

Using species other than *Saccharomyces* is also an option to reduce ethanol in wine. *Hanseniaspora uvarum,* a yeast that is commonly found in fresh grape must, has a 30% lower ethanol yield than *S. cerevisiae* (Ciani and Picciotti 1995). However, its use in the production of wine is questionable as many off flavors like ethyl acetate are produced at high concentrations. These results were confirmed by Ciani and Maccarelli (1997) who also showed that *Candida stellate* is a feasible option for lower ethanol fermentations. The ethanol yields reported in their study are not as low as *H.uvarum* however ethyl acetate production levels are similar to *S. cerevisiae* (Ciani and Maccarelli 1997).

In a recent publication it was shown that sequential inoculation using *Metschnikowia pulcherrima* yeast followed by inoculation with *S. cerevisiae* to complete alcoholic fermentation can reduce ethanol concentration by up to 1.6% (v/v) in Shiraz (Contreras et al. 2013).

2.3.4 Environmental manipulation

There are several studies which show that glycerol production is strain dependent (Rankine and Bridson 1971, Remize et al. 1999). Since some of these strains produce elevated glycerol levels, lower ethanol might be a direct consequence. However ethanol concentration has yet to be proven to be strain dependent in commercial wine yeast strains of the *S. cerevisiae* species. Sulphur dioxide, a commonly used chemical to treat grape must to prevent bacterial contamination and oxidation, has also been shown to increase glycerol formation (Rankine and Bridson 1971, Gardner et al. 1993). This is due to bisulphate binding to acetaldehyde, rendering it unavailable for ethanol production. As this reaction no longer takes place, intracellular NADH concentrations increase. As a consequence glycerol production increases to facilitate the re-oxidation of NADH back to NAD^+ (Figure 1).

Another study evaluated the impact of must pH and temperature of fermentations. The study was done using two *S. cerevisiae* strains, namely *Kalecik* and *Narince* (Yalcin and Ozbas 2008). The authors concluded that maximum glycerol production was obtained between pH 5.92 and 6.27, respectively. However, these pH values are never found in natural grape must. They also concluded that a fermentation temperature between 25°C and 30°C would yield a higher final glycerol concentration (Yalcin and Ozbas 2008). Interestingly, in another study the authors concluded that lower pH and temperature will result in a higher glycerol concentration in the *S. cerevisiae* strain T73 (Arroyo-López et al. 2010). These contradicting results may be due to the inherent differences found between wine yeast strains.

To our knowledge no study exists that systematically explores ethanol production levels of various yeast genotypes in controlled wine making conditions.

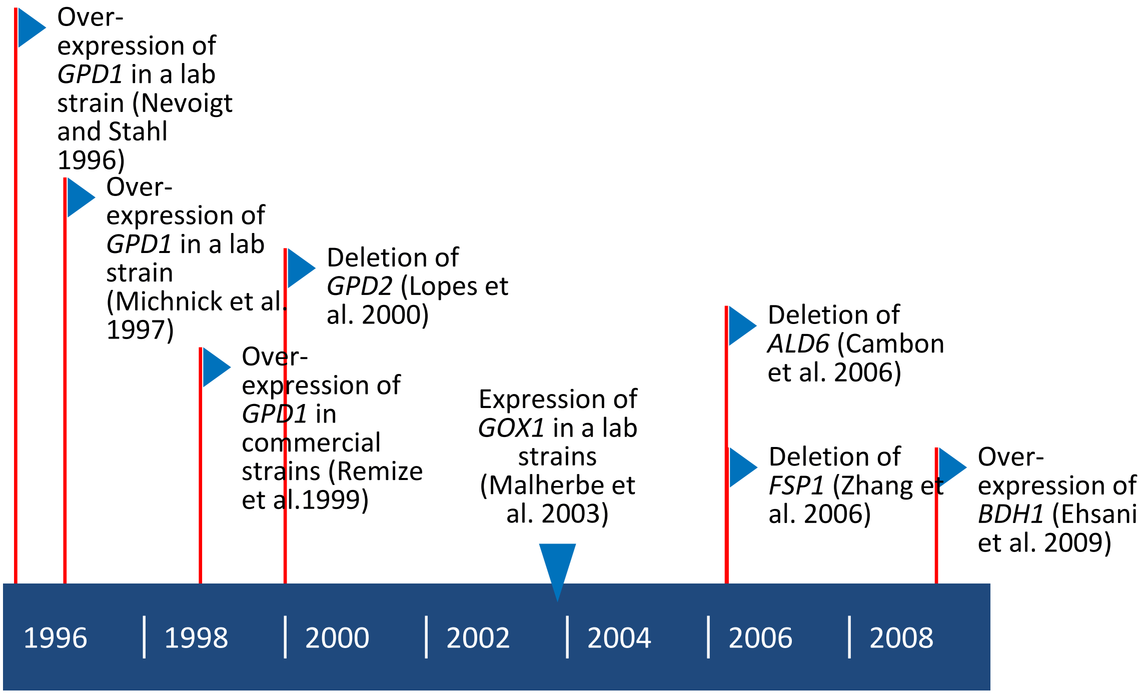

Figure 4. Timeline depicting some of the novel yeast strains generated in low ethanol wine production.

2.4 Conclusion

Currently there are several industrial or research prototype methods to decrease the ethanol concentration of wine. These methods range from viticultural and pre-fermentation treatments to post fermentation processes (distillation and filtration). Such methods have had some success, however many quality and cost issues still need to be resolved. Current research is largely focused on biological or GM approaches: Redirecting carbon towards glycerol has shown the potential to decrease the ethanol levels in wine. However, this increase in glycerol is accompanied by an increase in unwanted aroma compounds (Remize et al. 1999, Lopes et al. 2000, Cambon et al. 2006, Ehsani et al. 2009). The concentration of some of these compounds can be lowered through deletion and over-expression of certain genes, however the concentrations achieved never fall below the sensory threshold of these compounds. Redirecting carbon towards trehalose has also shown promise in terms of decreasing ethanol yields (Rossouw et al. 2013) but sensory analyses still has to be done to confirm that no unwanted aromas are produced. Even if unwanted aromas are eliminated in these strains, current legislation in South Africa prohibits their use in wine production. The ultimate goal of every low ethanol strategy is to find a practical, cost-effective approach in line with acceptable winemaking standards, and which does not negatively impact on the quality of the wine. This goal is yet to be realised, as researchers continue to address what may be one of the biggest scientific problems faced by the wine industry.

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Chapter 3

Research Results

Evaluating ethanol yields under various fermentation conditions

Chapter 3

Evaluating ethanol yields under various fermentation conditions

3.1 Introduction

For many social, economic and health-related reasons, the reduction of ethanol levels in wine has been one of the most pressing scientific pressing challenges in the wine sciences (Pickering 2000). Methods to achieve this target include viticultural and pre-fermentation treatments, but post-fermentation ethanol removal through methodologies such as spinning cone columns and reverse osmosis are currently the most popular tools. However, such systems are very expensive to run and their economic viability is questionable (García-Martín et al. 2010).

Several microbiological methods have been proposed in the past mostly focusing on redirecting carbon flux in fermenting yeast towards other compounds (such as glycerol) and away from ethanol. This can be achieved by genetic modification of certain genes controlling ethanol and glycerol production (Nevoigt and Stahl 1996, Michnick et al. 1997, Remize et al. 1999, Lopes et al. 2000, Cambon et al. 2006, Ehsani et al. 2009). Many of these methods were successful in terms of increasing glycerol and decreasing ethanol concentrations, however many off flavours such as acetic acid were also produced. Other genetic modifications were subsequently performed to decrease the production of these off flavours, with moderate success (Ehsani et al. 2009).

In several studies it has been shown that glycerol production is strain dependent and can also be environmentally manipulated by, for example increasing the fermentation temperature (Rankine and Bridson 1971, Remize et al. 1999, Yalcin and Ozbas 2008). Due to the fact that glycerol and ethanol are directly derived from a sugar molecule and that both re-oxidize NADH to NAD^+ their production is often inversely correlated. Therefore if glycerol production can be manipulated by changing the physico-chemical parameters of the fermenting must, ethanol production should potentially also be responsive to environmental signals. The aim of this project is to investigate whether factors such as commercial yeast strain, pH, temperature, yeast assimilable nitrogen (YAN), initial sugar concentration and grape cultivar could significantly influence the production of ethanol. These factors were selected since they are amenable to some control by the winemaker. Application of specific environmental settings may ultimately result in a lower ethanol wine after alcoholic fermentation. The added advantage would be that the use of such a 'parameter control' approach to lowering ethanol would not involve the use of controversial genetically modified organisms.

This study is divided into four objectives evaluating (1) the influence of yeast strain and (2) of must composition and fermentation conditions on ethanol yield, assessing results (3) in grape must experimentation and (4) follow the evolution of aroma compound production under these conditions.

To achieve these aims, 15 wine yeast strains that are popular in the South African wine industry were evaluated for their ethanol yield under nine different synthetic must conditions using a single factorial design. Based on the results from this part of the study two strains were selected which showed differences in ethanol yields under these conditions. These strains were then applied in the second objective to determine if the conditions tested may have a cumulative effect on ethanol yield in a more elaborate multi-factorial design. In this part of the study the impact of different factors were co-evaluated simultaneously. The third objective was then to determine whether the results obtained from the experiments in synthetic must were reproducible in grape must from four different cultivars (Sauvignon Blanc, Chardonnay, Shiraz and Cabernet Sauvignon). Finally the fourth objective investigated the effect of differences in selected parameters on the aroma profile of the wines produced.

Currently there is little information in how must composition and environmental factors interact with individual yeast strains to influence central carbon metabolism and associated metabolic regulatory circuits, and how precisely such interactions impact on the final production levels of carbon compounds (such as ethanol) in a specific must. This study therefore investigated the causative factors of high ethanol yields in specific wine musts in an attempt to provide specific guidelines on how to reduce ethanol yields. Ultimately the outcome of this research could provide winemakers with highly specific guidelines on how to achieve lower ethanol yields with existing wine yeast strains.

3.2 Materials and Methods

3.2.1 Strain and culture conditions

3.2.1.1 Freeze cultures

The 15 wine strains that were used in the study are summarized in Table 1. Strains were streaked out on YPD agar plates (Biolab, Gauteng, South Africa) and single colonies were then used to inoculate 5 mL YPD broth (Biolab, Gauteng, South Africa). These cultures were grown to saturation overnight at 30^{o}C, 1 ml of culture was then added to 1 ml 30% glycerol and stored at -80^{o}C. For each set of experiments new plates were streaked out from these stock cultures.

3.2.1.2 Culture conditions

Fresh YPD broth was used to zero the spectrophotometer (Powerwave$_x$, Bio-Tek Instruments). If the OD_{600} reading was more than 1.0, a 10X dilution with dH_2O was used, and blanks were also diluted similarly. Single colonies from plates streaked out from the freeze cultures were used to inoculate 5 ml YPD broth and grown to saturation overnight at 30^{o}C. Strains were inoculated into fresh YPD to an OD_{600} of 0.2 and incubated at 30^{o}C until an OD_{600} of approximately 1.5 was reached. Cells were centrifuged at 5000 rpm for 5 minutes and the supernatant was discarded. The cell pellet was resuspended in the corresponding wine must. This cell culture was then used to inoculate the different fermentations to an initial OD_{600} of 0.1. Fermentation vessels were sealed with a rubber stopper and a CO_2 outlet. OD_{600} readings were also taken at the end of fermentation, at either 10X or 20X dilution.

Table 1. Saccharomyces strains used in this study

Commercial Name	**Strain**
IWBT PR7	*S. cerevisiae and S. paradoxus* hybrid
VIN 200	*S. cerevisiae* hybrid
VIN 7	*S. cerevisiae* and *S. kudriavzevii* hybrid
VIN 13	*S. cerevisiae* hybrid
WE 14	*S. cerevisiae*
WE 372	*S. cerevisiae*
228	*S. cerevisiae*
AWRI 796	*S. cerevisiae*
EC1118	*S. cerevisiae bayanus*
N 96	*S. cerevisiae bayanus*
NT 45	*S. cerevisiae*
NT 50	*S. cerevisiae* hybrid
NT 112	*S. cerevisiae* hybrid
NT 116	*S. cerevisiae* hybrid
NT 202	*S. cerevisiae* hybrid

3.2.2 Must and fermentation treatments

3.2.2.1 Synthetic media

Synthetic wine must (MS300) was made as described in Table 2 and Table 3 (Bely et al. 1990). Base medium, amino acids and oligoelements were autoclaved separately. Vitamins and aerobic factors were filter sterilized separately. After addition of base medium, amino acids, oligoelements and vitamins, the pH of medium was adjusted to 3.4 using potassium hydroxide and the medium was filter sterilized.

Table 2. Synthetic must MS300 described by Bely et al. (1990)

		Gram per litre
Base Medium	Glucose	125
	Fructose	125
	Citric Acid	6
	Malic Acid	6
	KH_2PO_4	0.75
	K_2SO_4	0.5
	$MgSO_4.7H_2O$	0.25
	$CaCl_2.2H_2O$	0.155
	NaCl	0.2
Oligoelements	$MnSO_4.H_2O$	4
	$ZnSO_4.7H_2O$	4
	$CuSO_4.5H_2O$	1
	KI	1
	$CoCl_2.6H_2O$	0.4
	H_3BO_3	1 g
	$(NH_4)6Mo7O_{24}$	1 g
Vitamins	Myo-inositol	2.000
	Calcium Pantothenate	0.150
	Thiamine Hydrochloride	0.025
	Nicotinic Acid	0.200
	Pyridoxine	0.025
	Biotin	0.0003
Anaerobic factors	Ergosterol	1.500
	Oleic Acid	0.500

Table 3. Nitrogen Composition of MS300 media. As described by Bely et al. (1990)

		Milligrams per litre
Amino Acids	Tyrosine	18.326
	Tryptophane	179.333
	Isoleucine	32.725
	Aspartic Acid	44.506
	Glutamic acid	120.428
	Arginine	374.374
	Leucine	48.433
	Threonine	75.922
	Glycine	18.326
	Glutamine	505.274
	Alanine	145.299
	Valine	44.506
	Methionine	31.416
	Phenylalanine	37.961
	Serine	78.54
	Histidine	32.725
	Lysine	17.017
	Cysteine	13.09
	Proline	612.612
Ammonium Chloride	NH_4Cl	460

The total nitrogen concentration varied between 120 mg/L, 150 mg/L, 300 mg/L and 400 mg/L depending on the fermentation condition tested. However, amino acid/ammonium chloride ratios were kept the same. Total sugar concentrations were varied (150 g/L, 220 g/L, 250 g/L and 300 g/L) with glucose and fructose always added in equimolar amounts. The pH was also varied using potassium hydroxide (3.0, 3.4 and 4.0).

3.2.2.2 Grape derived must

Sauvignon Blanc, Chardonnay, Shiraz and Cabernet Sauvignon grapes were harvested when sugars reached appropriate concentration for cultivar from Welgevallen Experimental Wine Farm in Stellenbosch. Grapes were transported to the Institute for Wine Biotechnology, where they were destemmed, crushed and pressed by industry accepted methods. A total of 18 mL of 2.5% SO_2 was added to each 25 L crushed grapes (18 ppm final concentration).

Rapidase® was added to the white grape must and left at 4°C overnight for settling. The composition of all the different musts is summarized in Table 4.

3.2.2.3 Fermentations

Small scale fermentations of 80 mL were performed in 100 mL fermentation vessels (in triplicate) at 15°C, 20°C and 25°C. Fermentations were inoculated to an OD_{600} as described in section 3.2.1.2. Fermentations flasks were weighed daily to monitor CO_2 evolution, and synthetic wine must fermentations were sampled every fourth day (2 mL). Fermentations were stopped and sampled when there was no weight loss for three consecutive days.

Table 4. Different media types and fermentation temperatures used

Number	Name	Media Type	Sugar (g/L)	Nitrogen (mg/L)	pH	Temperature (°C)
1	Control	Synthetic (MS)	250	300	3.4	20
2	Low Nitrogen	Synthetic (MS)	250	120	3.4	20
3	High Nitrogen	Synthetic (MS)	250	400	3.4	20
4	Low Sugar	Synthetic (MS)	150	300	3.4	20
5	High Sugar	Synthetic (MS)	300	300	3.4	20
6	Low pH	Synthetic (MS)	250	300	3.0	20
7	High pH	Synthetic (MS)	250	300	4.0	20
8	Low Temperature	Synthetic (MS)	250	300	3.4	15
9	High Temperature	Synthetic (MS)	250	300	3.4	25
10	Low Nitrogen Low pH Low Temperature	Synthetic (MS)	220	150	3.0	15
11	Medium Nitrogen Low pH Low Temperature	Synthetic (MS)	220	300	3.0	15

Table 4 continued

12	High Nitrogen Low pH Low Temperature	Synthetic (MS)	220	400	3.0	15
13	Low Nitrogen Medium pH Low Temperature	Synthetic (MS)	220	150	3.4	15
14	Medium Nitrogen Medium pH Low Temperature	Synthetic (MS)	220	300	3.4	15
15	High Nitrogen Medium pH Low Temperature	Synthetic (MS)	220	400	3.4	15
16	Low Nitrogen High pH Low Temperature	Synthetic (MS)	220	150	4.0	15
17	Medium Nitrogen High pH Low Temperature	Synthetic (MS)	220	300	4.0	15
18	High Nitrogen High pH Low Temperature	Synthetic (MS)	220	400	4.0	15
19	Low Nitrogen Low pH Medium Temperature	Synthetic (MS)	220	150	3.0	20
20	Medium Nitrogen Low pH Medium Temperature	Synthetic (MS)	220	300	3.0	20
21	High Nitrogen Low pH Medium Temperature	Synthetic (MS)	220	400	3.0	20
22	Low Nitrogen Medium pH Medium	Synthetic (MS)	220	150	3.4	20

Table 4 continued

	Temperature					
23	Medium Nitrogen Medium pH Medium Temperature	Synthetic (MS)	220	300	3.4	20
24	High Nitrogen Medium pH Medium Temperature	Synthetic (MS)	220	400	3.4	20
25	Low Nitrogen High pH Medium Temperature	Synthetic (MS)	220	150	4.0	20
26	Medium Nitrogen High pH Medium Temperature	Synthetic (MS)	220	300	4.0	20
27	High Nitrogen High pH Medium Temperature	Synthetic (MS)	220	400	4.0	20
28	Low Nitrogen Low pH High Temperature	Synthetic (MS)	220	150	3.0	25
29	Medium Nitrogen Low pH High Temperature	Synthetic (MS)	220	300	3.0	25
30	High Nitrogen Low pH High Temperature	Synthetic (MS)	220	400	3.0	25
31	Low Nitrogen Medium pH High Temperature	Synthetic (MS)	220	150	3.4	25
32	Medium Nitrogen Medium pH High Temperature	Synthetic (MS)	220	300	3.4	25
33	High Nitrogen	Synthetic	220	400	3.4	25

Table 4 continued

	Medium pH High Temperature	(MS)				
34	Low Nitrogen High pH High Temperature	Synthetic (MS)	220	150	4.0	25
35	Medium Nitrogen High pH High Temperature	Synthetic (MS)	220	300	4.0	25
36	High Nitrogen High pH High Temperature	Synthetic (MS)	220	400	4.0	25
37	Control	Sauvignon Blanc	221.33	169.69	3.19	20
38	Low pH	Sauvignon Blanc	221.33	169.69	2.9	20
39	High pH	Sauvignon Blanc	221.33	4.0	3.19	20
40	High Nitrogen	Sauvignon Blanc	221.33	400	3.19	20
41	High Sugar	Sauvignon Blanc	300.00	169.69	3.19	20
42	Low Temperature	Sauvignon Blanc	221.33	169.69	3.19	15
43	High Temperature	Sauvignon Blanc	221.33	169.69	3.19	25
44	Control	Chardonnay	202.66	259.62	3.36	20
45	Low pH	Chardonnay	202.66	259.62	3.0	20
46	High pH	Chardonnay	202.66	259.62	4.0	20
47	High Nitrogen	Chardonnay	202.66	400.00	3.36	20
48	High Sugar	Chardonnay	300.00	259.62	3.36	20
49	Low Temperature	Chardonnay	202.66	259.62	3.36	15
50	High Temperature	Chardonnay	202.66	259.62	3.36	25
51	Control	Shiraz	227.76	172.49	3.55	20
52	Low pH	Shiraz	227.76	172.49	3.0	20
53	High pH	Shiraz	227.76	172.49	4.0	20
54	High Nitrogen	Shiraz	227.76	400.00	3.55	20
55	High Sugar	Shiraz	300.00	172.49	3.55	20
56	Low	Shiraz	227.76	172.49	3.55	15

Table 4 continued

	Temperature					
57	High Temperature	Shiraz	227.76	172.49	3.55	25
58	Control	Cabernet Sauvignon	238.02	163.17	3.49	20
59	Low pH	Cabernet Sauvignon	238.02	163.17	3.0	20
60	High pH	Cabernet Sauvignon	238.02	163.17	4.0	20
61	High Nitrogen	Cabernet Sauvignon	238.02	400.00	3.49	20
62	High Sugar	Cabernet Sauvignon	300.00	163.17	3.49	20
63	Low Temperature	Cabernet Sauvignon	238.02	163.17	3.49	15
64	High Temperature	Cabernet Sauvignon	238.02	163.17	3.49	25

3.2.3 Quantification of compounds

Ethanol and glycerol concentrations were quantified by High Performance Liquid Chromatography (HPLC) on an Aminex HPX-87H ion exchange column (Bio-Rad, California, USA) with 5 mM H_2SO_4 as the mobile phase. Samples and standards were prepared in accordance with the method described by Eyéghé-Bikong et al. (2012). Peak detection and quantification was performed by Agilent RID detectors. The HPChemstation software package was used to analyse and integrate the peaks.

Enzyme assays were used to determine residual glucose and fructose concentrations on the Arena 20XT enzyme robotic system (Thermo Electron Oy, Finland). Enytec^{TM} *Fluid* D-Glucose Id-N^{o}: 5140 (R-Biopharm AG, Germany) was used to determine glucose concentrations and Enytec^{TM} *Fluid* D-Fructose Id-N^{o}: 5120 (R-Biopharm AG, Germany) was used to determine fructose concentrations.

Grape must glucose and fructose concentrations were quantified before adjustment using the Winescan FT120 equipped with a purpose built Michelson interferometer (FOSS Analytical A/S, Hillerød, Denmark) to generate a Fourier transform mid infrared (FT-MIR) spectrum. In house adjustments were made using the Winescan FT120 2001 version 2.2.1 software.

Total YAN was determined by formol titration. Three drops of H_2O_2 were added to 50 ml of grape must and the pH was adjusted to 8.5 using NaOH. In the second step, 20 ml of formaldehyde was added and the pH titrated back to 8.5. By measuring the amount of 0.1 N NaOH added (ml) and multiplying by 28 mg/L the final YAN concentration was determined.

For the extraction of major aroma volatiles liquid-liquid extraction was used as described by Louw et al. (2010) with some minor modifications, 100 µl internal standard (4-methyl-2-pentanol) and 1 ml solvent (diethyl ether) was added to 5 ml synthetic wine after which the mixture was sonicated for 5 minutes. Samples were then centrifuged at 4000 rpm for 3 minutes. The diethyl ether layer was removed and added to Na_2SO_4 to remove residual water before the sample was transferred to a vial and crimped.

Gas chromatography (GC) equipped with a flame ionization detector (FID) and split/splitless injector was used to quantify the major aroma volatiles (Hewlett Packard 6890, Agilent, Little Falls, Wilmington, USA) according to the protocol described by Malherbe (2011). A J&B DBFFAP capillary GC column (Agilent, Little Falls, Wilmington, USA) with the dimensions of 60 m x 0.32 mm and a 0.5 µl coating film thickness and a hydrogen carrier gas flow rate of 3.3 ml/min was used to separate compounds. The extracted sample (3 µl) was injected into the column at an initial temperature of $33^{o}C$ and the temperature gradually increased to $200^{o}C$ according to the program described by Malherbe et al. (2001). A post run step was performed at $240^{o}C$ for 5 minutes after every run. After every 20 samples the column was cleaned by injecting 4 µl hexan. All samples were injected twice, peak integration and data collection was done using the HP ChemStations Rev. B01.03 [204] software.

3.2.4 Statistical analyses

All results were first interpreted by looking at the general impact to the ethanol yield depending on the must, temperature or strain used. Further validation was then done through statistical t-test evaluation (Statistica 12.0.1133.15 software package), either compared to other strains or a control.

3.3 Results

3.3.1 Ethanol yield of various strains under different synthetic must conditions

The first objective of this study was to investigate inherent ethanol yield differences in 15 wine yeast strains. These 15 wine yeast strains were fermented under nine different conditions (seven different synthetic musts MS300 and two different temperature settings). It was decided not to use final ethanol concentrations to compare strains as in many cases fermentations became stuck and thus did not go to dryness (<5 g/L). In some cases stuck fermentations were unavoidable, for example in the study of low nitrogen musts which did not contain enough YAN for complete alcoholic fermentation. Instead ethanol yield (grams ethanol produced per game sugar consumed) was used to compare strains, making it possible to compare ethanol production even if the fermentation did not go to complete dryness. It should be noted that in some cases of reduced sugar consumption ethanol yield data might also not be reliable as different phases of sugar consumption are being compared. But for the purpose of this study, ethanol yield data is more than adequate to compare ethanol production. All residual sugars and ethanol concentrations are listed in Table A12. The expected theoretical ethanol yield of *S. cerevisiae* is about 0.51 g.g^{-1} yet most fermentations will not reach this level.

For the purpose of this study the ethanol yields of different strains were evaluated in relation to one another. P-values were calculated in an all against comparison.

3.3.1.1 Effect of strain selection

When these 15 strains were fermented in standard synthetic MS300 must no large differences in ethanol yield were observed. Ethanol yield ranged between 0.35 and 0.40 g.g-1 (Figure 1). However, strain NT45 showed statistically significantly lower ethanol yield than IWBT PR7, VIN7, VIN13, WE14, 228 and AWRI796 (Figure 1; Table A1). VIN7, on the other hand, showed statistically significantly higher ethanol yield than VIN2000, WE14, AWRI796, EC1118, NT45 and NT112 (Figure 1; Table A1). Indicating that there is a small, yet statistically significant difference, in ethanol yield between strains.

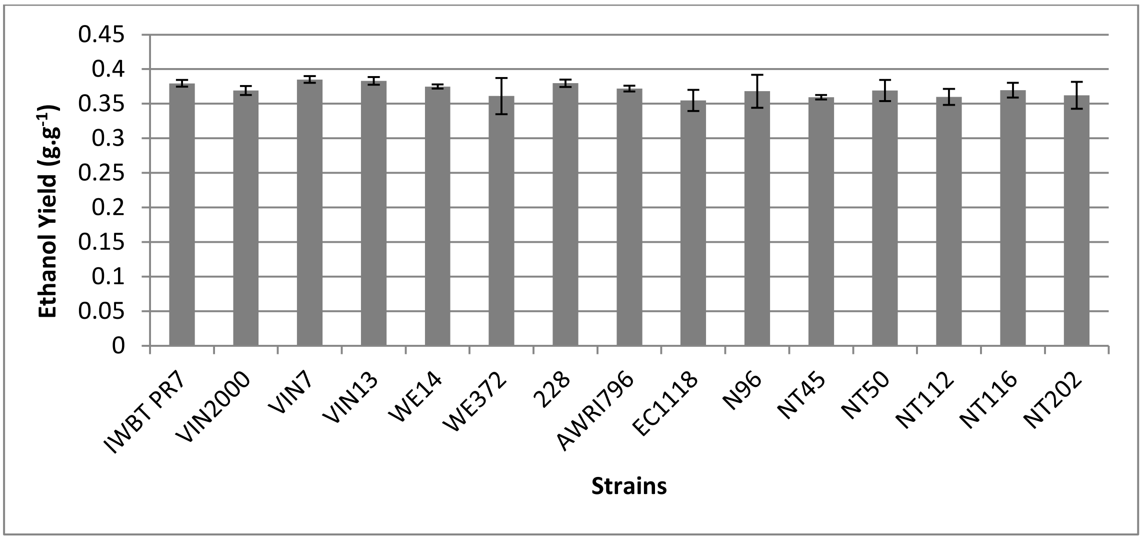

Figure 1. Ethanol yield of 15 industrial wine strains in MS300 synthetic wine must, fermented at 20°C.

3.3.1.2 Effect of nitrogen supplementation

In nitrogen deficient must where the YAN was 120 mg/L fermentations, VIN7 resulted in statistically significantly higher ethanol yields than fermentations with WE372, 228, AWRI796, EC1118 and NT112. The ethanol yield of EC1118 and NT112 were both statistically significantly less than those of six other strains (Figure 2; Table A2). However, many of these fermentations failed to reach dryness due to the low nitrogen content of the synthetic must (Table A12).

In MS must where the YAN was supplemented to 400 mg/L, the ethanol yield of VIN7 increased even further (Figure 3) compared to the nitrogen deficient must (Figure 2), with ethanol yields for VIN7 being statistically significantly higher than for nine of the other strains (Table A3). Therefore both above and below standard nitrogen levels seemed to increase the ethanol yield of VIN7 when compared to other strains fermented in the same must. However, the ethanol yields of fermentations conducted with EC1118 were increased (statistically significantly more than four other strains) when fermenting in MS must with increased YAN (Figure 3). In contrast, the ethanol yield for EC1118 was decreased in nitrogen deficient MS must, being statistically significantly lower than six other strains (Figure 2; Table A2). This shows that different strains (VIN7 and EC1118 in this case) behave differently with regard to ethanol yield relative to the total YAN in the must. In high nitrogen must NT50 also showed a notability lower ethanol yield being statistically significantly less than all stains except WE372 and NT45 (Table A3).

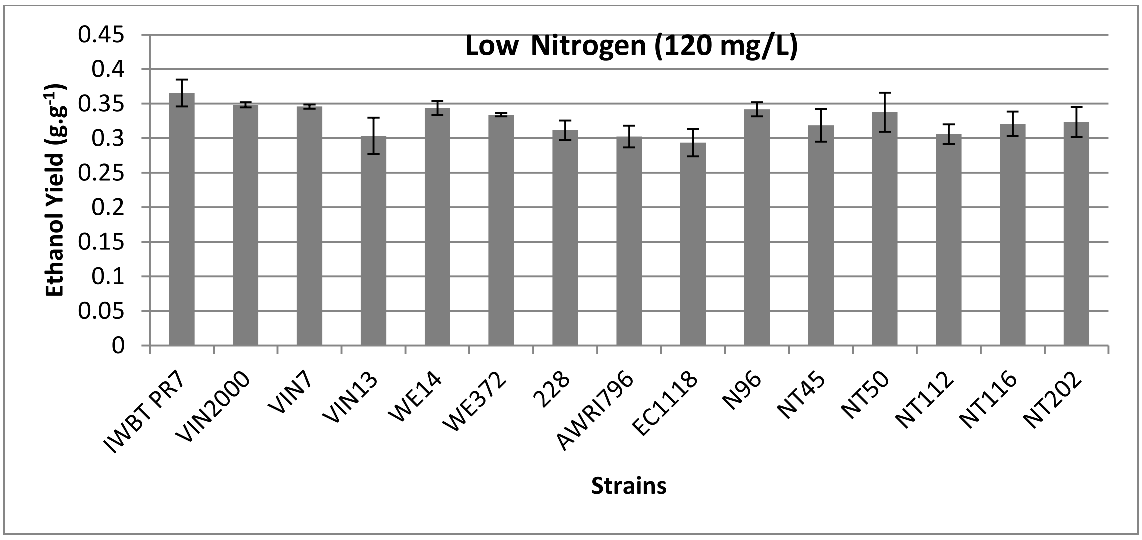

Figure 2. Ethanol yield of 15 industrial wine strains in nitrogen deficient (120 mg/L) MS synthetic wine must fermented at 20°C.

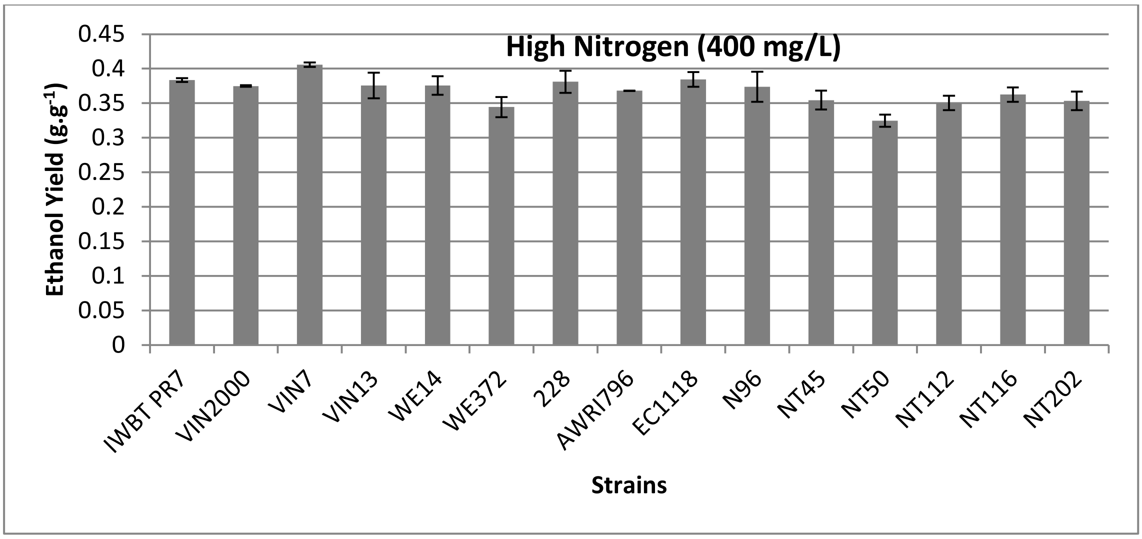

Figure 3. Ethanol yield of 15 industrial wine strains in high nitrogen (400 mg/L) MS synthetic wine must fermented at 20°C.

3.3.1.3 Effect of pH

When the pH of the MS300 synthetic wine must was lowered to 3.0 very small ethanol yield fluctuations were observed between strains (Figure 4). However, strain IWBT PR7 did show a higher ethanol yield, being statistically significantly higher than nine other strains. NT202 showed a slightly lower ethanol yield being significantly lower than five other strains (Table A4). The other 13 strains did not show significant differences amongst one another. This might be due to the fact that the pH of the synthetic must is already relatively low, thus a decrease of 0.4 may not have a significant effect on the yeast in terms of physiology and/or stress responses. A similar response is seen when the pH of the MS must is increased from 3.4 to 4.0 (Figure 5). Most strain produced a similar ethanol yield, however two strains did show a decreased ethanol yield namely WE372 and 228, being statistically significantly less than seven and nine other strains, respectively (Figure 5; Table A5).

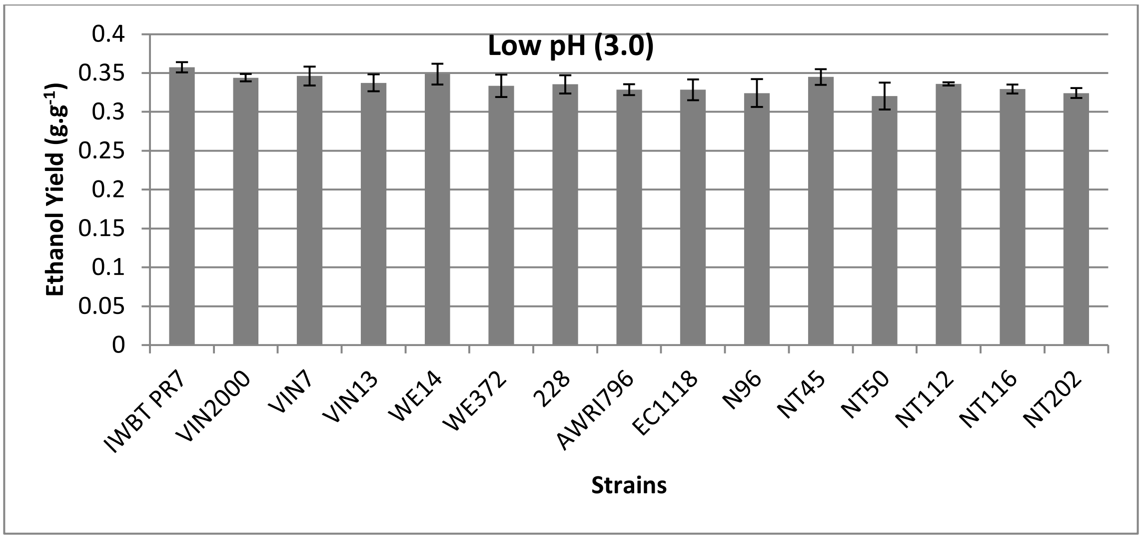

Figure 4. Ethanol yield of 15 industrial wine strains in low pH MS300 (pH 3.0) synthetic wine must fermented at 20°C.

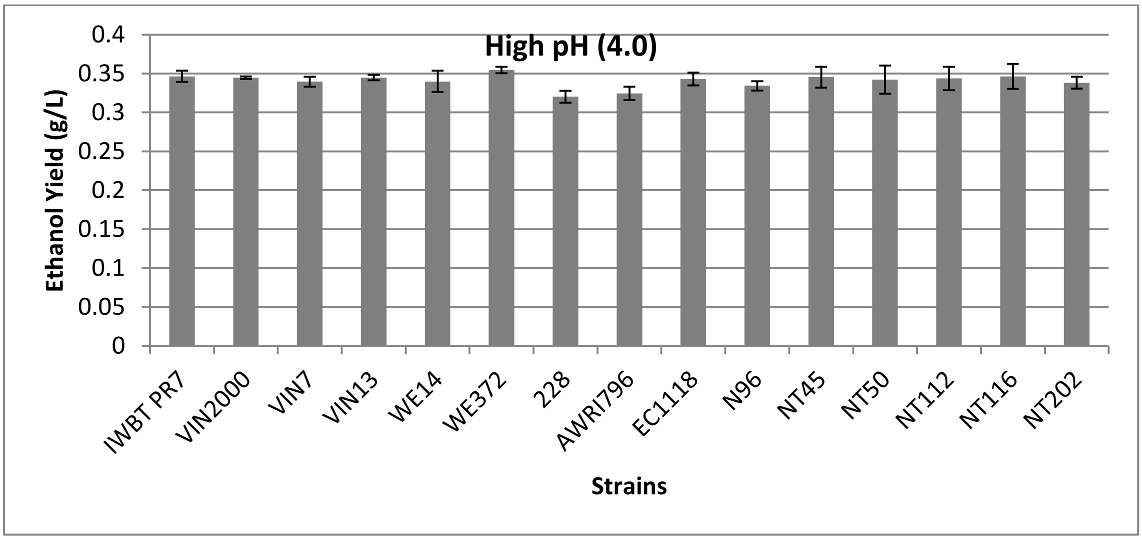

Figure 5. Ethanol yield of 15 industrial wine strains in high pH MS300 (pH 4.0) synthetic wine must fermented at 20°C.

3.3.1.4 Effect of sugar

When the initial sugar concentration was low (150 g/L), no statistically significant differences were observed (Figure 6; Table A6). However, when the initial sugar concentration was increased (300 g/L) NT202 did show a statistically significantly lower ethanol yield than IWBT PR7, VIN2000, VIN13, EC1118 and NT45. The ethanol yield of NT50 was also statistically significantly lower than four other strains (Figure 7; Table A7).

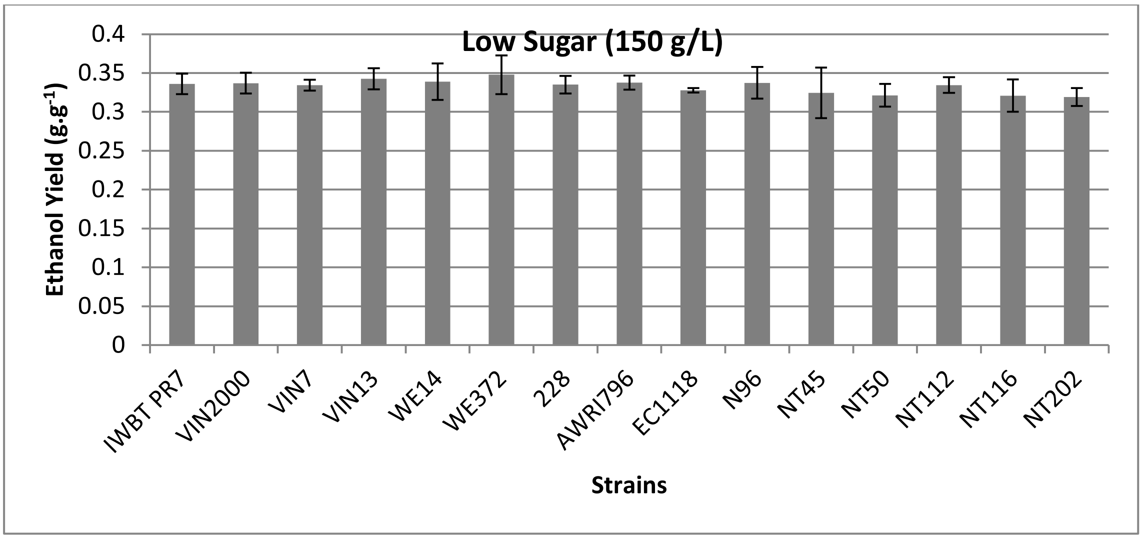

Figure 6. Ethanol yield of 15 industrial wine strains in low sugar MS300 (150 g/L) synthetic wine must fermented at 20°C.

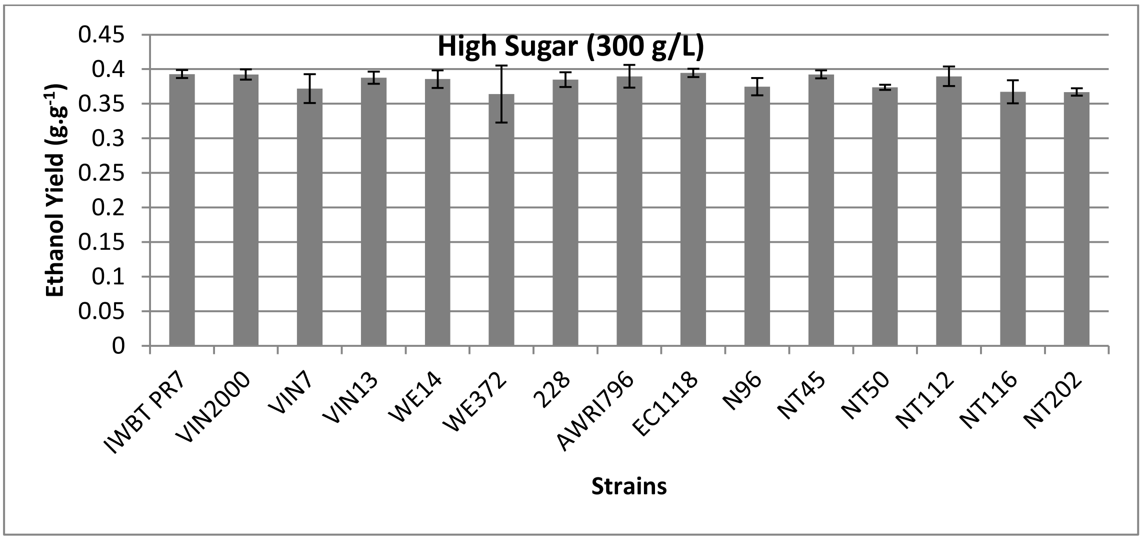

Figure 7. Ethanol yield of 15 industrial wine strains in high sugar MS300 (300 g/L) synthetic wine must fermented at 20°C.

3.3.2 Assessment of conditions that resulted in differences in ethanol yield

The two strains VIN13 and EC1118 showed fluctuations in ethanol yield under the different conditions of the single factorial experiments and it was therefore decided to further investigate ethanol yields under various combinations of fermentation settings using these two strains. Furthermore VIN13 and EC1118 have well annotated genomes and numerous transcriptomic and proteomic datasets available in public repositories thus making them a good choice in terms of correlating ethanol yield phenotypes to underlying molecular modalities. Thus the second objective of this study was to investigate the ethanol yield of the VIN13 and EC1118 strains in a more in-depth multi-factorial experimental design. The aim was to determine whether different must parameters could have a cumulative effect on ethanol yields.

Ethanol yield did not vary much under different wine must conditions. However, a combination of low nitrogen (150 mg/L) and low pH (3.0) resulted in a slightly lower ethanol yield for strain EC1118 (Figures 8 & 9). This was also true for VIN13 when fermented at 25°C but not at 20°C (Figures 10 & 11). However, upon further evaluation it was found that these differences were not statistically significant (Table A8). Overall no statistically significant trend was seen across both strains and all must composition settings (Table A8).

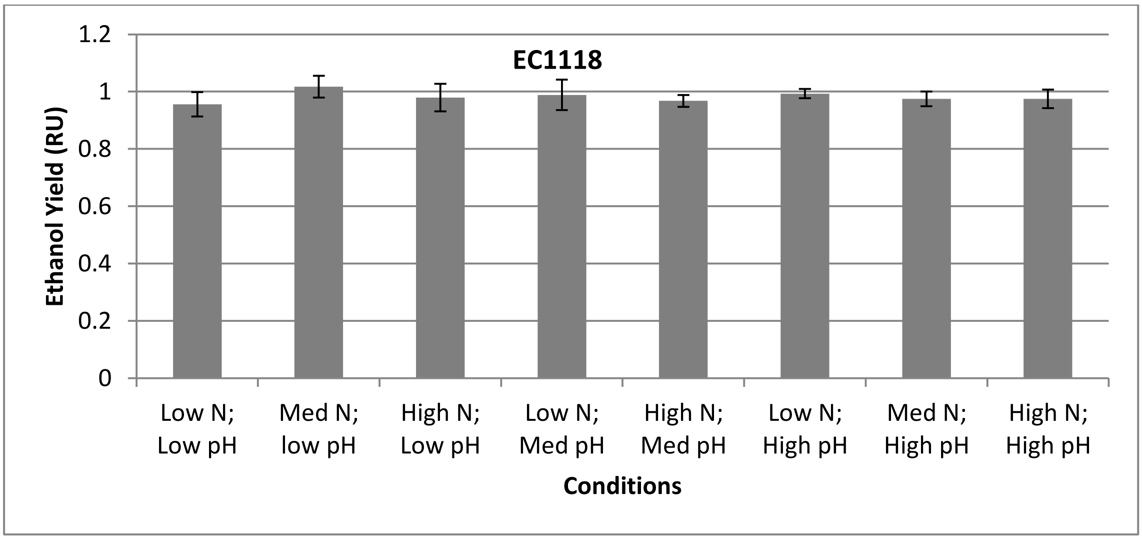

Figure 8. Ethanol yield of strain EC1118 under various pH and YAN concentrations. Fermentations were performed at 20°C. Ethanol yields were calculated relative to standard MS300 (which is set to 1). All values are the average of three repeats +/- standard deviation.

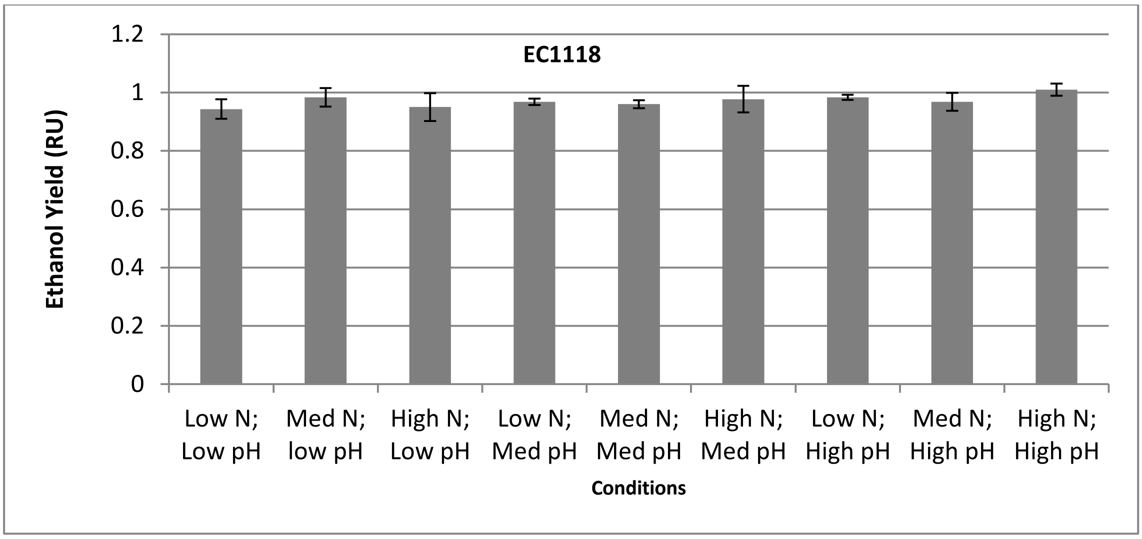

Figure 9. Ethanol yield of strain EC1118 under various pH and YAN concentrations. Fermentations were performed at 25°C. Ethanol yields were calculated relative to standard MS300 (which is set to 1). All values are the average of three repeats +/- standard deviation.

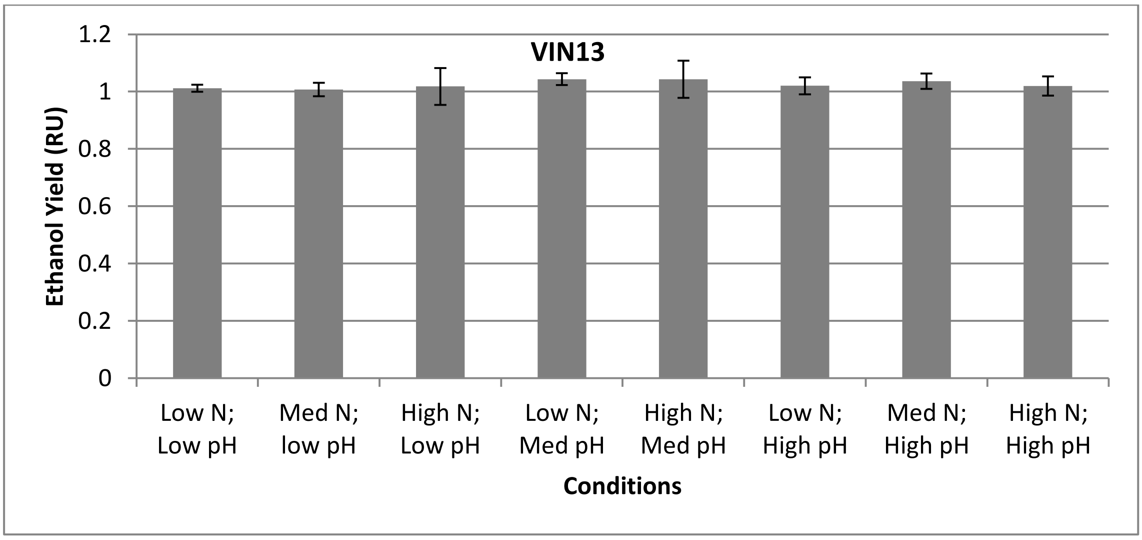

Figure 10. Ethanol yield of strain VIN13 under various pH and YAN concentrations. Fermentations were performed at 20°C. Ethanol yields were calculated relative to standard MS300 (which is set to 1). All values are the average of three repeats +/- standard deviation.

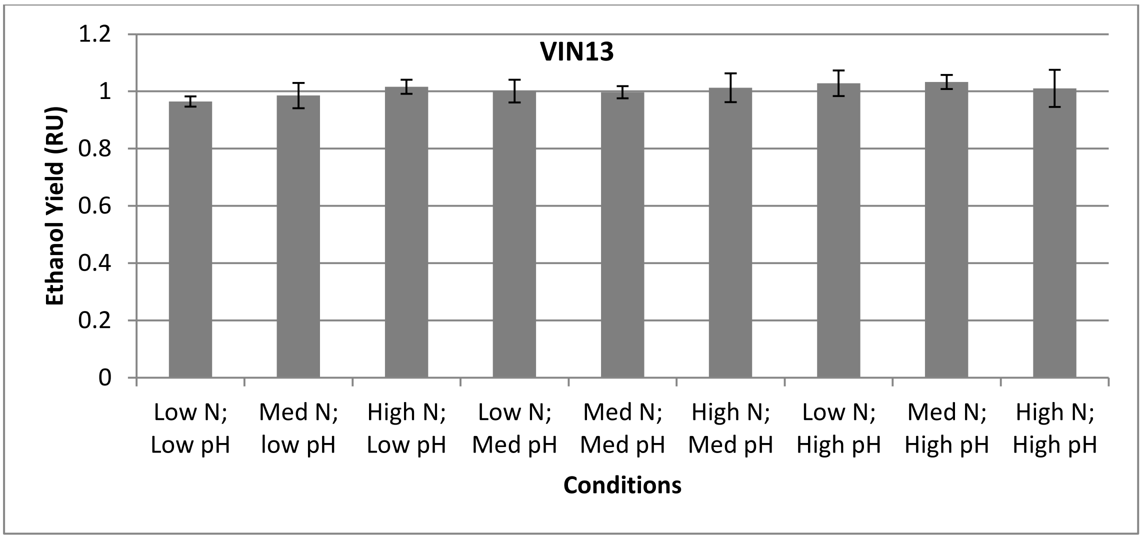

Figure 11. Ethanol yield of strain VIN13 under various pH and YAN concentrations. Fermentations were performed at 25°C. Ethanol yields were calculated relative to standard MS300 (which is set to 1). All values are the average of three repeats +/- standard deviation.

It is important to mention that many of the fermentations failed to go to dryness (<5 g/L). This was due to the fact that many of these conditions represented a relatively “harsh” environment for the yeast, particularly in combination with one another. This was particularly true for the low nitrogen (150 mg/L) and low pH (3.0) MS must (Figure 12). Many of the fermentations had to be stopped after 48 days, as they were struggling to reach dryness.

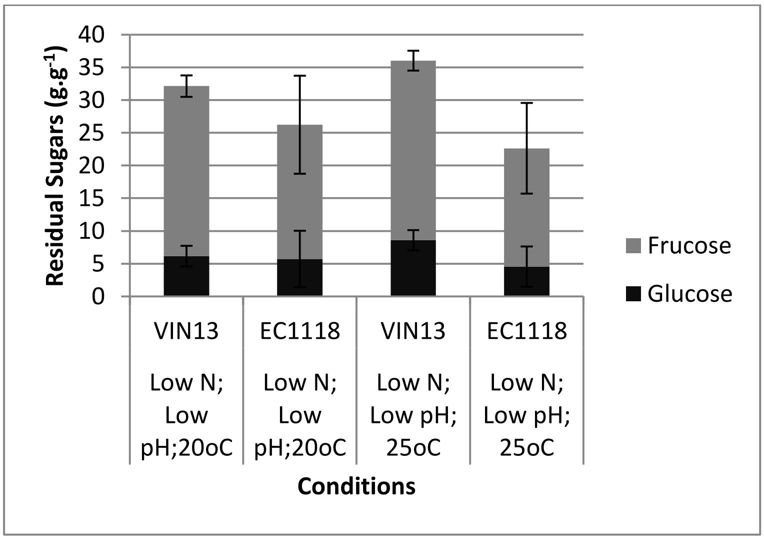

Figure 12. Glucose and fructose concentrations at the end of fermentation, for low Nitrogen (150 mg/L) and low pH (3.0) condition. All values are the average of three repeats +/- standard deviation.

3.3.3 Grape derived must

3.3.3.1 Ethanol yield in grape derived must

The third objective was to confirm and reassess the results from the synthetic must experiments in grape must using strains EC1118, VIN7, VIN13 and 228 in Sauvignon Blanc, Chardonnay, Shiraz and Cabernet Sauvignon wine musts. Since little difference in the ethanol yield was seen in the multi-factorial experiments (objective 2) it was decided to reproduce only the mono-factorial experimental designs (objective 1). However, only seven of the nine conditions could be adjusted in the grape must for practical reasons (sugar and nitrogen can only be supplemented, not removed from the must).

Both low and high (15°C and 25°C) temperature settings resulted in statistically significant higher ethanol yields compared to the control for EC1118 in the Chardonnay grape must (Figures 13; Table A9). VIN7 also had a significantly higher ethanol yield (for $p < 0.1$, Table A9) for high and low temperature settings in Chardonnay (Figure 14; Table A9). It thus seems that EC1118 and VIN7 behave similarly under high and low temperature settings. However, this trend is only evident for these specific conditions, and is not conserved for all four strains or cultivars. Interestingly the ethanol yield of strain 228 is almost identical (no statistically significant difference) when let to fermented at both the higher and lower temperature in Chardonnay (Figure 15; Table A9), and therefore does not behave the same way as EC1118 and VIN7. However, Strain 228 shows a statistically significant difference in ethanol yield when the pH is both lowered and increased (Figure 15; Table A9). When compared to the mono-factorial synthetic must data sets (objective one), strain 228 also showed a lower ethanol yield when fermented in a high pH must. To some degree this reaffirms the results obtained in the synthetic must experiments.

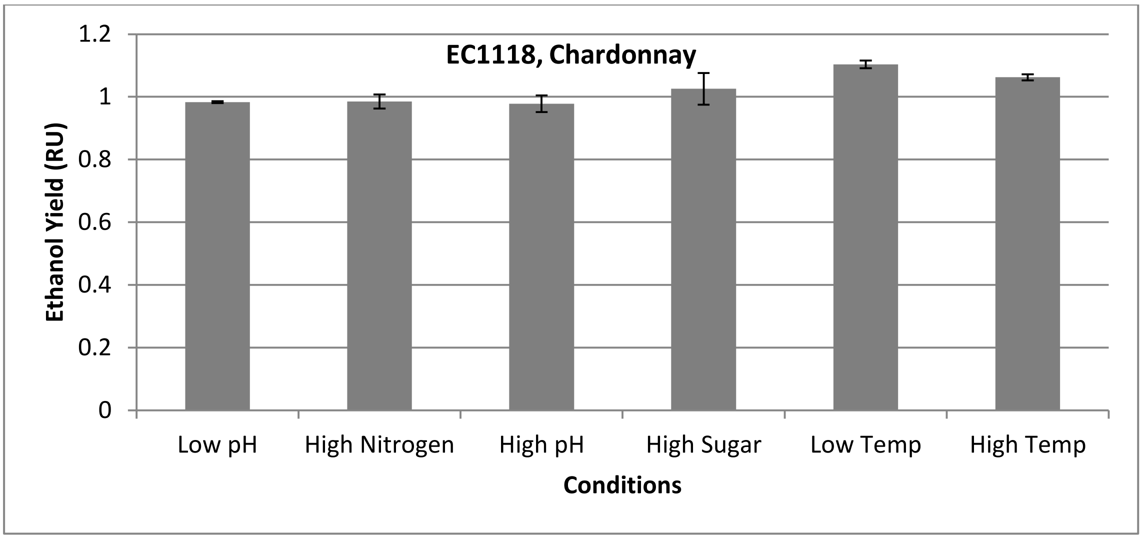

Figure 13. Ethanol Yield of EC1118 in Chardonnay grape must under six different conditions. Ethanol yields were calculated relative to unmodified grape must (which is set to 1). All values are the average of three repeats +/- standard deviation.

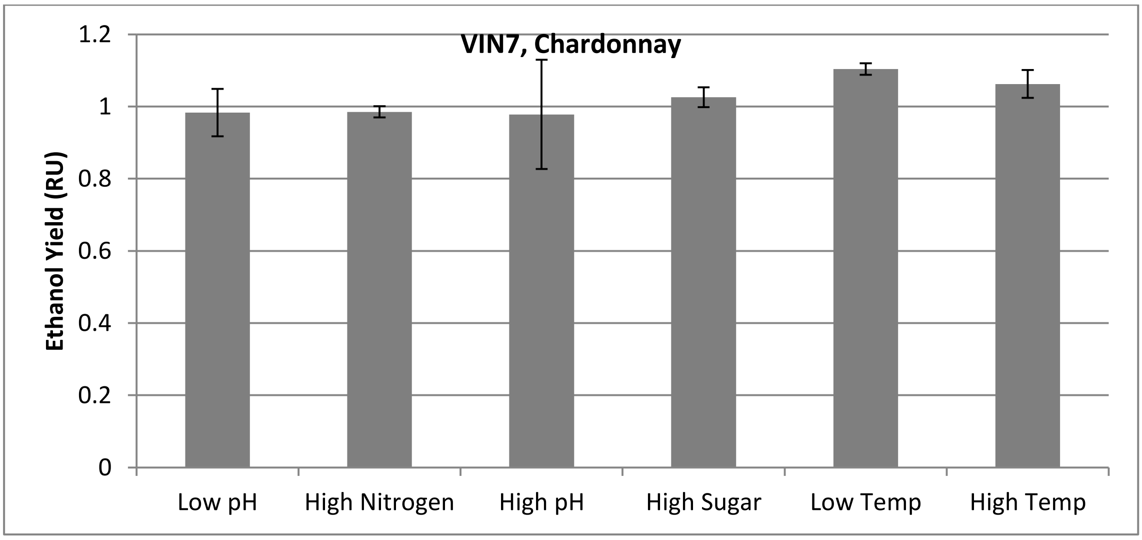

Figure 14. Ethanol yield of VIN7 in Chardonnay grape must under six different conditions. Ethanol yields were calculated relative to unmodified grape must (which is set to 1). All values are the average of three repeats +/- standard deviation.

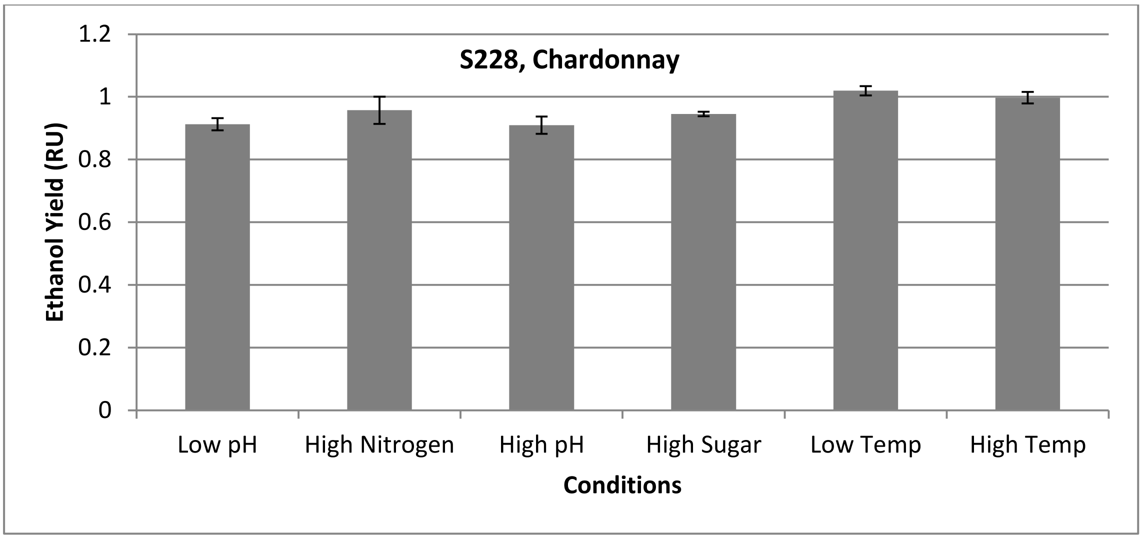

Figure 15. Ethanol yield of S228 in Chardonnay grape must under six different conditions. Ethanol yields were calculated relative to unmodified grape must (which is set to 1). All values are the average of three repeats +/- standard deviation.

Yeast strains were also found to behave differently in musts from different cultivars. When EC1118 was used to ferment Shiraz must the ethanol yield was statistically significantly lowered when temperature was decreased and increased (for $p < 0.1$, Table A9) showing an opposite trend compared to the Chardonnay data (Figures 13, 16 & A9).

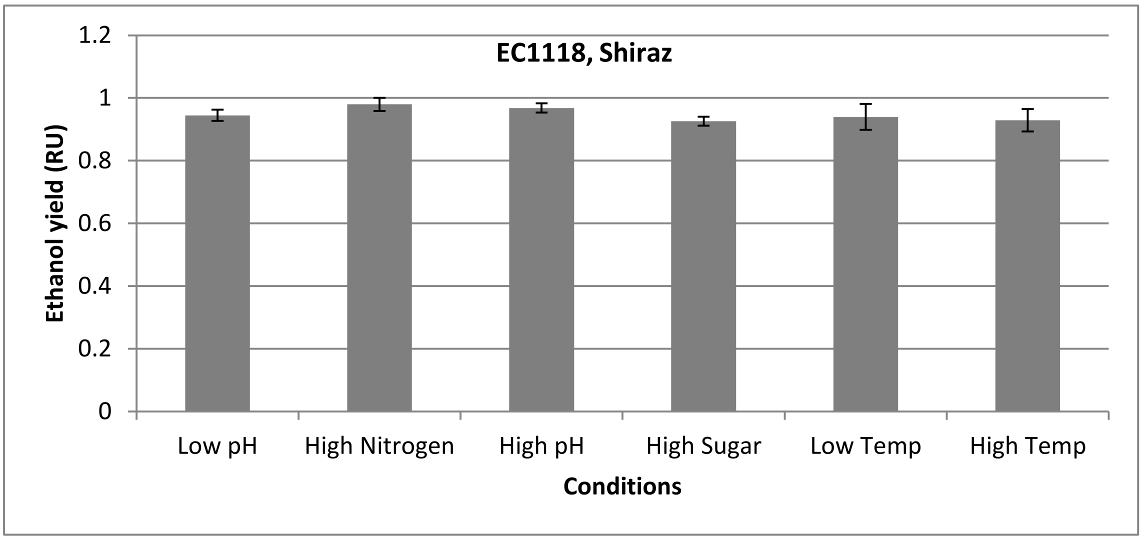

Figure 16. Ethanol yield of EC1118 in Shiraz grape must under six different conditions. Ethanol yields were calculated relative to unmodified grape must (which is set to 1). All values are the average of three repeats +/- standard deviation.

Increasing the sugar concentration of the grape must to 300 g/L decreased the ethanol yield in most cultivars and strains used. However, this might be due to the fact that these fermentations were unable to proceed to dryness.

Interestingly when the ethanol yield data were compared across all cultivars, all fermentations conducted in the Shiraz must showed a slightly elevated ethanol yield. This difference is very clear when strains EC1118, VIN7 and VIN13 were used (Figure 18, 19 & 20), furthermore these differences were all statistically significant (Table A10). However, when strain 228 was used the increased ethanol yield for the Shiraz fermentations were statistically significant compared to the Sauvignon Blanc and Cabernet Sauvignon fermentations but not for Chardonnay (Figure 17; Table A10).

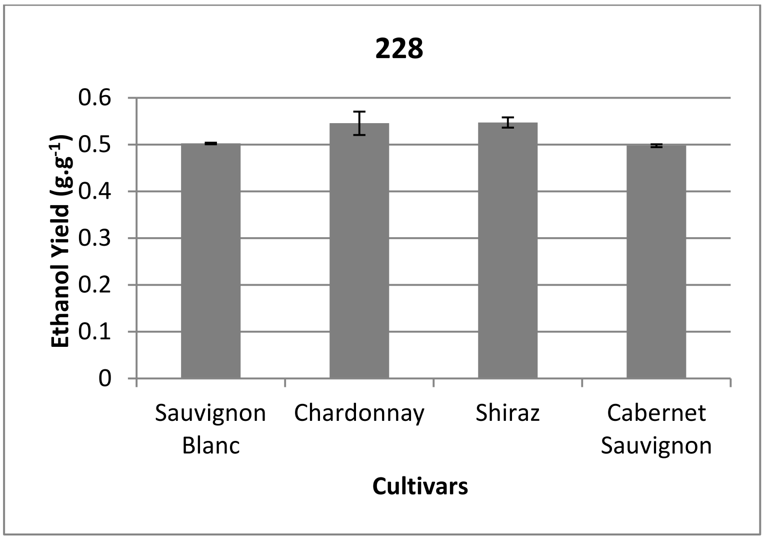

Figure 17. Ethanol yield of 228 in various grape cultivars. All values are the average of three repeats +/- standard deviation.

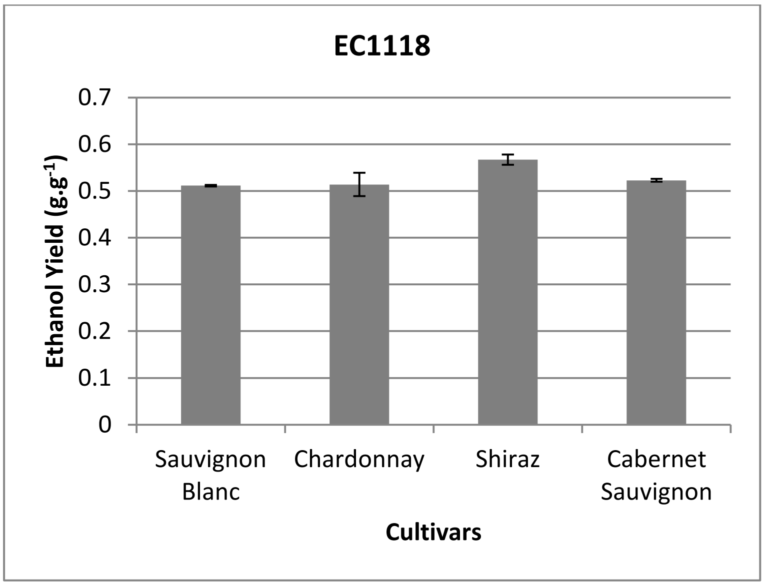

Figure 18. Ethanol yield of EC1118 in various grape cultivars. All values are the average of three repeats +/- standard deviation.

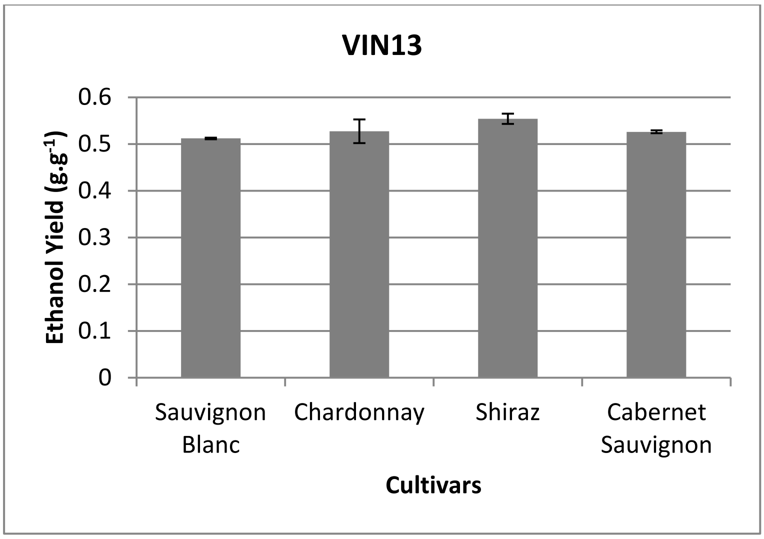

Figure 19. Ethanol yield of VIN13 in various grape cultivars. All values are the average of three repeats +/- standard deviation.

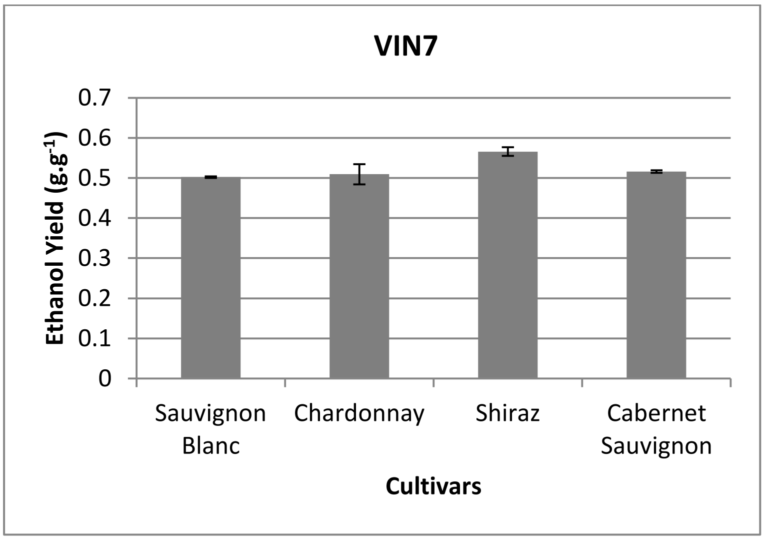

Figure 20. Ethanol yield of VIN7 in various grape cultivars. All values are the average of three repeats +/- standard deviation.

Overall, it should be noted that these differences in ethanol yield are very specific for each cultivar, strain and condition. No consistent trend was seen for all the four strains, or the seven conditions tested.

3.3.3.2 Glycerol yield in grape derived must

Glycerol yield seemed to be more condition dependent than ethanol yield. Both white cultivars, namely Sauvignon Blanc and Chardonnay, showed a consistent and statistically significant (for $p < 0.1$, Table A11) 1.2 fold increase in glycerol yield at higher fermenting temperatures of 25° C compared to 20 ° C (Figure 21 & 22; Table A11). This was not the case for red cultivars (Figure 23 & 24) which only showed a slight yet statistically significant increase in glycerol yield for VIN7 in Shiraz, but not when fermented in Cabernet Sauvignon (Table A11).

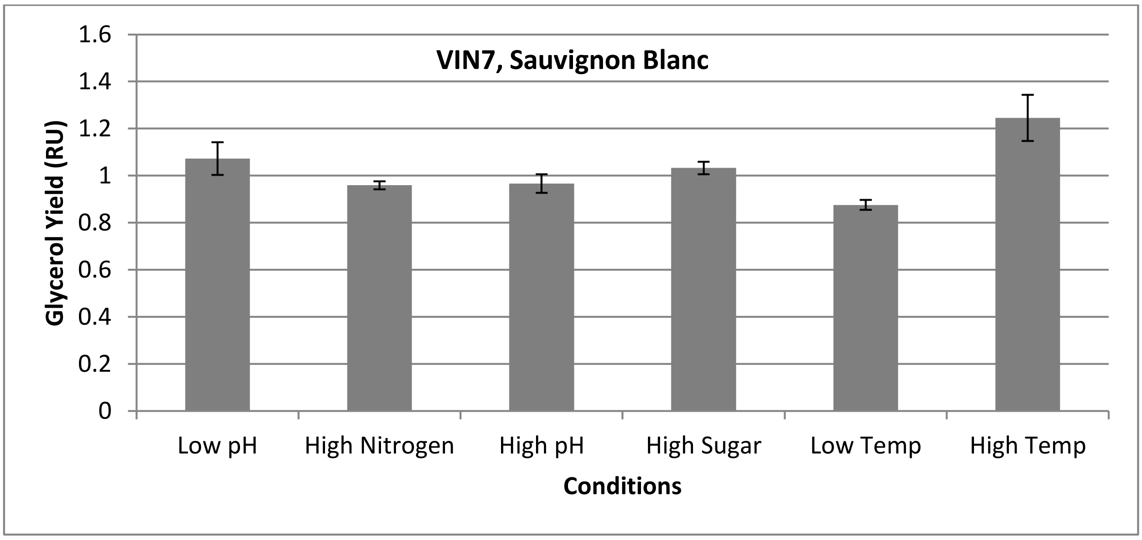

Figure 21. Glycerol yield of VIN7 in Sauvignon Blanc grape must in six different fermentation conditions. Glycerol yields were calculated relative to unmodified grape must (which is set to 1). All values are the average of three repeats +/- standard deviation.

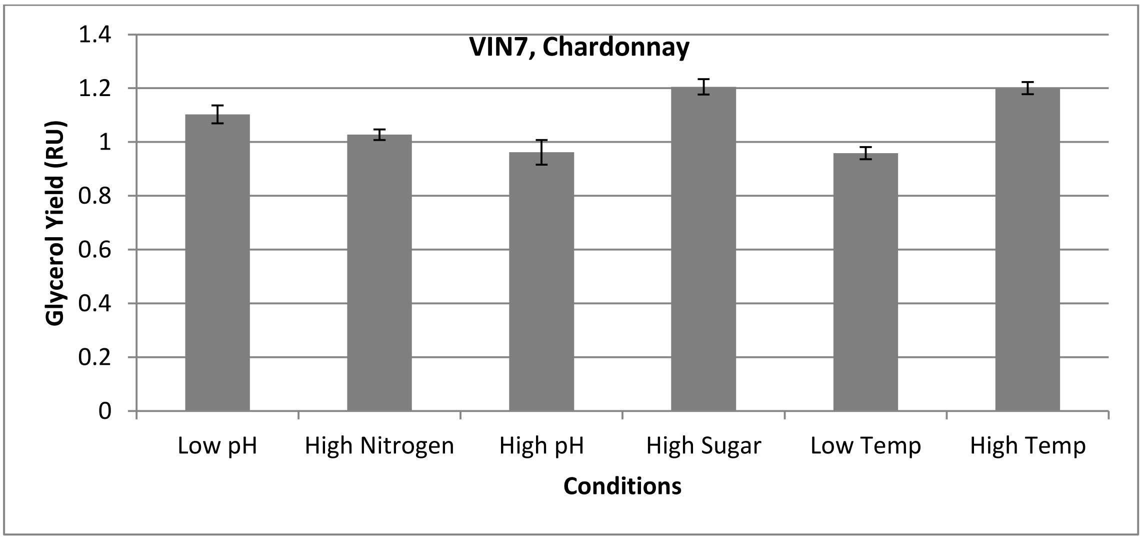

Figure 22. Glycerol yield of VIN7 in Chardonnay grape must in six different fermentation conditions. Glycerol yields were calculated relative to unmodified grape must (which is set to 1). All values are the average of three repeats +/- standard deviation.

Interestingly when VIN7 was used at a low fermenting temperature setting there was a statistically significant decrease in glycerol yield for all cultivars (Table A11). Only a small effect on glycerol production in the Chardonnay fermentations was seen (Figure 22). However, for the other three cultivars, namely Sauvignon Blanc, Shiraz and Cabernet Sauvignon a 0.8 fold decrease in glycerol yield was observed (Figures 21, 23 & 24). From these results it is clear that fermenting temperature has a statistically significant effect on glycerol production, which may also be cultivar or must specific.

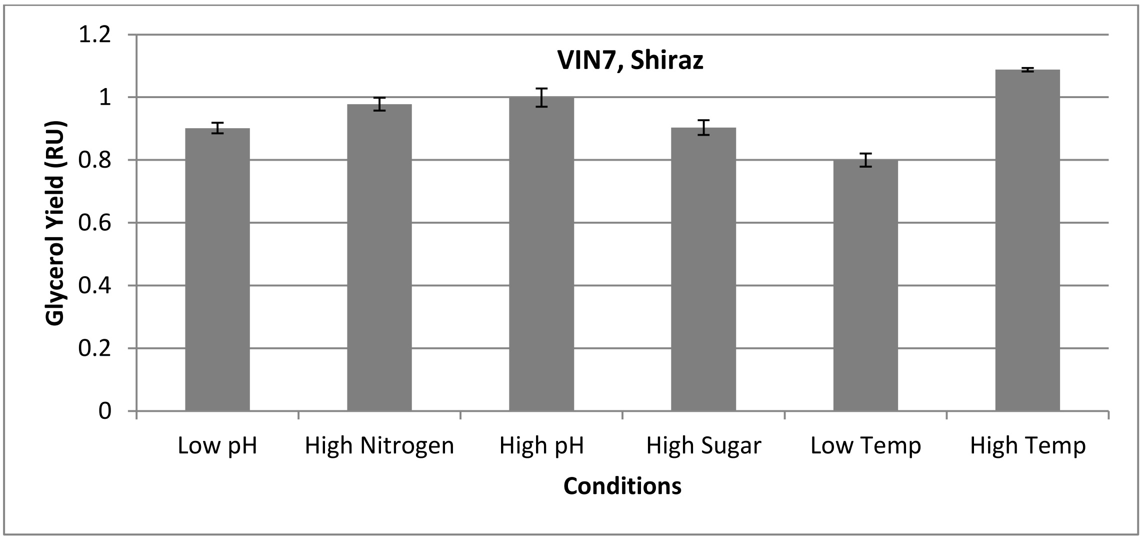

Figure 23. Glycerol yield of VIN7 in Shiraz grape must in six different fermentation conditions. Glycerol yields were calculated relative to unmodified grape must (which is set to 1). All values are the average of three repeats +/- standard deviation.

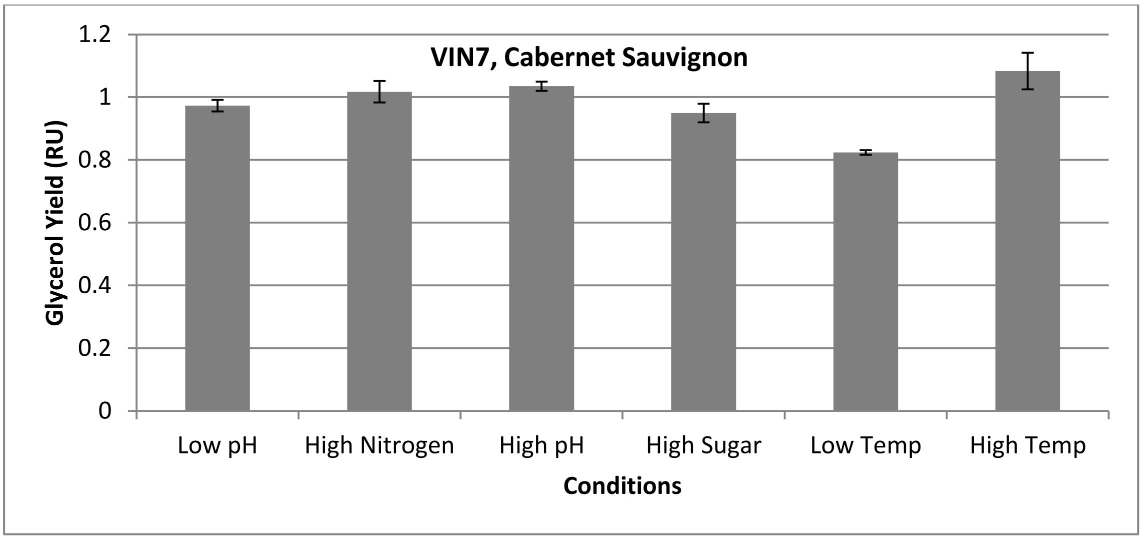

Figure 24. Glycerol yield of VIN7 in Cabernet Sauvignon grape must in six different fermentation conditions. Glycerol yields were calculated relative to unmodified grape must (which is set to 1). All values are the average of three repeats +/- standard deviation.

Another factor that increased glycerol production was increased sugar concentrations (300 g/L). Glycerol yield was statistically significantly increased in Chardonnay for all four yeast strains, namely 228, EC1118, VIN7 and VIN13 (Table A11). However, these results for the high sugar fermentations should be interpreted with caution since these fermentations were not able to reach dryness.

It is however clear that glycerol production is temperature dependent, and that increasing sugar concentrations could have an effect on glycerol production.

3.3.4 Aroma production

The fourth objective investigated the effect of wine pH on aroma compound production in two wine yeast strains, namely EC1118 and VIN7. Synthetic musts at various pH's (3.0, 3.4 and 4.0) were fermented to dryness after which samples were taken for analysis of volatile alcohols and esters.

Ethyl acetate concentrations did not show much variation between strains in medium and low pH musts (Figures 25 & 26). However, when both strains fermented in high pH must the difference between strains was close to 10 mg/L (Figure 27).

Interestingly VIN7 produced 3-ethoxy-1-propanol at a lower concentration than EC1118 at all three fermentation pH's. This shows that 3-ethoxy-1-propanol production is not only condition dependent but largely strain dependent.

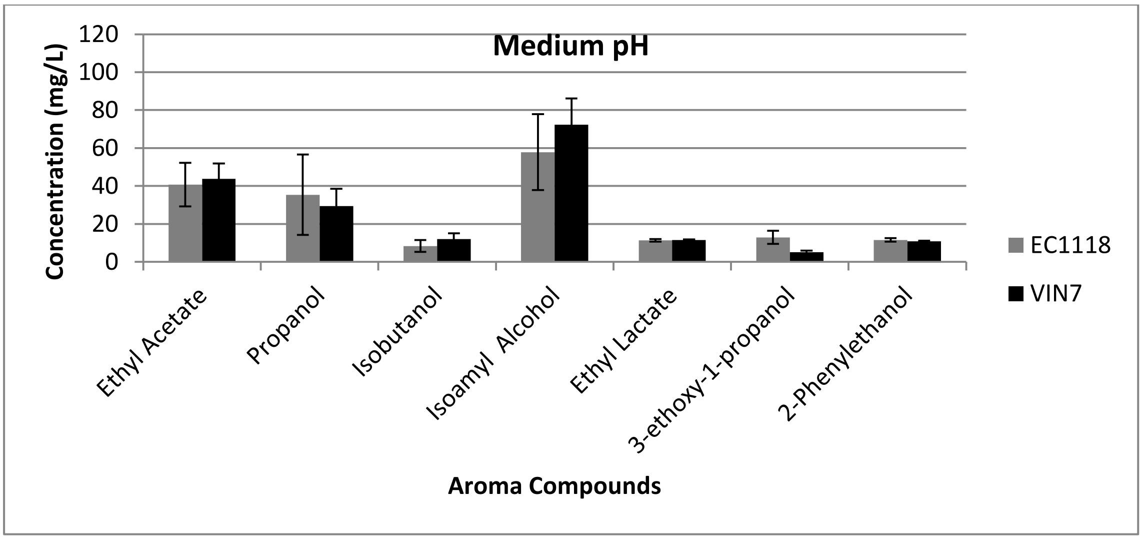

Figure 25. Seven of the 39 aroma compounds quantified on the GC-FID in synthetic wine at pH 3.4. All values are the average of three repeats +/- standard deviation.

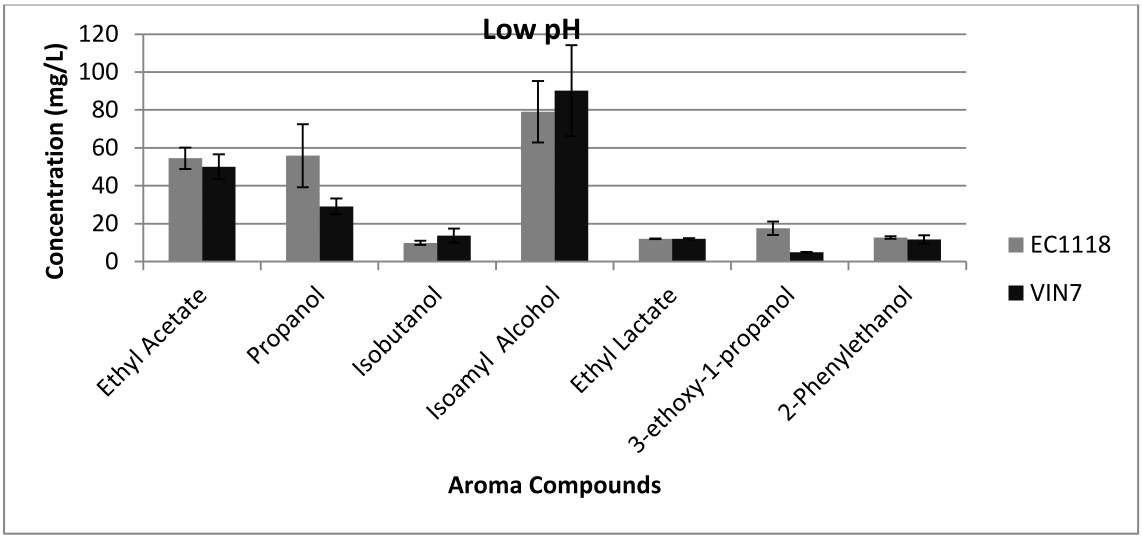

Figure 26. Seven of the 39 aroma compounds quantified on the GC-FID in synthetic wine at pH 3.0. All values are the average of three repeats +/- standard deviation.

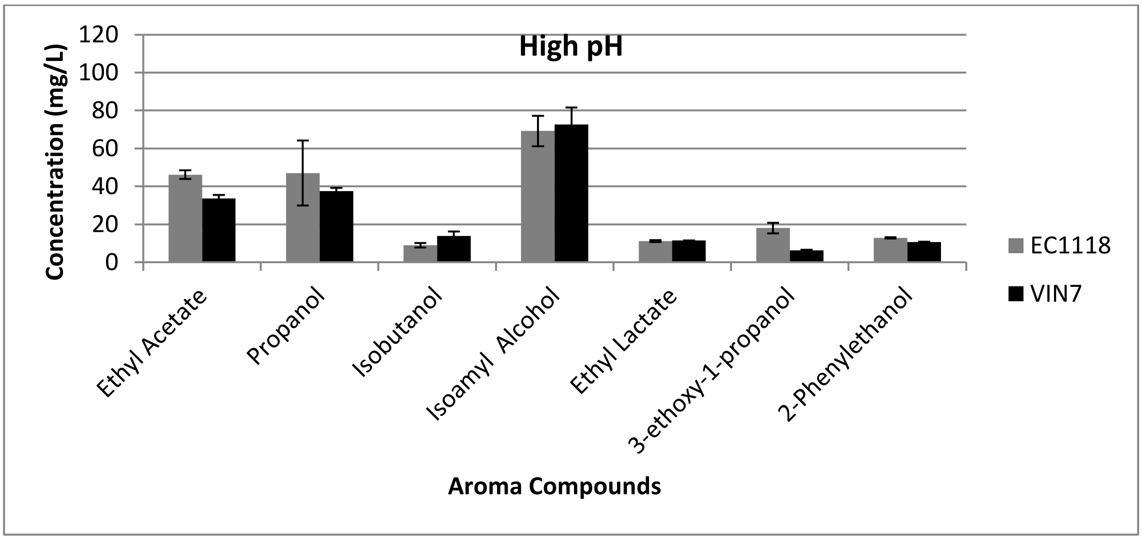

Figure 27. Seven of the 39 aroma compounds quantified on the GC-FID in synthetic wine at pH 4.0. All values are the average of three repeats +/- standard deviation.

3.4 Discussion

3.4.1 Ethanol yield of various strains in different wine must conditions

The first objective of this study investigated the ethanol yield of various stains under different fermentative conditions. A total of 15 strains were inoculated and let to ferment in standard MS300 synthetic must: NT45 showed lower ethanol yield while strain VIN7 showed a higher ethanol yield when compared to several other strains in the same must. This indicates that there is a statistically significant difference in ethanol yield depending on the genetic background of the strain used. When the same 15 strains fermented both low and high YAN MS must, VIN7 showed again a higher ethanol yield. VIN7 remained a higher ethanol yielding strain when it was fermented a high YAN MS must. This showed that VIN7 is a high ethanol yielding strain in a “normal” synthetic grape must, and this characteristic is retained when the YAN concentration of the must is increased or decreased. Interestingly EC1118 was a high ethanol yielding strain in must with increased YAN and became a lower ethanol yielding strain in a low YAN MS must. This shows that both strains (VIN7 and EC1118) behave differently depending on the YAN concentration of the must. The reasons for this might be twofold, firstly VIN7 is a *S. kudriavzevii* and *S. cerevisiae* hybrid, hence their difference in ethanol yield behaviour might be due to their genotypic differences. Secondly many of the fermentations conducted in low YAN must were not able to ferment to dryness (<5 g/L). This could have an effect on the ethanol yield as ethanol yields across different phases of sugar consumption are being compared with one another, which may introduce bias in the results.

When the pH of the MS300 was lowered to 3.0 smaller ethanol yield differences were observed between strains. Only two strains showed a difference in ethanol yield namely IWBT PR7 being statistically significant higher than nine other strains, and NT202 being statistically lower than five other strains. While the other 13 strains did not show statistically significant differences, this lack of difference in ethanol yields between strains might be due to the fact that ordinary MS300 already has a low pH thus a decrement of 0.4 might not have a significant effect on the yeast physiology and/or stress response. A similar response is seen when the pH was increased to 4.0: Only strains WE372 and 228 showed a slight yet statistically significant decrease in ethanol yield when compared to several other strains fermented in the same must. Increasing or decreasing the pH even further might result in a difference in ethanol yield, however, these pH values are no longer representative of the pH of grape must.

When the initial sugar concentration was varied, little to no difference was seen between strains. It is also important to note that the high sugar fermentations did not reach dryness (<5 g/L). The reason for this may be two-fold: the ethanol concentration could increase to toxic levels, due to the high initial sugar levels, or the yeast could be compromised due to the increased osmotic pressure resulting from high sugar concentration. Therefore it cannot be said with certainty that high initial sugar concentrations did not induce a change in ethanol yield between strains.

3.4.2 Low ethanol yielding wine must conditions

The second objective was to investigate the strains EC1118 and VIN13 with regard to ethanol yields under various combinations of fermentation conditions in a more in-depth multifactorial experimental design.

Overall, no statistically significant difference was observed between the conditions tested in objective two. EC1118 showed a slight decrease in ethanol yield when fermented in MS must containing a low YAN concentration and a low pH. This was also true for VIN13 when fermented at a higher temperature of 25^{o}C. It was concluded that wine must composition could have an effect on the ethanol yield. However, this effect is moderate and not consistently statistically relevant.

Many of these fermentations did not reach dryness, possibly due to the fact that these conditions represented a “harsh” environment for the yeast. This is especially true for the low nitrogen and low pH MS must, therefore it cannot be concluded with complete certainty that this combination of conditions would reliably yield a lower ethanol concentration.

Further studies should be done in terms of testing more conditions and including additional level settings (i.e. more and smaller increments in the different experimental factors). Another reason that might explain why no consistent trend was seen in the multivariate design is that carbon metabolism is central to energy generation in yeast, thus any small changes could result in cell death. Therefore it would be very hard to induce a substantial change to this pathway as it is the main means for redox balancing to ensure continued energy generation for cellular metabolism and biomass formation.

3.4.3 Grape derived must

3.4.3.1 Ethanol yield in grape derived must

The third objective was to confirm some of the results from the synthetic must experiments in grape derived must. Strains EC1118, VIN7, VIN13 and 228 were used to ferment Chardonnay, Sauvignon Blanc, Shiraz and Cabernet Sauvignon grape must. The pH, nitrogen, sugar and fermenting temperature were modified.

No predictable differences were seen. For example, EC1118 and VIN7 showed a slightly higher ethanol yield when fermented at lower and higher temperature in Chardonnay. This was not observed in any of the other cultivars or strains. Strain 228 showed a decrease in ethanol yield when pH was either increased or decreased, which was also not seen in the other cultivars or strains. This to some extent confirms what was seen in the synthetic must (objective 1), where ethanol yield was decreased when pH was increased. However, these findings were observed only in Chardonnay fermentations and not in the other three cultivars.

Strains also behaved differently in musts from different cultivars. EC1118 yielded less ethanol in Shiraz, when temperature was increased an opposite effect compared to Chardonnay, where ethanol yield was increased. This indicates that the cultivar used is also having an effect on the ethanol yield.

Further investigation showed strains 228, EC1118, VIN7 and VIN13 has an increased ethanol yield when used to ferment unmodified Shiraz must, indicating that the increase is independent of strain used. Further studies will have to be done in order to determine what the reason for this trend might be, repeats across vintages and vineyards will have to be carried out.

Increasing the initial sugar concentration decreased the ethanol yield in all four strains and all four cultivars. However, all failed to reach dryness. This could be attributed to an increase in osmotic stress due to the extra sugar present, which could in turn lead to an increased glycerol production (and lowered flux to ethanol) as the yeast are producing more glycerol to compensate for the increase in osmotic pressure.

3.4.3.2 Glycerol yield in grape derived must

Glycerol yield appeared to be more condition dependent than ethanol yield based on the results from our experiments. When the fermentation temperature was increased a consistent increase in glycerol yield was observed (1.2 fold increase in white cultivars). The temperature dependence of glycerol production has been reported previously (Rankine and Bridson 1971, Torija et al. 2002, Yalcin and Ozbas 2008). The red cultivars also showed an increase in glycerol yield, but not to the same extent as the white cultivars. When temperature was decreased a consistent 0.8 fold decrease in glycerol yield was observed in Sauvignon Blanc, Shiraz and Cabernet Sauvignon must, while the Chardonnay must only showed a slight decrease.

When sugar levels were increased in Chardonnay a 1.2 fold increase in glycerol yield was observed with all four strains. However, there was no correlation between the increase in glycerol and decrease in ethanol yield in Chardonnay. This shows that while glycerol might be slightly increased, not enough carbon is diverted away from ethanol to have a measurable effect on the final ethanol concentration.

3.4.4 Aroma production

The fourth objective investigated the aroma production of EC1118 and VIN7 under various wine must conditions. Most aroma compounds were produced in similar quantities. However, inter-strain differences were evident for certain volatiles such as ethyl acetate depending on the initial pH of the must.

Interestingly there is a strain difference between EC1118 and VIN7 in 3-ethoxy-1-propanol production. A similar concentration in each strain is seen under various pH’s. This shows that 3-ethoxy-1-propanol production is strain dependent and not condition dependent, with EC1118 always producing less. The odor activity value (OAV) value for 3-ethoxy-1-propanol (which imparts a fruity aroma) is 0.10 mg/L, thus all the values obtained were above this threshold. Hence inoculation of EC1118 as opposed to VIN7 (regardless of pH, and possible other environmental conditions as well) may result in a wine with a more fruity aroma.

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Chapter 4

General discussion and conclusions

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This is the first systematic study evaluating the impact of individual and combined variations in several environmental parameters on the yield of the major products of alcoholic fermentation in wine fermentative conditions. The parameters evaluated, yeast strain selection, pH, fermenting temperature, initial sugar concentration, yeast assimilable nitrogen and cultivar were selected because they are at least amenable to control by the wine maker.

Ethanol yields of the 15 strains in standard synthetic grape must showed some variation between one another. It was further found that many strains behave differently under different must conditions, with EC1118, 228, VIN7 and NT45 showing the highest fluctuations in ethanol yield. However, these differences were unpredictable and no overall trend was seen. Further investigations showed that these conditions did not have a cumulative effect on EC1118 and VIN13 in multivariate experiments.

Interestingly VIN7 and EC1118 behaved differently depending on the YAN concentration of the must. VIN7 is a *S. kudriavzevii* and *S. cerevisiae* hybrid while EC1118 is a *S. cerevisiae bayanus* strain, hence their difference in ethanol yield behaviour might be due to their genotypic differences. This poses an interesting question regarding the use of other non-*cerevisiae* species to reduce ethanol yield. Further research would have to be done to identify low ethanol yielding non-*cerevisiae* species and then potentially breeding them with *S. cerevisiae*.

In the grape must experiments it was observed that temperature and pH can have an effect on the ethanol yield when strains EC1118, VIN7, VIN13 and 228 were used. However, these differences are specific and no real trend was seen across conditions or strains used. Interestingly Shiraz grape must did show a slightly higher ethanol yield regardless of strain used. It is likely that the 2013 Shiraz must used in our experiments may have some unknown, or presently undefined 'factor' or combination of factors, which is responsible for this observation. However, further research will have to be conducted to determine what the responsible factor/s is/are.

Glycerol yield seemed to be more condition dependent, especially when temperature was varied which confirms previous findings (Rankine and Bridson 1971, Torija et al. 2002, Yalcin and Ozbas 2008). No correlation between an increase in glycerol and a decrease in ethanol was seen. Hence, while temperature does increase the glycerol yield, not enough carbon is directed towards this end product to significantly reduce ethanol yields.

While an ethanol yield difference can be induced by varying must composition or fermenting temperature, no overall predictable trend was observed in the 15 strain screened. It would therefore not be a viable option to winemakers in lowering the ethanol of wine. These strains used are all very diverse, and some are hybrid strains. This diversity could be the reason that a change in ethanol yield is so specific, and that no general response is seen across strains. The fact that VIN7 and EC1118 behave differently depending on the YAN further substantiates this argument.

Other methods will have to explored to find a microbial solution to the current ethanol problem faced by the wine industry. For example, the utilization of non-*Saccharomyces* yeasts is an option that should be further investigated. Species like *H. uvarum* and *C. stellate* have already been shown to yield less ethanol than *S. cerevisiae* (Ciani and Picciotti 1995, Ciani and Maccarelli 1997). A combination (co- or sequential inoculation) of various non-*Saccharomyces* and *Saccharomyces* species might yield a lower ethanol wine with no negative impact, and potentially even a positive impact, on sensorial wine quality (Tora and Vazquez 2002). Contreras et al (2013) has already shown that sequential inoculation could be a viable option. Hybridizing *S. cerevisiae* with other low ethanol yielding non-*Saccharomyces* species, in an attempt to develop a strain which shares both the low ethanol yielding and low off flavor production characteristics of the two parental species is another option. Significant genetic diversity exists between different strains of non-*Saccharomyces* species, thus studies to date which have concluded that certain species are unsuitable for winemaking may have incorrectly extended the findings based on a small selection of strains to cover the entire species. Suitability for wine fermentation, as well as ethanol yield characteristics should be evaluated on a strain by strain basis, thus considerable work remains to be done in this field.

Another method that could potentially produce a low ethanol yielding yeast strain is directed evolution. Selective pressures other than ethanol will have to be used, for example osmotic stress. Glycerol is produced by yeast to counteract osmotic pressure, and strains that produce more glycerol may be selected for.

Our data suggest that strains can be induced to yield different amount of ethanol by changing certain conditions. However, these changes are inconsistent, and as such would not be a viable option to reduce ethanol in wine at a commercial level.

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APPENDIX

Table A1. Comparative P-values of 15 strains ethanol yields in MS300 synthetic must.

	IWBT PR7	VIN2000	VIN7	VIN13	WE14	WE372	S228	AWRI796	EC1118	N96	NT45	NT50	NT112	NT116	NT202
IWBT PR7	1.000000	0.095206	0.230061	0.442115	0.233728	0.294031	0.947587	0.105901	0.056858	0.467637	0.004095	0.329137	0.053153	0.217636	0.208186
VIN2000	0.095206	1.000000	0.028737	0.050800	0.249580	0.624309	0.097059	0.595449	0.209878	0.944598	0.082485	0.999750	0.287625	0.956594	0.577978
VIN7	0.230061	0.028737	1.000000	0.681897	0.037426	0.191132	0.270833	0.023432	0.031397	0.297034	0.001718	0.161583	0.025331	0.084085	0.119072
VIN13	0.442115	0.050800	0.681897	1.000000	0.091671	0.224032	0.493385	0.050532	0.040256	0.351288	0.003487	0.212697	0.035211	0.124338	0.147299
WE14	0.233728	0.249580	0.037426	0.091671	1.000000	0.411957	0.237624	0.365188	0.090947	0.655712	0.004196	0.563790	0.094839	0.458327	0.324182
WE372	0.294031	0.624309	0.191132	0.224032	0.411957	1.000000	0.289037	0.517023	0.741409	0.743598	0.918584	0.661551	0.945171	0.622999	0.959883
S228	0.947587	0.097059	0.270833	0.493385	0.237624	0.289037	1.000000	0.111050	0.056508	0.458867	0.004949	0.321391	0.053281	0.213477	0.204375
AWRI796	0.105901	0.595449	0.023432	0.050532	0.365188	0.517023	0.111050	1.000000	0.138105	0.807356	0.016075	0.790409	0.166061	0.757376	0.444015
EC1118	0.056858	0.209878	0.031397	0.040256	0.090947	0.741409	0.056508	0.138105	1.000000	0.462141	0.644434	0.313016	0.677519	0.241093	0.643325
N96	0.467637	0.944598	0.297034	0.351288	0.655712	0.743598	0.458867	0.807356	0.462141	1.000000	0.562087	0.951362	0.614716	0.926837	0.747696
NT45	0.004095	0.082485	0.001718	0.003487	0.004196	0.918584	0.004949	0.016075	0.644434	0.562087	1.000000	0.334654	0.952022	0.184804	0.828186
NT50	0.329137	0.999750	0.161583	0.212697	0.563790	0.661551	0.321391	0.790409	0.313016	0.951362	0.334654	1.000000	0.441418	0.971017	0.640832
NT112	0.053153	0.287625	0.025331	0.035211	0.094839	0.945171	0.053281	0.166061	0.677519	0.614716	0.952022	0.441418	1.000000	0.338584	0.874429
NT116	0.217636	0.956594	0.084085	0.124338	0.458327	0.622999	0.213477	0.757376	0.241093	0.926837	0.184804	0.971017	0.338584	1.000000	0.584749
NT202	0.208186	0.577978	0.119072	0.147299	0.324182	0.959883	0.204375	0.444015	0.643325	0.747696	0.828186	0.640832	0.874429	0.584749	1.000000

Table A2. Comparative P-values of 15 strains ethanol yields in low nitrogen (120 mg/L) MS synthetic must.

	IWBT PR7	**VIN2000**	**VIN7**	**VIN13**	**WE14**	**WE372**	**S228**	**AWRI796**	**EC1118**	**N96**	**NT45**	**NT50**	**NT112**	**NT116**	**NT202**
IWBT PR7	1.000000	0.207512	0.157803	0.030536	0.162142	0.050293	0.017550	0.011936	0.010730	0.132412	0.056400	0.235450	0.012678	0.042026	0.067226
VIN2000	0.207512	1.000000	0.401491	0.043211	0.506253	0.004826	0.011640	0.007858	0.008965	0.339808	0.096509	0.558208	0.007201	0.057157	0.122543
VIN7	0.157803	0.401491	1.000000	0.050929	0.764982	0.007296	0.014538	0.009427	0.010460	0.535116	0.118713	0.655009	0.008763	0.073470	0.153994
VIN13	0.030536	0.043211	0.050929	1.000000	0.068965	0.115410	0.665775	0.948429	0.622195	0.079796	0.498315	0.198541	0.893452	0.401798	0.368592
WE14	0.162142	0.506253	0.764982	0.068965	1.000000	0.186528	0.032200	0.018656	0.017052	0.804588	0.164441	0.749219	0.019600	0.122154	0.217037
WE372	0.050293	0.004826	0.007296	0.115410	0.186528	1.000000	0.051319	0.025894	0.023724	0.285938	0.319698	0.832122	0.026855	0.263027	0.446447
S228	0.017550	0.011640	0.014538	0.665775	0.032200	0.051319	1.000000	0.490378	0.263652	0.039785	0.674824	0.222010	0.653068	0.521534	0.468681
AWRI796	0.011936	0.007858	0.009427	0.948429	0.018656	0.025894	0.490378	1.000000	0.574130	0.022241	0.372762	0.129783	0.777965	0.250428	0.243697
EC1118	0.010730	0.008965	0.010460	0.622195	0.017052	0.023724	0.263652	0.574130	1.000000	0.019727	0.226435	0.089108	0.418891	0.149044	0.150440
N96	0.132412	0.339808	0.535116	0.079796	0.804588	0.285938	0.039785	0.022241	0.019727	1.000000	0.195842	0.839772	0.023750	0.151450	0.261691
NT45	0.056400	0.096509	0.118713	0.498315	0.164441	0.319698	0.674824	0.372762	0.226435	0.195842	1.000000	0.417034	0.466845	0.910908	0.807329
NT50	0.235450	0.558208	0.655009	0.198541	0.749219	0.832122	0.222010	0.129783	0.089108	0.839772	0.417034	1.000000	0.155191	0.423415	0.523768
NT112	0.012678	0.007201	0.008763	0.893452	0.019600	0.026855	0.653068	0.777965	0.418891	0.023750	0.466845	0.155191	1.000000	0.323012	0.306693
NT116	0.042026	0.057157	0.073470	0.401798	0.122154	0.263027	0.521534	0.250428	0.149044	0.151450	0.910908	0.423415	0.323012	1.000000	0.871630
NT202	0.067226	0.122543	0.153994	0.368592	0.217037	0.446447	0.468681	0.243697	0.150440	0.261691	0.807329	0.523768	0.306693	0.871630	1.000000

Table A3. Comparative P-values of 15 strains ethanol yields in high nitrogen (400 mg/L) MS synthetic must

	IWBT PR7	**VIN2000**	**VIN7**	**VIN13**	**WE14**	**WE372**	**S228**	**AWRI796**	**EC1118**	**N96**	**NT45**	**NT50**	**NT112**	**NT116**	**NT202**
IWBT PR7	1.000000	0.058861	0.018413	0.614573	0.506145	0.064743	0.857517	0.016896	0.910776	0.592987	0.099711	0.003138	0.024422	0.076322	0.060033
VIN2000	0.058861	1.000000	0.006226	0.964878	0.945124	0.097311	0.640690	0.014015	0.317448	0.945994	0.170393	0.004644	0.050278	0.208509	0.122925
VIN7	0.018413	0.006226	1.000000	0.153501	0.091618	0.028197	0.167798	0.003746	0.079255	0.145556	0.035936	0.001254	0.005974	0.012065	0.014455
VIN13	0.614573	0.964878	0.153501	1.000000	0.996344	0.202898	0.780481	0.630215	0.533418	0.928439	0.325852	0.022953	0.136988	0.374486	0.213546
WE14	0.506145	0.945124	0.091618	0.996344	1.000000	0.154723	0.747853	0.512069	0.469485	0.919892	0.258585	0.013166	0.094475	0.299771	0.168590
WE372	0.064743	0.097311	0.028197	0.202898	0.154723	1.000000	0.138333	0.146488	0.035872	0.200914	0.550769	0.144654	0.619261	0.192280	0.526020
S228	0.857517	0.640690	0.167798	0.780481	0.747853	0.138333	1.000000	0.371628	0.792735	0.713788	0.215969	0.013239	0.074626	0.203606	0.125891
AWRI796	0.016896	0.014015	0.003746	0.630215	0.512069	0.146488	0.371628	1.000000	0.132434	0.754464	0.293423	0.006944	0.104346	0.521603	0.239783
EC1118	0.910776	0.317448	0.079255	0.533418	0.469485	0.035872	0.792735	0.132434	1.000000	0.486967	0.068230	0.001687	0.016459	0.063166	0.035418
N96	0.592987	0.945994	0.145556	0.928439	0.919892	0.200914	0.713788	0.754464	0.486967	1.000000	0.357674	0.022428	0.169164	0.468606	0.242460
NT45	0.099711	0.170393	0.035936	0.325852	0.258585	0.550769	0.215969	0.293423	0.068230	0.357674	1.000000	0.055349	0.727037	0.497104	0.940592
NT50	0.003138	0.004644	0.001254	0.022953	0.013166	0.144654	0.013239	0.006944	0.001687	0.022428	0.055349	1.000000	0.030310	0.008369	0.036397
NT112	0.024422	0.050278	0.005974	0.136988	0.094475	0.619261	0.074626	0.104346	0.016459	0.169164	0.727037	0.030310	1.000000	0.221813	0.771663
NT116	0.076322	0.208509	0.012065	0.374486	0.299771	0.192280	0.203606	0.521603	0.063166	0.468606	0.497104	0.008369	0.221813	1.000000	0.404155
NT202	0.060033	0.122925	0.014455	0.213546	0.168590	0.526020	0.125891	0.239783	0.035418	0.242460	0.940592	0.036397	0.771663	0.404155	1.000000

Table A4. Comparative P-values of 15 strains ethanol yields in low pH (3.00) MS300 synthetic must.

	IWBT PR7	VIN2000	VIN7	VIN13	WE14	WE372	S228	AWRI796	EC1118	N96	NT45	NT50	NT112	NT116	NT202
IWBT PR7	1.000000	0.046620	0.226831	0.052600	0.175660	0.058690	0.047180	0.006500	0.028320	0.038860	0.143393	0.025808	0.005442	0.004857	0.003148
VIN2000	0.046620	1.000000	0.794857	0.390936	0.859236	0.298342	0.307863	0.035837	0.133536	0.137491	0.895018	0.085615	0.058396	0.027488	0.012729
VIN7	0.226831	0.794857	1.000000	0.405011	0.747087	0.310852	0.333917	0.095321	0.166970	0.153230	0.901659	0.102528	0.225706	0.094959	0.049585
VIN13	0.052600	0.390936	0.405011	1.000000	0.658739	0.733801	0.845273	0.306318	0.428006	0.338244	0.426396	0.224465	0.843754	0.323006	0.145860
WE14	0.175660	0.859236	0.747087	0.658739	1.000000	0.496579	0.555333	0.211851	0.292432	0.243569	0.812485	0.167110	0.493910	0.220850	0.117521
WE372	0.058690	0.298342	0.310852	0.733801	0.496579	1.000000	0.869899	0.615800	0.682170	0.519235	0.323067	0.367004	0.783938	0.660853	0.360190
S228	0.047180	0.307863	0.333917	0.845273	0.555333	0.869899	1.000000	0.433663	0.541040	0.415209	0.346278	0.280794	0.936699	0.464660	0.219129
AWRI796	0.006500	0.035837	0.095321	0.306318	0.211851	0.615800	0.433663	1.000000	0.997649	0.717125	0.081085	0.491038	0.152508	0.884914	0.473548
EC1118	0.028320	0.133536	0.166970	0.428006	0.292432	0.682170	0.541040	0.997649	1.000000	0.753126	0.164714	0.551021	0.394042	0.930416	0.637034
N96	0.038860	0.137491	0.153230	0.338244	0.243569	0.519235	0.415209	0.717125	0.753126	1.000000	0.154244	0.799728	0.318801	0.660479	0.998896
NT45	0.143393	0.895018	0.901659	0.426396	0.812485	0.323067	0.346278	0.081085	0.164714	0.154244	1.000000	0.100733	0.203159	0.078206	0.038375
NT50	0.025808	0.085615	0.102528	0.224465	0.167110	0.367004	0.280794	0.491038	0.551021	0.799728	0.100733	1.000000	0.195461	0.441234	0.734543
NT112	0.005442	0.058396	0.225706	0.843754	0.493910	0.783938	0.936699	0.152508	0.394042	0.318801	0.203159	0.195461	1.000000	0.125481	0.036199
NT116	0.004857	0.027488	0.094959	0.323006	0.220850	0.660853	0.464660	0.884914	0.930416	0.660479	0.078206	0.441234	0.125481	1.000000	0.353098
NT202	0.003148	0.012729	0.049585	0.145860	0.117521	0.360190	0.219129	0.473548	0.637034	0.998896	0.038375	0.734543	0.036199	0.353098	1.000000

Table A5. Comparative P-values of 15 strains ethanol yields in high pH (4.00) MS300 synthetic must.

	IWBT PR7	VIN2000	VIN7	VIN13	WE14	WE372	S228	AWRI796	EC1118	N96	NT45	NT50	NT112	NT116	NT202
IWBT PR7	1.000000	0.704837	0.292942	0.741162	0.648114	0.175221	0.012458	0.027800	0.609615	0.087751	0.913865	0.727753	0.789521	0.996593	0.244901
VIN2000	0.704837	1.000000	0.256662	0.970231	0.770538	0.018944	0.005395	0.015849	0.734672	0.041545	0.928590	0.826153	0.913584	0.863119	0.218212
VIN7	0.292942	0.256662	1.000000	0.293868	0.700507	0.028676	0.027472	0.070094	0.608840	0.343444	0.536521	0.828322	0.693195	0.535404	0.807364
VIN13	0.741162	0.970231	0.293868	1.000000	0.769956	0.038329	0.007117	0.019369	0.740100	0.057802	0.938948	0.822724	0.908067	0.872747	0.244150
WE14	0.648114	0.770538	0.700507	0.769956	1.000000	0.158805	0.043289	0.086343	0.974307	0.306040	0.797387	0.971504	0.931610	0.757559	0.588314
WE372	0.175221	0.018944	0.028676	0.038329	0.158805	1.000000	0.002372	0.005521	0.094645	0.008434	0.328035	0.319926	0.300977	0.448038	0.031303
S228	0.012458	0.005395	0.027472	0.007117	0.043289	0.002372	1.000000	0.551068	0.023707	0.064701	0.046557	0.124228	0.073921	0.062444	0.044202
AWRI796	0.027800	0.015849	0.070094	0.019369	0.086343	0.005521	0.551068	1.000000	0.053266	0.179837	0.084156	0.200363	0.128253	0.104737	0.107449
EC1118	0.609615	0.734672	0.608840	0.740100	0.974307	0.094645	0.023707	0.053266	1.000000	0.206612	0.797745	0.951956	0.945855	0.756757	0.495430
N96	0.087751	0.041545	0.343444	0.057802	0.306040	0.008434	0.064701	0.179837	0.206612	1.000000	0.255297	0.508845	0.371087	0.284867	0.520203
NT45	0.913865	0.928590	0.536521	0.938948	0.797387	0.328035	0.046557	0.084156	0.797745	0.255297	1.000000	0.817501	0.887577	0.939896	0.458379
NT50	0.727753	0.826153	0.828322	0.822724	0.971504	0.319926	0.124228	0.200363	0.951956	0.508845	0.817501	1.000000	0.920372	0.780133	0.738328
NT112	0.789521	0.913584	0.693195	0.908067	0.931610	0.300977	0.073921	0.128253	0.945855	0.371087	0.887577	0.920372	1.000000	0.841039	0.602294
NT116	0.996593	0.863119	0.535404	0.872747	0.757559	0.448038	0.062444	0.104737	0.756757	0.284867	0.939896	0.780133	0.841039	1.000000	0.465763
NT202	0.244901	0.218212	0.807364	0.244150	0.588314	0.031303	0.044202	0.107449	0.495430	0.520203	0.458379	0.738328	0.602294	0.465763	1.000000

Table A6. Comparative P-values of 15 strains ethanol yields in low sugar (150 g/L) MS300 synthetic must.

	IWBT PR7	**VIN2000**	**VIN7**	**VIN13**	**WE14**	**WE372**	**S228**	**AWRI796**	**EC1118**	**N96**	**NT45**	**NT50**	**NT112**	**NT116**	**NT202**
IWBT PR7	1.000000	0.933136	0.853167	0.574308	0.863526	0.509413	0.928415	0.865596	0.344183	0.923475	0.599858	0.262620	0.884570	0.346019	0.172027
VIN2000	0.933136	1.000000	0.777264	0.634723	0.909030	0.545590	0.859104	0.945272	0.310027	0.975245	0.572592	0.242957	0.814362	0.323483	0.159457
VIN7	0.853167	0.777264	1.000000	0.397770	0.753250	0.419964	0.927904	0.639171	0.208825	0.815107	0.636221	0.236056	0.977763	0.349080	0.127079
VIN13	0.574308	0.634723	0.397770	1.000000	0.833050	0.768139	0.496893	0.623316	0.134120	0.733128	0.423598	0.135925	0.453255	0.202950	0.084926
WE14	0.863526	0.909030	0.753250	0.833050	1.000000	0.719419	0.811346	0.932372	0.438275	0.943531	0.632381	0.362456	0.780669	0.429561	0.283602
WE372	0.509413	0.545590	0.419964	0.768139	0.719419	1.000000	0.466364	0.544956	0.239901	0.608853	0.381535	0.188102	0.442956	0.225134	0.147683
S228	0.928415	0.859104	0.927904	0.496893	0.811346	0.466364	1.000000	0.770006	0.336792	0.868309	0.623262	0.265283	0.954964	0.357847	0.165873
AWRI796	0.865596	0.945272	0.639171	0.623316	0.932372	0.544956	0.770006	1.000000	0.141591	0.987510	0.536691	0.172800	0.710312	0.268598	0.095241
EC1118	0.344183	0.310027	0.208825	0.134120	0.438275	0.239901	0.336792	0.141591	1.000000	0.462658	0.869854	0.492956	0.328986	0.601148	0.284644
N96	0.923475	0.975245	0.815107	0.733128	0.943531	0.608853	0.868309	0.987510	0.462658	1.000000	0.590957	0.328649	0.836325	0.381654	0.252525
NT45	0.599858	0.572592	0.636221	0.423598	0.632381	0.381535	0.623262	0.536691	0.869854	0.590957	1.000000	0.887010	0.636953	0.881797	0.809797
NT50	0.262620	0.242957	0.236056	0.135925	0.362456	0.188102	0.265283	0.172800	0.492956	0.328649	0.887010	1.000000	0.266935	0.978708	0.859646
NT112	0.884570	0.814362	0.977763	0.453255	0.780669	0.442956	0.954964	0.710312	0.328986	0.836325	0.636953	0.266935	1.000000	0.365143	0.161941
NT116	0.346019	0.323483	0.349080	0.202950	0.429561	0.225134	0.357847	0.268598	0.601148	0.381654	0.881797	0.978708	0.365143	1.000000	0.912483
NT202	0.172027	0.159457	0.127079	0.084926	0.283602	0.147683	0.165873	0.095241	0.284644	0.252525	0.809797	0.859646	0.161941	0.912483	1.000000

Table A7. Comparative P-values of 15 strains ethanol yields in high sugar (300 g/L) MS300 synthetic must.

	IWBT PR7	VIN2000	VIN7	VIN13	WE14	WE372	S228	AWRI796	EC1118	N96	NT45	NT50	NT112	NT116	NT202
IWBT PR7	1.000000	0.920479	0.168234	0.439276	0.922747	0.298634	0.318461	0.766826	0.760067	0.083713	0.921990	0.008616	0.728059	0.067518	0.004589
VIN2000	0.920479	1.000000	0.185123	0.522144	0.885045	0.309669	0.378110	0.813886	0.715169	0.103040	0.988731	0.017883	0.783250	0.077995	0.008394
VIN7	0.168234	0.185123	1.000000	0.294234	0.228110	0.787528	0.393421	0.309420	0.145891	0.849552	0.176317	0.879215	0.288842	0.786617	0.711759
VIN13	0.439276	0.522144	0.294234	1.000000	0.598672	0.389802	0.743784	0.858333	0.330839	0.215812	0.481024	0.066587	0.847510	0.137604	0.025112
WE14	0.922747	0.885045	0.228110	0.598672	1.000000	0.311983	0.475928	0.770300	0.963446	0.188433	0.887269	0.115354	0.749989	0.125994	0.057993
WE372	0.298634	0.309669	0.787528	0.389802	0.311983	1.000000	0.448740	0.375809	0.276751	0.693866	0.305954	0.706519	0.369222	0.906370	0.913996
S228	0.318461	0.378110	0.393421	0.743784	0.475928	0.448740	1.000000	0.689828	0.247823	0.347143	0.345772	0.170193	0.664201	0.204246	0.061199
AWRI796	0.766826	0.813886	0.309420	0.858333	0.770300	0.375809	0.689828	1.000000	0.661127	0.275939	0.801962	0.177726	0.994996	0.174837	0.084022
EC1118	0.760067	0.715169	0.145891	0.330839	0.963446	0.276751	0.247823	0.661127	1.000000	0.068475	0.689888	0.007033	0.611474	0.057906	0.003937
N96	0.083713	0.103040	0.849552	0.215812	0.188433	0.693866	0.347143	0.275939	0.068475	1.000000	0.089816	0.914338	0.240841	0.576800	0.375743
NT45	0.921990	0.988731	0.176317	0.481024	0.887269	0.305954	0.345772	0.801962	0.689888	0.089816	1.000000	0.009516	0.767522	0.071179	0.004950
NT50	0.008616	0.017883	0.879215	0.066587	0.115354	0.706519	0.170193	0.177726	0.007033	0.914338	0.009516	1.000000	0.132894	0.550013	0.134835
NT112	0.728059	0.783250	0.288842	0.847510	0.749989	0.369222	0.664201	0.994996	0.611474	0.240841	0.767522	0.132894	1.000000	0.153121	0.058814
NT116	0.067518	0.077995	0.786617	0.137604	0.125994	0.906370	0.204246	0.174837	0.057906	0.576800	0.071179	0.550013	0.153121	1.000000	0.966075
NT202	0.004589	0.008394	0.711759	0.025112	0.057993	0.913996	0.061199	0.084022	0.003937	0.375743	0.004950	0.134835	0.058814	0.966075	1.000000

Table A8. P-values of various must and fermentative conditions (P-values calculated using ordinary MS300 synthetic must as Control).

Nitrogen	pH	Temperature	Strain	P-value
Low	Low	Medium	EC1118	0.268608
Medium	Low	Medium	EC1118	0.278641
High	Low	Medium	EC1118	0.760576
Low	Medium	Medium	EC1118	0.387527
High	Medium	Medium	EC1118	0.765575
Low	High	Medium	EC1118	0.050714
Medium	High	Medium	EC1118	0.871542
High	High	Medium	EC1118	0.515353
Low	Low	High	EC1118	0.090784
Medium	Low	High	EC1118	0.795433
High	Low	High	EC1118	0.250218
Low	Medium	High	EC1118	0.097048
Medium	Medium	High	EC1118	0.057402
High	Medium	High	EC1118	0.695895
Low	High	High	EC1118	0.589060
Medium	High	High	EC1118	0.350636
High	High	High	EC1118	0.201858
Low	Low	Medium	VIN13	0.911163
Medium	Low	Medium	VIN13	0.859401
High	Low	Medium	VIN13	0.988316
Low	Medium	Medium	VIN13	0.597535
High	Medium	Medium	VIN13	0.892057
Low	High	Medium	VIN13	0.608521
Medium	High	Medium	VIN13	0.850610
High	High	Medium	VIN13	0.845635
Low	Low	High	VIN13	0.446726
Medium	Low	High	VIN13	0.649236
High	Low	High	VIN13	0.966613
Low	Medium	High	VIN13	0.803475
Medium	Medium	High	VIN13	0.752169
High	Medium	High	VIN13	0.929617
Low	High	High	VIN13	0.897014
Medium	High	High	VIN13	0.843630
High	High	High	VIN13	0.912712

Table A9. P-values of ethanol yield for various conditions, strains and cultivars tested. (P-values calculated using unmodified grape must as the Control).

Condition	Strain	Cultivar			
		Sauvignon Blanc	Chardonnay	Shiraz	Cabernet Sauvignon
Low pH	S228	0.034768	0.038896	0.087562	0.352513
Low pH	EC1118	0.748354	0.196141	0.006876	0.193480
Low pH	VIN7	0.667032	0.640321	0.012619	0.661142
Low pH	VIN13	0.049838	0.074306	0.011700	0.077376
High Nitrogen	S228	0.896681	0.304868	0.363388	0.367299
High Nitrogen	EC1118	0.231581	0.426911	0.348095	0.332786
High Nitrogen	VIN7	0.315500	0.597296	0.087532	0.415919
High Nitrogen	VIN13	0.194373	0.527934	0.250026	0.077598
High pH	S228	0.870147	0.042798	0.141094	0.171517
High pH	EC1118	0.649770	0.296718	0.026333	0.561457
High pH	VIN7	0.115888	0.397368	0.140508	0.461800
High pH	VIN13	0.005572	0.046546	0.375023	0.123202
High Sugar	S228	0.061964	0.112129	0.017993	0.026870
High Sugar	EC1118	0.021048	0.453104	0.001182	0.067734
High Sugar	VIN7	0.089979	0.037282	0.022585	0.031781
High Sugar	VIN13	0.007556	0.403400	0.004946	0.003292
Low Temperature	S228	0.441618	0.526541	0.548752	0.031871
Low Temperature	EC1118	0.160745	0.001236	0.065618	0.746716
Low Temperature	VIN7	0.284073	0.008390	0.123210	0.487824
Low Temperature	VIN13	0.313132	0.007958	0.211054	0.331107
High Temperature	S228	0.041672	0.920569	0.131684	0.406927
High Temperature	EC1118	0.671389	0.006625	0.027649	0.141040
High Temperature	VIN7	0.706872	0.051771	0.005618	0.905578
High Temperature	VIN13	0.061280	0.261452	0.000425	0.039969

Table A10 P-values for different cultivars and strains compared to Shiraz.

	Sauvignon Blanc	Chardonnay	Shiraz	Cabernet Sauvignon
228	0.002193	0.920634	1.000000	0.009578
EC1118	0.000566	0.000797	1.000000	0.008750
VIN7	0.000265	0.006372	1.000000	0.003025
VIN13	0.000145	0.009037	1.000000	0.001112

Table A11. P-values for glycerol yield of various conditions, strains and cultivars tested. (P-values calculated using unmodified grape must as the control).

Condition	Strain	Cultivar			
		Sauvignon Blanc	Chardonnay	Shiraz	Cabernet Sauvignon
Low pH	S228	0.091179	0.006277	0.056473	0.459648
Low pH	EC1118	0.333320	0.452146	0.001504	0.036344
Low pH	VIN7	0.150169	0.006327	0.004378	0.414451
Low pH	VIN13	0.001895	0.001415	0.004261	0.522737
High Nitrogen	S228	0.038309	0.034711	0.572517	0.345342
High Nitrogen	EC1118	0.112572	0.648643	0.242438	0.758916
High Nitrogen	VIN7	0.033030	0.091679	0.290865	0.647222
High Nitrogen	VIN13	0.175498	0.033860	0.060257	0.962162
High pH	S228	0.635990	0.278679	0.263713	0.249100
High pH	EC1118	0.567344	0.321976	0.076062	0.687771
High pH	VIN7	0.238106	0.224874	0.964053	0.301765
High pH	VIN13	0.022808	0.769557	0.445374	0.744541
High Sugar	S228	0.791786	0.006931	0.363529	0.059553
High Sugar	EC1118	0.601844	0.015481	0.016022	0.761991
High Sugar	VIN7	0.130787	0.000263	0.007857	0.197890
High Sugar	VIN13	0.706343	0.000117	0.045808	0.008401
Low Temperature	S228	0.086398	0.080610	0.015159	0.034144
Low Temperature	EC1118	0.001046	0.076698	0.001649	0.026504
Low Temperature	VIN7	0.001024	0.041009	0.000417	0.003434
Low Temperature	VIN13	0.006986	0.186152	0.000762	0.328493
High Temperature	S228	0.010098	0.000616	0.014786	0.884998
High Temperature	EC1118	0.003548	0.001468	0.022138	0.031148
High Temperature	VIN7	0.012694	0.000127	0.003637	0.130479
High Temperature	VIN13	0.051794	0.000053	0.026713	0.013308

Table A12. Complete data set of first three objectives.

	Name	Media Type	Strain	Repeat	Glucose (g/L)	Fructose (g/L)	Ethanol (g/L)	Ethanol Yield	Average Ethanol yield	Standard Deviation
1	Control	Synthetic	IWBT PR7	A	0.159	2.812	91.68539	0.384464	0.37948	0.004788
2	Control	Synthetic	IWBT PR7	B	0.241	4.559	88.72284	0.374917		
3	Control	Synthetic	IWBT PR7	C	2.824	18.5	83.43974	0.37906		
4	Control	Synthetic	VIN2000	A	0.13	1.796	89.97301	0.375638	0.369272	0.006568
5	Control	Synthetic	VIN2000	B	0.129	1.754	88.55647	0.369657		
6	Control	Synthetic	VIN2000	C	0.081	1.282	87.03503	0.36252		
7	Control	Synthetic	VIN7	A	0.013	0.225	91.55361	0.379562	0.385061	0.004875
8	Control	Synthetic	VIN7	B	0.014	0.267	93.27585	0.386771		
9	Control	Synthetic	VIN7	C	0.016	0.296	93.76562	0.388852		
10	Control	Synthetic	VIN13	A	0.77	7.309	89.60223	0.383953	0.383148	0.005714
11	Control	Synthetic	VIN13	B	0.141	2.914	92.59549	0.388417		
12	Control	Synthetic	VIN13	C	0.015	0.493	90.85184	0.377075		
13	Control	Synthetic	WE14	A	0.015	0.801	89.52352	0.372037	0.374894	0.003036
14	Control	Synthetic	WE14	B	0.272	6.229	88.82883	0.378082		
15	Control	Synthetic	WE14	C	0.047	2.193	89.5976	0.374561		
16	Control	Synthetic	WE372	A	0.046	4.332	92.18137	0.388838	0.361075	0.025981
17	Control	Synthetic	WE372	B	6.254	32.459	68.39175	0.337348		
18	Control	Synthetic	WE372	C	0.024	3.875	84.8136	0.357038		
19	Control	Synthetic	228	A	0.995	12.032	85.90495	0.376084	0.379768	0.005274
20	Control	Synthetic	228	B	1.639	17.138	85.90809	0.38581		
21	Control	Synthetic	228	C	1.304	16.507	84.40256	0.377411		
22	Control	Synthetic	AWRI796	A	0.022	2.142	88.00675	0.367794	0.371858	0.004163
23	Control	Synthetic	AWRI796	B	0.038	2.581	88.76389	0.371665		
24	Control	Synthetic	AWRI796	C	0.043	3.342	89.53829	0.376114		
25	Control	Synthetic	EC1118	A	0.926	7.572	86.1224	0.369705	0.354916	0.015312
26	Control	Synthetic	EC1118	B	0.067	1.662	85.31841	0.355912		
27	Control	Synthetic	EC1118	C	0.043	1.273	81.43565	0.339131		
28	Control	Synthetic	N96	A	0.025	0.837	90.55457	0.376394	0.368215	0.023863
29	Control	Synthetic	N96	B	0.016	0.469	93.23116	0.386913		
30	Control	Synthetic	N96	C	0.064	1.614	81.84211	0.341338		
31	Control	Synthetic	NT45	A	0.961	7.25	82.94651	0.355634	0.359428	0.003402
32	Control	Synthetic	NT45	B	0.196	2.662	85.99809	0.360445		
33	Control	Synthetic	NT45	C	0.114	1.926	86.71463	0.362206		
34	Control	Synthetic	NT50	A	6.485	24.981	78.24143	0.372613	0.369275	0.015185
35	Control	Synthetic	NT50	B	0.018	0.474	84.98443	0.352699		
36	Control	Synthetic	NT50	C	0.087	2.293	91.44608	0.382513		
37	Control	Synthetic	NT112	A	0.038	2.211	84.18592	0.351951	0.359873	0.011546
38	Control	Synthetic	NT112	B	0.168	5.034	83.75999	0.354548		
39	Control	Synthetic	NT112	C	1.989	16.072	83.34993	0.373121		
40	Control	Synthetic	NT116	A	0.115	3.061	86.19986	0.361773	0.369688	0.010571
41	Control	Synthetic	NT116	B	0.279	5.21	90.06314	0.381692		
42	Control	Synthetic	NT116	C	0.199	4.505	86.5527	0.365598		

43	Control	Synthetic	NT202	A	0.047	1.838	89.43388	0.373323	0.362079	0.019525
44	Control	Synthetic	NT202	B	0.013	0.575	81.77953	0.339533		
45	Control	Synthetic	NT202	C	0.015	0.989	89.77668	0.373381		
46	Low Nitrogen	Synthetic	IWBT PR7	A	0.159	2.812	87.2879	0.361328	0.365497	0.019419
47	Low Nitrogen	Synthetic	IWBT PR7	B	0.241	4.559	83.55187	0.348501		
48	Low Nitrogen	Synthetic	IWBT PR7	C	2.824	18.5	86.31166	0.386662		
49	Low Nitrogen	Synthetic	VIN2000	A	0.13	1.796	85.43704	0.352143	0.348373	0.003581
50	Low Nitrogen	Synthetic	VIN2000	B	0.129	1.754	84.43722	0.34796		
51	Low Nitrogen	Synthetic	VIN2000	C	0.081	1.282	83.90233	0.345017		
52	Low Nitrogen	Synthetic	VIN7	A	0.013	0.225	85.18967	0.348697	0.345795	0.00314
53	Low Nitrogen	Synthetic	VIN7	B	0.014	0.267	83.65168	0.342462		
54	Low Nitrogen	Synthetic	VIN7	C	0.016	0.296	84.56016	0.346226		
55	Low Nitrogen	Synthetic	VIN13	A	0.77	7.309	71.29634	0.301506	0.303566	0.026329
56	Low Nitrogen	Synthetic	VIN13	B	0.141	2.914	79.90093	0.330865		
57	Low Nitrogen	Synthetic	VIN13	C	0.015	0.493	67.92256	0.278327		
58	Low Nitrogen	Synthetic	WE14	A	0.015	0.801	80.9843	0.33227	0.343824	0.010193
59	Low Nitrogen	Synthetic	WE14	B	0.272	6.229	82.7585	0.347659		
60	Low Nitrogen	Synthetic	WE14	C	0.047	2.193	85.18154	0.351545		
61	Low Nitrogen	Synthetic	WE372	A	0.046	4.332	80.85553	0.336662	0.334183	0.002467
62	Low Nitrogen	Synthetic	WE372	B	6.254	32.459	68.28085	0.331729		
63	Low Nitrogen	Synthetic	WE372	C	0.024	3.875	80.41429	0.334158		
64	Low Nitrogen	Synthetic	228	A	0.995	12.032	73.91094	0.319243	0.311582	0.014014
65	Low Nitrogen	Synthetic	228	B	1.639	17.138	72.26786	0.320096		
66	Low Nitrogen	Synthetic	228	C	1.304	16.507	66.97946	0.295408		
67	Low Nitrogen	Synthetic	AWRI796	A	0.022	2.142	69.06108	0.284926	0.302347	0.01576
68	Low Nitrogen	Synthetic	AWRI796	B	0.038	2.581	76.356	0.315615		
69	Low Nitrogen	Synthetic	AWRI796	C	0.043	3.342	73.91552	0.306498		
70	Low Nitrogen	Synthetic	EC1118	A	0.926	7.572	72.43574	0.306868	0.293444	0.019703
71	Low Nitrogen	Synthetic	EC1118	B	0.067	1.662	73.48618	0.30264		
72	Low Nitrogen	Synthetic	EC1118	C	0.043	1.273	65.87269	0.270824		
73	Low Nitrogen	Synthetic	N96	A	0.025	0.837	82.53464	0.338695	0.341626	0.010182
74	Low Nitrogen	Synthetic	N96	B	0.016	0.469	81.32864	0.33323		
75	Low Nitrogen	Synthetic	N96	C	0.064	1.614	85.72086	0.352952		
76	Low Nitrogen	Synthetic	NT45	A	0.961	7.25	76.80085	0.324966	0.318714	0.023473
77	Low Nitrogen	Synthetic	NT45	B	0.196	2.662	70.7536	0.292747		
78	Low Nitrogen	Synthetic	NT45	C	0.114	1.926	82.07092	0.338428		
79	Low Nitrogen	Synthetic	NT50	A	6.485	24.981	78.89172	0.370244	0.337886	0.028247
80	Low Nitrogen	Synthetic	NT50	B	0.018	0.474	77.6462	0.318151		
81	Low Nitrogen	Synthetic	NT50	C	0.087	2.293	78.76792	0.325264		
82	Low Nitrogen	Synthetic	NT112	A	0.038	2.211	77.38695	0.319388	0.306025	0.014059
83	Low Nitrogen	Synthetic	NT112	B	0.168	5.034	69.73567	0.291361		
84	Low Nitrogen	Synthetic	NT112	C	1.989	16.072	69.60435	0.307324		
85	Low Nitrogen	Synthetic	NT116	A	0.115	3.061	75.16183	0.311396	0.320737	0.017725

86	Low Nitrogen	Synthetic	NT116	B	0.279	5.21	74.02059	0.309635		
87	Low Nitrogen	Synthetic	NT116	C	0.199	4.505	81.82916	0.341179		
88	Low Nitrogen	Synthetic	NT202	A	0.047	1.838	72.82701	0.300118	0.323526	0.021744
89	Low Nitrogen	Synthetic	NT202	B	0.013	0.575	79.8638	0.327367		
90	High Nitrogen	Synthetic	NT202	C	0.015	0.989	83.55791	0.343094		
91	High Nitrogen	Synthetic	IWBT PR7	B	0.068	2.159	91.31403	0.385395	0.383388	0.002839
92	High Nitrogen	Synthetic	IWBT PR7	C	0.108	10.26	87.25779	0.38138		
93	High Nitrogen	Synthetic	VIN2000	B	0.093	1.982	89.06827	0.375676	0.374873	0.001136
94	High Nitrogen	Synthetic	VIN2000	C	0.024	0.621	89.22238	0.37407		
95	High Nitrogen	Synthetic	VIN7	B	0.016	0.711	96.15057	0.403255	0.405551	0.003246
96	High Nitrogen	Synthetic	VIN7	C	0.006	0.528	97.32397	0.407846		
97	High Nitrogen	Synthetic	VIN13	B	0.071	2.656	91.89816	0.388681	0.375528	0.018601
98	High Nitrogen	Synthetic	VIN13	C	0.616	10.227	82.73741	0.362375		
99	High Nitrogen	Synthetic	WE14	B	0.307	8.299	88.78343	0.385082	0.375612	0.013394
100	High Nitrogen	Synthetic	WE14	C	0.558	12.213	82.89138	0.366141		
101	High Nitrogen	Synthetic	WE372	B	0.001	1.789	84.18438	0.35465	0.344416	0.014474
102	High Nitrogen	Synthetic	WE372	C	0.123	9.819	76.60131	0.334181		
103	High Nitrogen	Synthetic	228	B	4.397	22.9	78.33434	0.369735	0.381048	0.015999
104	High Nitrogen	Synthetic	228	C	2.834	18.489	85.47205	0.392362		
105	High Nitrogen	Synthetic	AWRI796	B	0.0008	0.88	87.73741	0.368208	0.368124	0.000118
106	High Nitrogen	Synthetic	AWRI796	C	0.92	12.496	83.08415	0.368041		
107	High Nitrogen	Synthetic	EC1118	A	4.179	17.921	85.96044	0.396016	0.384368	0.010617
108	High Nitrogen	Synthetic	EC1118	B	5.055	20.767	81.46568	0.381857		
109	High Nitrogen	Synthetic	EC1118	C	6.285	22.444	78.96161	0.375232		
110	High Nitrogen	Synthetic	N96	A	0.386	4.953	92.49612	0.39558	0.37368	0.021751
111	High Nitrogen	Synthetic	N96	B	2.961	15.16	82.532	0.373377		
112	High Nitrogen	Synthetic	N96	C	0.1281	2.483	83.28559	0.352082		
113	High Nitrogen	Synthetic	NT45	B	0.635	5.693	84.78224	0.36413	0.354439	0.013706
114	High Nitrogen	Synthetic	NT45	C	0.111	2.8	81.44721	0.344747		
115	High Nitrogen	Synthetic	NT50	A	0.0047	0.461	75.18682	0.314988	0.324718	0.008763
116	High Nitrogen	Synthetic	NT50	B	0.0172	1.105	77.88148	0.327177		
117	High Nitrogen	Synthetic	NT50	C	0.0347	1.33	78.94637	0.331989		
118	High Nitrogen	Synthetic	NT112	A	0.005	0.986	84.88713	0.356411	0.350396	0.010311
119	High Nitrogen	Synthetic	NT112	B	1.563	12.188	76.29959	0.338489		
120	High Nitrogen	Synthetic	NT112	C	0.153	5.57	83.17152	0.356286		
121	High Nitrogen	Synthetic	NT116	A	0.069	5.34	82.03522	0.350947	0.362564	0.010308
122	High Nitrogen	Synthetic	NT116	B	0.042	4.416	85.93276	0.366131		
123	High Nitrogen	Synthetic	NT116	C	2.381	16.867	81.5039	0.370615		
124	High Nitrogen	Synthetic	NT202	A	0.674	9.553	84.4549	0.368902	0.353437	0.013473
125	High Nitrogen	Synthetic	NT202	B	0.181	5.88	80.24147	0.344233		
126	High Nitrogen	Synthetic	NT202	C	2.576	17.257	76.14641	0.347177		
127	Low Sugar	Synthetic	IWBT PR7	A	0.009	0.097	51.6781	0.347494	0.335955	0.013038
128	Low Sugar	Synthetic	IWBT PR7	B	0.01	0.207	47.82285	0.32181		

129	Low Sugar	Synthetic	IWBT PR7	C	0.001	0.112	50.34714	0.33856		
130	Low Sugar	Synthetic	VIN2000	A	0.002	0.01	51.58843	0.346672	0.336921	0.013458
131	Low Sugar	Synthetic	VIN2000	B	0.001	0.103	47.82285	0.321566		
132	Low Sugar	Synthetic	VIN2000	C	0.003	0.006	50.97238	0.342525		
133	Low Sugar	Synthetic	VIN7	A	0.003	0	50.1257	0.336822	0.334265	0.007071
134	Low Sugar	Synthetic	VIN7	B	0.003	0.022	48.54841	0.326271		
135	Low Sugar	Synthetic	VIN7	C	0.002	0.013	50.55008	0.339701		
136	Low Sugar	Synthetic	VIN13	A	0.001	0.342	52.22864	0.351756	0.342559	0.013443
137	Low Sugar	Synthetic	VIN13	B	0	0.128	51.86312	0.348789		
138	Low Sugar	Synthetic	VIN13	C	0.001	0.184	48.62412	0.327132		
139	Low Sugar	Synthetic	WE14	A	0.003	0.079	#DIV/0!	#DIV/0!	0.3389	0.023493
140	Low Sugar	Synthetic	WE14	B	0.002	0.009	52.90423	0.355511		
141	Low Sugar	Synthetic	WE14	C	0.003	0.019	47.95664	0.322288		
142	Low Sugar	Synthetic	WE372	A	0.003	0.014	53.24832	0.357838	0.347728	0.024988
143	Low Sugar	Synthetic	WE372	B	0.002	0.685	54.22901	0.366077		
144	Low Sugar	Synthetic	WE372	C	0.003	0.294	47.41971	0.319269		
145	Low Sugar	Synthetic	228	A	0.003	0.153	51.70181	0.34777	0.335004	0.011255
146	Low Sugar	Synthetic	228	B	0.001	0.36	49.10047	0.330728		
147	Low Sugar	Synthetic	228	C	0.002	0.054	48.57423	0.326513		
148	Low Sugar	Synthetic	AWRI796	A	0.003	0.015	49.85278	0.335022	0.337603	0.008963
149	Low Sugar	Synthetic	AWRI796	B	0.003	0.066	49.12049	0.330214		
150	Low Sugar	Synthetic	AWRI796	C	0.002	0.218	51.65029	0.347573		
151	Low Sugar	Synthetic	EC1118	A	0.002	0.11	49.19955	0.330841	0.327709	0.00275
152	Low Sugar	Synthetic	EC1118	B	0.011	0.057	48.583	0.326598		
153	Low Sugar	Synthetic	EC1118	C	0.002	0.021	48.46242	0.325689		
154	Low Sugar	Synthetic	N96	A	0.002	0.01	46.90278	0.315184	0.337388	0.020477
155	Low Sugar	Synthetic	N96	B	0.002	0.053	52.89108	0.355528		
156	Low Sugar	Synthetic	N96	C	0.002	0.03	50.80475	0.341451		
157	Low Sugar	Synthetic	NT45	A	0.001	0.017	44.05947	0.296089	0.324405	0.032659
158	Low Sugar	Synthetic	NT45	B	0.001	0.04	47.16269	0.316993		
159	Low Sugar	Synthetic	NT45	C	0	0.107	53.5574	0.360133		
160	Low Sugar	Synthetic	NT50	A	0.002	0.036	46.30918	0.31125	0.321282	0.014513
161	Low Sugar	Synthetic	NT50	B	0.011	0.5	50.11785	0.337922		
162	Low Sugar	Synthetic	NT50	C	0.002	0.027	46.8213	0.314673		
163	Low Sugar	Synthetic	NT112	A	0.001	0.04	48.45786	0.325698	0.334477	0.01019
164	Low Sugar	Synthetic	NT112	B	0.002	0.114	51.40058	0.345651		
165	Low Sugar	Synthetic	NT112	C	0.001	0.189	49.35828	0.332082		
166	Low Sugar	Synthetic	NT116	A	0.003	0.003	49.70764	0.334019	0.320867	0.020728
167	Low Sugar	Synthetic	NT116	B	0.001	0.074	44.17395	0.296972		
168	Low Sugar	Synthetic	NT116	C	0.002	0.014	49.34559	0.331609		
169	Low Sugar	Synthetic	NT202	A	0.002	0.096	46.51564	0.312763	0.319264	0.011527
170	Low Sugar	Synthetic	NT202	B	0.009	0.06	46.47903	0.312456		
171	Low Sugar	Synthetic	NT202	C	0.002	0.015	49.48879	0.332573		
172	High Sugar	Synthetic	IWBT	A	0.61	10.6711	98.48518	0.387623	0.392945	0.005806

			PR7							
173	High Sugar	Synthetic	IWBT PR7	B	1.49	18.5203	96.19347	0.392074		
174	High Sugar	Synthetic	IWBT PR7	C	1.411	15.8499	99.02397	0.399138		
175	High Sugar	Synthetic	VIN2000	A	1.538	8.3315	99.53487	0.38959	0.392368	0.007388
176	High Sugar	Synthetic	VIN2000	B	0.074	1.103	102.177	0.386772		
177	High Sugar	Synthetic	VIN2000	C	0.047	0.81	105.9958	0.400742		
178	High Sugar	Synthetic	VIN7	A	0.016	0.606	104.7553	0.395701	0.371952	0.020848
179	High Sugar	Synthetic	VIN7	B	0.015	0.235	94.55365	0.356664		
180	High Sugar	Synthetic	VIN7	C	0.758	11.2031	92.10663	0.363491		
181	High Sugar	Synthetic	VIN13	A	0.773	8.1408	99.96721	0.389824	0.387715	0.008818
182	High Sugar	Synthetic	VIN13	B	2.223	17.0194	97.2854	0.395287		
183	High Sugar	Synthetic	VIN13	C	1.84	14.6045	94.09676	0.378033		
184	High Sugar	Synthetic	WE14	A	0.189	13.3486	103.4184	0.410687	0.385676	0.012567
185	High Sugar	Synthetic	WE14	B	1.058	18.6897	96.90755	0.394562		
186	High Sugar	Synthetic	WE14	C	0.281	15.501	94.03686	0.37679		
187	High Sugar	Synthetic	WE372	A	10.7857	83.1017	66.71804	0.389098	0.364268	0.041212
188	High Sugar	Synthetic	WE372	B	0.012	7.7431	99.69371	0.387009		
189	High Sugar	Synthetic	WE372	C	5.9418	30.7287	72.42365	0.316696		
190	High Sugar	Synthetic	228	A	5.3771	26.7324	92.65748	0.397252	0.384899	0.010776
191	High Sugar	Synthetic	228	B	5.2977	27.4159	87.80659	0.377432		
192	High Sugar	Synthetic	228	C	3.2784	22.221	91.14842	0.380013		
193	High Sugar	Synthetic	AWRI796	A	1.061	16.8248	98.3832	0.397556	0.389759	0.016385
194	High Sugar	Synthetic	AWRI796	B	0.159	7.7258	95.50411	0.370932		
195	High Sugar	Synthetic	AWRI796	C	4.9681	43.16	87.06268	0.40079		
196	High Sugar	Synthetic	EC1118	A	0.753	6.413	100.4058	0.388884	0.394519	0.005986
197	High Sugar	Synthetic	EC1118	B	1.061	9.5189	100.3489	0.393871		
198	High Sugar	Synthetic	EC1118	C	1.024	8.6699	102.4699	0.400803		
199	High Sugar	Synthetic	N96	A	0.087	1.834	101.759	0.386278	0.374787	0.012435
200	High Sugar	Synthetic	N96	B	0.041	1.143	99.46051	0.3765		
201	High Sugar	Synthetic	N96	C	5.1641	14.8389	88.71574	0.361585		
202	High Sugar	Synthetic	NT45	A	0.293	3.3788	102.5734	0.391974	0.39245	0.005823
203	High Sugar	Synthetic	NT45	B	0.014	0.386	102.5058	0.386879		
204	High Sugar	Synthetic	NT45	C	0.011	0.307	105.6165	0.398496		
205	High Sugar	Synthetic	NT50	A	0.013	0.415	98.19294	0.370641	0.373931	0.003642
206	High Sugar	Synthetic	NT50	B	0.079	1.833	98.34547	0.373307		
207	High Sugar	Synthetic	NT50	C	0.061	1.696	99.59906	0.377844		
208	High Sugar	Synthetic	NT112	A	0.096	2.728	104.6914	0.398776	0.389676	0.014022
209	High Sugar	Synthetic	NT112	B	0.694	8.5063	101.623	0.396724		
210	High Sugar	Synthetic	NT112	C	1.572	12.8927	93.7149	0.373528		
211	High Sugar	Synthetic	NT116	A	0.765	9.4697	97.68101	0.382881	0.367484	0.016735
212	High Sugar	Synthetic	NT116	B	0.191	4.4882	96.42377	0.369898		
213	High Sugar	Synthetic	NT116	C	0.016	0.468	92.61851	0.349673		
214	High Sugar	Synthetic	NT202	A	0.055	2.051	98.15703	0.372867	0.367026	0.005257

215	High Sugar	Synthetic	NT202	B	0.889	10.1452	92.96419	0.365538		
216	High Sugar	Synthetic	NT202	C	0.832	10.0784	92.2804	0.362673		
217	Low pH	Synthetic	IWBT PR7	A	0.416	6.705	84.0216	0.356647	0.357362	0.006493
218	Low pH	Synthetic	IWBT PR7	B	2.369	14.282	82.32613	0.364182		
219	Low pH	Synthetic	IWBT PR7	C	0.138	3.963	83.81231	0.351256		
220	Low pH	Synthetic	VIN2000	A	0.082	1.574	83.50912	0.346435	0.343986	0.004914
221	Low pH	Synthetic	VIN2000	B	0.257	2.817	83.19965	0.347194		
222	Low pH	Synthetic	VIN2000	C	1.444	6.16	79.54277	0.338329		
223	Low pH	Synthetic	VIN7	A	0.014	0.278	86.37759	0.356319	0.346076	0.012065
224	Low pH	Synthetic	VIN7	B	0.014	0.153	84.67924	0.349133		
225	Low pH	Synthetic	VIN7	C	0.347	6.275	78.56424	0.332777		
226	Low pH	Synthetic	VIN13	A	0.239	4.097	77.44331	0.324883	0.337331	0.01094
227	Low pH	Synthetic	VIN13	B	3.624	16.373	76.09912	0.341693		
228	Low pH	Synthetic	VIN13	C	1.556	9.089	80.15886	0.345418		
229	Low pH	Synthetic	WE14	A	1.643	13.753	74.9565	0.329751	0.348602	0.013474
230	Low pH	Synthetic	WE14	B	3.33	23.51	73.19552	0.339074		
231	Low pH	Synthetic	WE14	C	0.129	4.7	85.19184	0.35813		
232	Low pH	Synthetic	WE372	A	1.599	22.334	75.12929	0.343408	0.333534	0.01434
233	Low pH	Synthetic	WE372	B	0.405	13.154	77.93569	0.340108		
234	Low pH	Synthetic	WE372	C	0.454	18.47	70.95871	0.317085		
235	Low pH	Synthetic	228	A	7.119	33.558	66.36294	0.328478	0.335402	0.011749
236	Low pH	Synthetic	228	B	17.514	58.308	58.23802	0.348968		
237	Low pH	Synthetic	228	C	11.807	45.117	61.07873	0.328761		
238	Low pH	Synthetic	AWRI796	A	1.441	12.72	73.72697	0.322589	0.328518	0.007072
239	Low pH	Synthetic	AWRI796	B	0.411	8.685	76.30262	0.32662		
240	Low pH	Synthetic	AWRI796	C	1.399	12.249	77.04364	0.336346		
241	Low pH	Synthetic	EC1118	A	0.052	1.504	75.58652	0.313438	0.328546	0.013363
242	Low pH	Synthetic	EC1118	B	0.263	3.764	80.86981	0.338819		
243	Low pH	Synthetic	EC1118	C	1.143	5.834	78.58827	0.33338		
244	Low pH	Synthetic	N96	A	0.017	0.514	73.53559	0.303643	0.324214	0.017815
245	Low pH	Synthetic	N96	B	0.412	4.68	79.4821	0.334497		
246	Low pH	Synthetic	N96	C	0.114	2.488	80.31615	0.334502		
247	Low pH	Synthetic	NT45	A	0.044	1.22	82.94749	0.343547	0.344887	0.009963
248	Low pH	Synthetic	NT45	B	0.399	4.812	84.41917	0.355453		
249	Low pH	Synthetic	NT45	C	4.13	19.514	73.53193	0.335663		
250	Low pH	Synthetic	NT50	A	0.016	0.212	72.88174	0.300567	0.32032	0.017366
251	Low pH	Synthetic	NT50	B	0.016	0.38	80.73621	0.33319		
252	Low pH	Synthetic	NT50	C	0.019	0.498	79.24574	0.327203		
253	Low pH	Synthetic	NT112	A	2.23	15.003	75.26229	0.333793	0.335983	0.001925
254	Low pH	Synthetic	NT112	B	0.045	2.108	81.00582	0.336745		
255	Low pH	Synthetic	NT112	C	0.332	5.992	79.75885	0.337411		
256	Low pH	Synthetic	NT116	A	0.015	0.49	78.19562	0.322851	0.329324	0.00565
257	Low pH	Synthetic	NT116	B	0.017	0.485	80.37945	0.331863		

258	Low pH	Synthetic	NT116	C	0.07	2.902	79.89444	0.333259		
259	Low pH	Synthetic	NT202	A	0.139	6.689	77.2024	0.327294	0.324198	0.006295
260	Low pH	Synthetic	NT202	B	0.026	1.822	79.08556	0.328346		
261	Low pH	Synthetic	NT202	C	0.039	2.654	76.07395	0.316954		
262	High pH	Synthetic	IWBT PR7	A	0.018	0.466	81.86668	0.354482	0.346434	0.007306
263	High pH	Synthetic	IWBT PR7	B	0.074	2.527	77.85242	0.340218		
264	High pH	Synthetic	IWBT PR7	C	0.016	0.394	79.61037	0.344601		
265	High pH	Synthetic	VIN2000	A	0.029	0.514	79.7876	0.345567	0.34468	0.001532
266	High pH	Synthetic	VIN2000	B	0.047	0.767	79.6923	0.34556		
267	High pH	Synthetic	VIN2000	C	0.044	0.68	79.11224	0.342911		
268	High pH	Synthetic	VIN7	A	0.026	0.692	78.29223	0.339348	0.339638	0.006425
269	High pH	Synthetic	VIN7	B	0.012	0.204	80.04751	0.346203		
270	High pH	Synthetic	VIN7	C	0.016	0.294	77.04725	0.333362		
271	High pH	Synthetic	VIN13	A	0.031	1.379	80.17752	0.348565	0.344769	0.003595
272	High pH	Synthetic	VIN13	B	0.062	1.793	79.04953	0.344327		
273	High pH	Synthetic	VIN13	C	0.019	0.288	78.90948	0.341415		
274	High pH	Synthetic	WE14	A	0.017	0.649	80.36683	0.348262	0.339887	0.013973
275	High pH	Synthetic	WE14	B	0.077	3.935	79.54381	0.349767		
276	High pH	Synthetic	WE14	C	0.016	0.663	76.14988	0.330007		
277	High pH	Synthetic	WE372	A	0.015	1.346	81.95887	0.356233	0.354418	0.004154
278	High pH	Synthetic	WE372	B	0.012	0.522	82.51247	0.357355		
279	High pH	Synthetic	WE372	C	0.019	1.759	80.30189	0.349665		
280	High pH	Synthetic	228	A	0.015	0.521	74.93536	0.324542	0.320147	0.0076
281	High pH	Synthetic	228	B	0.263	5.797	70.17427	0.311371		
282	High pH	Synthetic	228	C	0.459	8.066	72.33921	0.324527		
283	High pH	Synthetic	AWRI796	A	0.029	2.142	75.71896	0.330275	0.324448	0.008579
284	High pH	Synthetic	AWRI796	B	0.014	0.651	72.59864	0.314598		
285	High pH	Synthetic	AWRI796	C	0.043	2.372	75.22584	0.328473		
286	High pH	Synthetic	EC1118	A	0.2	2.702	77.32971	0.338379	0.342949	0.008106
287	High pH	Synthetic	EC1118	B	0.028	0.574	78.05699	0.338158		
288	High pH	Synthetic	EC1118	C	0.047	1.127	81.12163	0.352308		
289	High pH	Synthetic	N96	A	0.041	1.085	78.16367	0.339391	0.33422	0.005925
290	High pH	Synthetic	N96	B	0.113	2.099	76.90653	0.335515		
291	High pH	Synthetic	N96	C	0.21	3.113	74.76365	0.327755		
292	High pH	Synthetic	NT45	A	0.03	0.52	80.45867	0.348484	0.345421	0.013369
293	High pH	Synthetic	NT45	B	0.028	0.589	76.35014	0.330785		
294	High pH	Synthetic	NT45	C	0.018	0.363	82.48332	0.356992		
295	High pH	Synthetic	NT50	A	0.025	0.798	80.69337	0.349915	0.342212	0.018164
296	High pH	Synthetic	NT50	B	0.014	0.254	82.12225	0.355256		
297	High pH	Synthetic	NT50	C	0.02	0.56	74.21104	0.321466		
298	High pH	Synthetic	NT112	A	0.268	4.791	74.53278	0.329248	0.343665	0.015134
299	High pH	Synthetic	NT112	B	0.063	2.259	82.34788	0.359426		
300	High pH	Synthetic	NT112	C	0.291	4.797	77.4822	0.342321		

301	High pH	Synthetic	NT116	A	0.02	0.802	81.82741	0.354831	0.346388	0.016023
302	High pH	Synthetic	NT116	B	0.026	1.176	82.05917	0.356423		
303	High pH	Synthetic	NT116	C	0.095	3.251	74.79131	0.327909		
304	High pH	Synthetic	NT202	A	0.018	1.222	79.52282	0.345464	0.33814	0.007608
305	High pH	Synthetic	NT202	B	0.013	0.486	76.27146	0.330276		
306	High pH	Synthetic	NT202	C	0.016	0.792	78.10791	0.338681		
307	Low Temperature	Synthetic	IWBT PR7	A	0.017	0.654	90.95543	0.37224	0.369242	0.005608
308	Low Temperature	Synthetic	IWBT PR7	B	0.206	4.285	89.64709	0.372713		
309	Low Temperature	Synthetic	IWBT PR7	C	0.958	10.392	84.76776	0.362772		
310	Low Temperature	Synthetic	VIN2000	A	0.177	1.956	88.39329	0.363932	0.37769	0.013361
311	Low Temperature	Synthetic	VIN2000	B	3.46	11.962	89.68327	0.390615		
312	Low Temperature	Synthetic	VIN2000	C	0.074	1.252	92.24276	0.378523		
313	Low Temperature	Synthetic	VIN7	A	0.117	2.74	94.10553	0.388609	0.3946	0.009637
314	Low Temperature	Synthetic	VIN7	B	0.004	0.303	99.28283	0.405716		
315	Low Temperature	Synthetic	VIN7	C	0.026	1.256	94.92866	0.389475		
316	Low Temperature	Synthetic	VIN13	A	1.468	10.843	92.11146	0.395828	0.35449	0.042831
317	Low Temperature	Synthetic	VIN13	B	15.328	2.584	70.47235	0.310307		
318	Low Temperature	Synthetic	VIN13	C	2.465	15.362	81.18288	0.357335		
319	Low Temperature	Synthetic	WE14	A	1.036	15.8	85.73323	0.375725	0.38283	0.014184
320	Low Temperature	Synthetic	WE14	B	0.003	0.554	96.0385	0.39286		
321	Low Temperature	Synthetic	WE14	C	0.006	1.126	90.92047	0.372801		
322	Low Temperature	Synthetic	WE372	A	11.07	34.713	61.97721	0.311077	0.335283	0.024941
323	Low Temperature	Synthetic	WE372	B	7.298	34.468	67.85979	0.333872		
324	Low Temperature	Synthetic	WE372	C	1.621	30.698	76.76249	0.360899		
325	Low Temperature	Synthetic	228	A	8.869	2.034	48.46817	0.207028	0.315512	0.094457
326	Low Temperature	Synthetic	228	B	3.399	25.351	82.07974	0.37953		
327	Low Temperature	Synthetic	228	C	4.144	25.152	77.65458	0.359977		
328	Low Temperature	Synthetic	AWRI796	A	0.717	14.446	87.21323	0.379429	0.372175	0.015769
329	Low Temperature	Synthetic	AWRI796	B	1.185	13.411	81.58851	0.354085		
330	Low Temperature	Synthetic	AWRI796	C	0.487	12.478	88.87845	0.383011		
331	Low Temperature	Synthetic	EC1118	A	0.056	1.826	89.76455	0.369196	0.37712	0.007337
332	Low Temperature	Synthetic	EC1118	B	0.264	3.967	91.13411	0.378486		
333	Low Temperature	Synthetic	EC1118	C	1.294	9.48	89.87411	0.383679		
334	Low Temperature	Synthetic	N96	A	2.175	12.401	80.68343	0.350126	0.366329	0.014848
335	Low Temperature	Synthetic	N96	B	4.003	18.361	84.44866	0.379284		
336	Low Temperature	Synthetic	N96	C	0.033	1.26	90.0751	0.369578		
337	Low Temperature	Synthetic	NT45	A	2.899	14.208	82.59154	0.362387	0.320953	0.048449
338	Low	Synthetic	NT45	B	19.827	2.087	59.72059	0.267682		

	Temperature									
339	Low Temperature	Synthetic	NT45	C	9.813	2.562	77.42074	0.332789		
340	Low Temperature	Synthetic	NT50	A	58.237	1.499	30.83693	0.166433	0.20928	0.049805
341	Low Temperature	Synthetic	NT50	B	25.951	2.119	57.25755	0.263924		
342	Low Temperature	Synthetic	NT50	C	54.613	1.569	37.29145	0.197482		
343	Low Temperature	Synthetic	NT112	A	0.062	2.61	87.45604	0.360874	0.363122	0.007407
344	Low Temperature	Synthetic	NT112	B	2.055	17.966	80.34595	0.357099		
345	Low Temperature	Synthetic	NT112	C	0.027	1.958	90.26033	0.371393		
346	Low Temperature	Synthetic	NT116	A	0.268	6.467	91.2192	0.38282	0.320183	0.068446
347	Low Temperature	Synthetic	NT116	B	27.717	2.244	53.14554	0.247124		
348	Low Temperature	Synthetic	NT116	C	6.957	29.655	68.89953	0.330604		
349	Low Temperature	Synthetic	NT202	A	0.008	0.755	87.01866	0.356263	0.328224	0.057669
350	Low Temperature	Synthetic	NT202	B	23.556	2.333	57.3891	0.261898		
351	Low Temperature	Synthetic	NT202	C	0.234	6.335	87.39413	0.366512		
352	Low N; Low pH; Low Temp	Synthetic	Vin13	A	22.455	54.416	71.62899	0.513183	0.498504	0.013667
353	Low N; Low pH; Low Temp	Synthetic	Vin13	B	27.22	61.883	61.90868	0.486145		
354	Low N; Low pH; Low Temp	Synthetic	Vin13	C	24.265	58.742	66.21193	0.496185		
355	Low N; Low pH; Low Temp	Synthetic	EC1118	A	23.62	53.72	64.7123	0.465191	0.48298	0.026465
356	Low N; Low pH; Low Temp	Synthetic	EC1118	B	27.307	58.486	61.45475	0.470355		
357	Low N; Low pH; Low Temp	Synthetic	EC1118	C	23.192	53.874	71.5584	0.513394		
358	Med N; low pH; Low Temp	Synthetic	Vin13	A	32.025	69.079	52.95353	0.491621	0.5197	0.033178
359	Med N; low pH; Low Temp	Synthetic	Vin13	B	35.762	73.909	50.67974	0.511168		
360	Med N; low pH; Low Temp	Synthetic	Vin13	C	29.294	65.026	63.69534	0.556311		
361	Med N; low pH; Low Temp	Synthetic	EC1118	A	18.276	46.09	72.23052	0.500038	0.51051	0.012767
362	Med N; low pH; Low Temp	Synthetic	EC1118	B	32.492	62.363	57.75093	0.50676		
363	Med N; low pH; Low Temp	Synthetic	EC1118	C	28.682	60.089	62.99151	0.524732		
364	High N; Low pH; Low Temp	Synthetic	Vin13	A	41.814	78.569	45.98133	0.509122	0.494964	0.020408
365	High N; Low pH; Low Temp	Synthetic	Vin13	B	42.437	78.706	42.23157	0.471571		
366	High N; Low pH; Low Temp	Synthetic	Vin13	C	32.701	69.664	54.6214	0.504199		
367	High N; Low pH; Low Temp	Synthetic	EC1118	A	34.472	62.882	60.1729	0.530887	0.513744	0.016774
368	High N; Low pH; Low Temp	Synthetic	EC1118	B	42.589	72.759	48.91253	0.512979		
369	High N; Low pH; Low Temp	Synthetic	EC1118	C	27.668	57.122	62.62235	0.497366		
370	Low N; Med pH; Low Temp	Synthetic	Vin13	A	12.45	41.702	80.77837	0.519631	0.519806	0.010557
371	Low N; Med pH; Low Temp	Synthetic	Vin13	B	16.552	55.471	72.98042	0.530449		
372	Low N; Med pH; Low Temp	Synthetic	Vin13	C	8.991	38.605	82.51729	0.509337		
373	Low N; Med pH; Low Temp	Synthetic	EC1118	A	21.903	52.64	73.60879	0.544999	0.520008	0.024051
374	Low N; Med pH; Low Temp	Synthetic	EC1118	B	14.047	43.042	75.80413	0.497023		

375	Low N; Med pH; Low Temp	Synthetic	EC1118	C	13.546	39.172	81.26821	0.518004		
376	Med N; Med pH; Low Temp	Synthetic	Vin13	A	10.167	42.678	69.11448	0.43915	0.501551	0.054196
377	Med N; Med pH; Low Temp	Synthetic	Vin13	B	25.42	67.793	61.85899	0.528645		
378	Med N; Med pH; Low Temp	Synthetic	Vin13	C	13.116	44.74	81.80161	0.536857		
379	Med N; Med pH; Low Temp	Synthetic	EC1118	A	10.125	35.273	84.6345	0.513467	0.506088	0.006402
380	Med N; Med pH; Low Temp	Synthetic	EC1118	B	3.533	16.594	95.43404	0.502019		
381	Med N; Med pH; Low Temp	Synthetic	EC1118	C	7.175	28.467	87.77771	0.502778		
382	High N; Med pH; Low Temp	Synthetic	Vin13	A	21.109	58.891	65.98687	0.504881	0.506245	0.015056
383	High N; Med pH; Low Temp	Synthetic	Vin13	B	25.981	65.957	61.98515	0.521936		
384	High N; Med pH; Low Temp	Synthetic	Vin13	C	8.949	38.027	80.53774	0.491918		
385	High N; Med pH; Low Temp	Synthetic	EC1118	A	8.736	31.303	82.68453	0.484501	0.502687	0.017316
386	High N; Med pH; Low Temp	Synthetic	EC1118	B	9.719	33.523	84.49513	0.504581		
387	High N; Med pH; Low Temp	Synthetic	EC1118	C	7.373	28.513	90.72341	0.518977		
388	Low N; High pH; Low Temp	Synthetic	Vin13	A	0.0057	0.038	104.3439	0.497402	0.520799	0.025929
389	Low N; High pH; Low Temp	Synthetic	Vin13	B	1.556	16.253	105.3527	0.548676		
390	Low N; High pH; Low Temp	Synthetic	Vin13	C	0.0727	2.201	107.1609	0.516319		
391	Low N; High pH; Low Temp	Synthetic	EC1118	A	3.504	18.494	93.18613	0.496136	0.493717	0.004379
392	Low N; High pH; Low Temp	Synthetic	EC1118	B	0.043	2.516	101.2814	0.488662		
393	Low N; High pH; Low Temp	Synthetic	EC1118	C	2.116	12.487	96.89742	0.496353		
394	Med N; High pH; Low Temp	Synthetic	Vin13	A	6.162	36.887	82.07835	0.48738	0.508181	0.018207
395	Med N; High pH; Low Temp	Synthetic	Vin13	B	0.02	3.708	108.273	0.521224		
396	Med N; High pH; Low Temp	Synthetic	Vin13	C	0.1046	2.916	107.5399	0.515938		
397	Med N; High pH; Low Temp	Synthetic	EC1118	A	0.0448	0.819	102.0075	0.484383	0.504226	0.017588
398	Med N; High pH; Low Temp	Synthetic	EC1118	B	2.71	15.697	99.97931	0.517895		
399	Med N; High pH; Low Temp	Synthetic	EC1118	C	0.163	3.437	106.0898	0.5104		
400	High N; High pH; Low Temp	Synthetic	Vin13	A	0.0046	0.7074	106.7049	0.508152	0.508105	0.007171
401	High N; High pH; Low Temp	Synthetic	Vin13	B	0.0115	0.319	105.3753	0.500911		
402	High N; High pH; Low Temp	Synthetic	Vin13	C	0.0191	1.336	107.8644	0.515252		
403	High N; High pH; Low Temp	Synthetic	EC1118	A	0.0448	0.885	102.4833	0.488555	0.516415	0.024263
404	High N; High pH; Low Temp	Synthetic	EC1118	B	0.181	3.509	110.3159	0.532906		
405	High N; High pH; Low Temp	Synthetic	EC1118	C	3.112	18.289	99.90788	0.527784		
406	Low N; Low pH; Med Temp	Synthetic	Vin13	A	4.831	24.206	92.52749	0.493712	0.497811	0.006013
407	Low N; Low pH; Med Temp	Synthetic	Vin13	B	5.742	26.285	91.29008	0.495006		
408	Low N; Low pH; Med Temp	Synthetic	Vin13	C	7.909	27.446	91.4006	0.504714		
409	Low N; Low pH; Med Temp	Synthetic	EC1118	A	10.716	29.134	82.03558	0.46453	0.489305	0.021724
410	Low N; Low pH; Med Temp	Synthetic	EC1118	B	3.346	16.695	97.8678	0.498288		
411	Low N; Low pH;	Synthetic	EC1118	C	3.098	15.693	99.83638	0.505097		

	Med Temp									
412	Med N; low ; Med Temp	Synthetic	Vin13	A	8.766	29.374	83.75368	0.490717	0.49551	0.011501
413	Med N; low ; Med Temp	Synthetic	Vin13	B	6.656	24.194	86.70141	0.48718		
414	Med N; low ; Med Temp	Synthetic	Vin13	C	8.38	28.65	87.37585	0.508632		
415	Med N; low ; Med Temp	Synthetic	EC1118	A	3.862	18.844	93.11876	0.500343	0.520792	0.0193
416	Med N; low ; Med Temp	Synthetic	EC1118	B	0.508	6.041	105.8552	0.523344		
417	Med N; low ; Med Temp	Synthetic	EC1118	C	2.877	14.893	102.9143	0.538689		
418	High N; Low pH; Med Temp	Synthetic	Vin13	A	13.554	41.539	74.19761	0.476833	0.500985	0.03181
419	High N; Low pH; Med Temp	Synthetic	Vin13	B	26.678	65.803	63.48574	0.537027		
420	High N; Low pH; Med Temp	Synthetic	Vin13	C	18.206	47.47	70.92964	0.489096		
421	High N; Low pH; Med Temp	Synthetic	EC1118	A	5.736	21.459	88.03188	0.47973	0.501291	0.02465
422	High N; Low pH; Med Temp	Synthetic	EC1118	B	16.827	42.243	80.08443	0.528164		
423	High N; Low pH; Med Temp	Synthetic	EC1118	C	9.648	28.098	85.78055	0.495979		
424	Low N; Med pH; Med Temp	Synthetic	Vin13	A	1.11	11.155	102.1234	0.517499	0.513498	0.010225
425	Low N; Med pH; Med Temp	Synthetic	Vin13	B	1.426	12.337	102.0567	0.521117		
426	Low N; Med pH; Med Temp	Synthetic	Vin13	C	3.629	21.782	92.44298	0.501877		
427	Low N; Med pH; Med Temp	Synthetic	EC1118	A	3.053	14.706	91.06731	0.474689	0.506094	0.027239
428	Low N; Med pH; Med Temp	Synthetic	EC1118	B	0.764	5.851	106.2212	0.523282		
429	Low N; Med pH; Med Temp	Synthetic	EC1118	C	0.021	0.673	108.6991	0.520312		
430	Med N; Med pH; Med Temp	Synthetic		A	110.1217	100.1057				
431	Med N; Med pH; Med Temp	Synthetic	Vin13	B	3.418	19.884	91.37886	0.488852		
432	Med N; Med pH; Med Temp	Synthetic	Vin13	C	1.543	12.935	96.73981	0.494203		
433	Med N; Med pH; Med Temp	Synthetic	Vin13	A	4.006	20.553	91.61744	0.493447		
434	Med N; Med pH; Med Temp	Synthetic	EC1118	B	3.757	16.72	97.01197	0.511261		
435	Med N; Med pH; Med Temp	Synthetic	EC1118	C	6.855	25.247	88.03409	0.494226		
436	Med N; Med pH; Med Temp	Synthetic	EC1118	A	1.348	10.231	105.386	0.530516		
437	High N; Med pH; Med Temp	Synthetic	Vin13	B	0.4	5.822	102.2876	0.500242	0.513315	0.031725
438	High N; Med pH; Med Temp	Synthetic	Vin13	C	7.907	41.545	88.60266	0.549487		
439	High N; Med pH; Med Temp	Synthetic	Vin13	A	5.87	24.086	88.60249	0.490215		
440	High N; Med pH; Med Temp	Synthetic	EC1118	B	5.411	21.67	91.10874	0.496189	0.49546	0.01042
441	High N; Med pH; Med Temp	Synthetic	EC1118	C	5.946	23.131	91.80894	0.505497		
442	High N; Med pH; Med Temp	Synthetic	EC1118	A	0.908	6.699	98.4372	0.484695		
443	Low N; High pH; Med Temp	Synthetic	Vin13	B	0.005	0.116	102.0121	0.486465	0.502283	0.014586
444	Low N; High pH; Med Temp	Synthetic	Vin13	C	0.014	0.513	107.829	0.515202		
445	Low N; High pH; Med Temp	Synthetic	Vin13	A	0.011	0.432	105.7742	0.505181		
446	Low N; High pH; Med Temp	Synthetic	EC1118	B	0.006	0.074	104.9276	0.50027	0.508406	0.008161
447	Low N; High pH; Med Temp	Synthetic	EC1118	C	0.12	1.925	105.6239	0.508353		

448	Low N; High pH; Med Temp	Synthetic	EC1118	A	0.512	4.986	105.5522	0.516593		
449	Med N; High pH; Med Temp	Synthetic	Vin13	B	0.01	0.128	107.4382	0.508419	0.509912	0.013186
450	Med N; High pH; Med Temp	Synthetic	Vin13	C	0.006	1.183	104.6156	0.497536		
451	Med N; High pH; Med Temp	Synthetic	Vin13	A	0.013	0.431	110.5241	0.52378		
452	Med N; High pH; Med Temp	Synthetic	EC1118	B	0.014	0.015	102.3942	0.4843	0.499214	0.013272
453	Med N; High pH; Med Temp	Synthetic	EC1118	C	0.02	0.146	107.6999	0.509724		
454	Med N; High pH; Med Temp	Synthetic	EC1118	A	0.3	3.358	104.6508	0.503617		
455	High N; High pH; Med Temp	Synthetic	Vin13	B	0.005	0.319	102.3594	0.486559	0.501741	0.016291
456	High N; High pH; Med Temp	Synthetic	Vin13	C	0.003	0.764	104.9054	0.499714		
457	High N; High pH; Med Temp	Synthetic	Vin13	A	0.005	0.133	109.2704	0.518951		
458	High N; High pH; Med Temp	Synthetic	EC1118	B	0.003	0.016	102.2613	0.485389	0.498918	0.016544
459	High N; High pH; Med Temp	Synthetic	EC1118	C	0.013	0.056	104.0515	0.494004		
460	High N; High pH; Med Temp	Synthetic	EC1118	A	0.008	0.017	108.9943	0.517363		
461	Low N; Low pH; High Temp	Synthetic	Vin13	B	10.367	29.099	83.04551	0.469229	0.474831	0.008783
462	Low N; Low pH; High Temp	Synthetic	Vin13	C	7.607	26.218	85.89017	0.470312		
463	Low N; Low pH; High Temp	Synthetic	Vin13	A	7.818	26.907	88.12775	0.484954		
464	Low N; Low pH; High Temp	Synthetic	EC1118	B	3.223	15.585	92.64236	0.468741	0.482946	0.016898
465	Low N; Low pH; High Temp	Synthetic	EC1118	C	2.319	12.756	101.0158	0.501633		
466	Low N; Low pH; High Temp	Synthetic	EC1118	A	8.08	25.901	87.30421	0.478463		
467	Med N; low pH; High Temp	Synthetic	Vin13	B	11.162	29.663	77.76275	0.462898	0.485027	0.021499
468	Med N; low pH; High Temp	Synthetic	Vin13	C	9.339	28.051	86.71342	0.505836		
469	Med N; low pH; High Temp	Synthetic	Vin13	A	9.718	28.938	82.75684	0.486347		
470	Med N; low pH; High Temp	Synthetic	EC1118	B	4.643	19.85	91.18166	0.494684	0.503294	0.016251
471	Med N; low pH; High Temp	Synthetic	EC1118	C	4.269	19.208	96.75411	0.522039		
472	Med N; low pH; High Temp	Synthetic	EC1118	A	6.261	22.62	88.73659	0.493159		
473	High N; Low pH; High Temp	Synthetic	Vin13	B	14.995	39.663	76.6302	0.491093	0.500123	0.012058
474	High N; Low pH; High Temp	Synthetic	Vin13	C	20.677	52.775	70.51927	0.513817		
475	High N; Low pH; High Temp	Synthetic	Vin13	A	21.832	51.085	68.26485	0.495459		
476	High N; Low pH; High Temp	Synthetic	EC1118	B	9.898	27.208	80.27126	0.462413	0.486453	0.024488
477	High N; Low pH; High Temp	Synthetic	EC1118	C	23.387	51.716	69.33871	0.511366		
478	High N; Low pH; High Temp	Synthetic	EC1118	A	21.796	50.074	67.41201	0.485579		
479	Low N; Med pH; High Temp	Synthetic	Vin13	B	0.252	4.365	96.51586	0.470836	0.492716	0.019366
480	Low N; Med pH; High Temp	Synthetic	Vin13	C	2.735	16.229	95.2551	0.499656		
481	Low N; Med pH; High Temp	Synthetic	Vin13	A	0.731	8.427	101.7583	0.507656		
482	Low N; Med pH; High Temp	Synthetic	EC1118	B	1.284	7.847	98.20369	0.489857	0.495585	0.005482
483	Low N; Med pH; High Temp	Synthetic	EC1118	C	0.102	1.613	103.138	0.496117		
484	Low N; Med pH;	Synthetic	EC1118	A	1.049	7.456	100.7073	0.500781		

	High Temp										
485	Med N; Med pH; High Temp	Synthetic	Vin13	B	3.884	18.465	91.5783	0.487434	0.490767	0.010551
486	Med N; Med pH; High Temp	Synthetic	Vin13	C	7.392	29.367	87.18207	0.502582		
487	Med N; Med pH; High Temp	Synthetic	Vin13	A	7.485	29.208	83.69317	0.482286		
488	Med N; Med pH; High Temp	Synthetic	EC1118	B	1.588	9.067	96.48229	0.483445	0.491552	0.007055
489	Med N; Med pH; High Temp	Synthetic	EC1118	C	1.87	10.382	97.97899	0.494905		
490	Med N; Med pH; High Temp	Synthetic	EC1118	A	5.812	21.081	90.98963	0.496304		
491	High N; Med pH; High Temp	Synthetic	Vin13	B	6.132	23.26	85.49077	0.471528	0.498318	0.02468
492	High N; Med pH; High Temp	Synthetic	Vin13	C	0.667	7.912	101.7265	0.5033		
493	High N; Med pH; High Temp	Synthetic	Vin13	A	7.282	25.796	92.38496	0.520127		
494	High N; Med pH; High Temp	Synthetic	EC1118	B	3.421	14.477	92.47106	0.479622	0.500231	0.023278
495	High N; Med pH; High Temp	Synthetic	EC1118	C	4.206	17.011	93.90508	0.495591		
496	High N; Med pH; High Temp	Synthetic	EC1118	A	4.237	17.459	99.31662	0.525479		
497	Low N; High pH; High Temp	Synthetic	Vin13	B	0.042	1.55	100.2927	0.481645	0.506201	0.021834
498	Low N; High pH; High Temp	Synthetic	Vin13	C	0.234	5.877	104.6123	0.513534		
499	Low N; High pH; High Temp	Synthetic	Vin13	A	0.017	0.183	109.7211	0.523425		
500	Low N; High pH; High Temp	Synthetic	EC1118	B	0.033	0.446	105.1029	0.502061	0.503445	0.00447
501	Low N; High pH; High Temp	Synthetic	EC1118	C	0.029	0.373	106.4778	0.508442		
502	Low N; High pH; High Temp	Synthetic	EC1118	A	1.732	10.472	98.77531	0.49983		
503	Med N; High pH; High Temp	Synthetic	Vin13	B	0.025	1.226	104.9231	0.499146	0.508301	0.012281
504	Med N; High pH; High Temp	Synthetic	Vin13	C	0.86	10.783	100.6061	0.5035		
505	Med N; High pH; High Temp	Synthetic	Vin13	A	0.368	6.443	106.8776	0.522258		
506	Med N; High pH; High Temp	Synthetic	EC1118	B	0.01	0.163	101.0659	0.478343	0.495717	0.015766
507	Med N; High pH; High Temp	Synthetic	EC1118	C	0.195	2.442	104.3463	0.499696		
508	Med N; High pH; High Temp	Synthetic	EC1118	A	0.087	1.085	107.0581	0.509111		
509	High N; High pH; High Temp	Synthetic	Vin13	B	0.806	9.694	103.746	0.518217	0.49732	0.031706
510	High N; High pH; High Temp	Synthetic	Vin13	C	0.02	0.823	96.70896	0.460837		
511	High N; High pH; High Temp	Synthetic	Vin13	A	0.085	2.813	106.5816	0.512905		
512	High N; High pH; High Temp	Synthetic	EC1118	B	0.053	0.812	109.8068	0.523306	0.516924	0.010507
513	High N; High pH; High Temp	Synthetic	EC1118	C	0.178	1.685	105.4192	0.504797		
514	High N; High pH; High Temp	Synthetic	EC1118	A	0.067	0.989	109.5736	0.52267		
515	Control	Sauvignon Blanc	228	A	1.39	15.224	106.4703	0.502874	0.502264	0.001733
516	Control	Sauvignon Blanc	228	B	1.463	15.072	106.666	0.50361		
517	Control	Sauvignon Blanc	228	C	0	6.873	110.8008	0.500309		
518	Control	Sauvignon Blanc	EC1118	A	0	1.799	118.0437	0.521075	0.511338	0.00907
519	Control	Sauvignon Blanc	EC1118	B	0	0.05	114.8585	0.503131		
520	Control	Sauvignon Blanc	EC1118	C	0.092	2.192	115.2438	0.509807		

521	Control	Sauvignon Blanc	VIN 7	A	0.005	0.35	115.7513	0.50772	0.502093	0.006326
522	Control	Sauvignon Blanc	VIN 7	B	0.006	0.376	112.8941	0.495246		
523	Control	Sauvignon Blanc	VIN 7	C	0.008	0.411	114.7145	0.503313		
524	Control	Sauvignon Blanc	VIN 13	A	0.008	1.489	117.1029	0.516234	0.512004	0.004189
525	Control	Sauvignon Blanc	VIN 13	B	0.024	2.071	114.8989	0.507857		
526	Control	Sauvignon Blanc	VIN 13	C	0.005	1.221	116.2629	0.51192		
527	Low pH	Sauvignon Blanc	228	A	0.029	3.5639	113.4624	0.50485	0.507028	0.001973
528	Low pH	Sauvignon Blanc	228	B	0.015	2.3423	114.694	0.50754		
529	Low pH	Sauvignon Blanc	228	C	0.039	4.3318	113.9309	0.508695		
530	Low pH	Sauvignon Blanc	EC1118	A	0.007	0.61	116.5718	0.511907	0.508546	0.010755
531	Low pH	Sauvignon Blanc	EC1118	B	0.001	0.184	113.2805	0.496512		
532	Low pH	Sauvignon Blanc	EC1118	C	0.001	0.394	117.8962	0.517219		
533	Low pH	Sauvignon Blanc	VIN 7	A	0.02	0.689	105.2832	0.462522	0.494461	0.027803
534	Low pH	Sauvignon Blanc	VIN 7	B	0.006	0.23	115.7874	0.507613		
535	Low pH	Sauvignon Blanc	VIN 7	C	0.005	0.26	117.0577	0.513247		
536	Low pH	Sauvignon Blanc	VIN 13	A	0.008	0.138	114.5499	0.50199	0.50284	0.00388
537	Low pH	Sauvignon Blanc	VIN 13	B	0.003	0.138	115.7127	0.507075		
538	Low pH	Sauvignon Blanc	VIN 13	C	0.012	0.188	113.9447	0.499456		
539	High Nitrogen	Sauvignon Blanc	228	A	0.185	3.5483	113.3184	0.504524	0.502923	0.008064
540	High Nitrogen	Sauvignon Blanc	228	B	0.126	2.8105	114.9696	0.510067		
541	High Nitrogen	Sauvignon Blanc	228	C	0.23	4.2179	110.6414	0.494178		
542	High Nitrogen	Sauvignon Blanc	EC1118	A	0.029	1.212	115.8539	0.510152	0.501959	0.007115
543	High Nitrogen	Sauvignon Blanc	EC1118	B	0.024	1.14	112.9808	0.497332		
544	High Nitrogen	Sauvignon Blanc	EC1118	C	0.011	0.812	113.3918	0.498393		
545	High Nitrogen	Sauvignon Blanc	VIN 7	A	0.004	0.237	116.3066	0.509901	0.509416	0.009077
546	High Nitrogen	Sauvignon Blanc	VIN 7	B	0.004	0.258	114.0624	0.500108		
547	High Nitrogen	Sauvignon Blanc	VIN 7	C	0.004	0.241	118.207	0.518241		
548	High Nitrogen	Sauvignon Blanc	VIN 13	A	0.012	1.331	113.9212	0.501867	0.504821	0.006802
549	High Nitrogen	Sauvignon Blanc	VIN 13	B	0	0.368	113.9836	0.499994		
550	High Nitrogen	Sauvignon Blanc	VIN 13	C	0.069	2.502	115.728	0.5126		
551	High pH	Sauvignon Blanc	228	A	0.379	6.9128	110.9368	0.501872	0.502446	0.000498
552	High pH	Sauvignon Blanc	228	B	0.533	8.0181	110.4858	0.502696		
553	High pH	Sauvignon Blanc	228	C	0.176	5.03	112.1838	0.502769		
554	High pH	Sauvignon Blanc	EC1118	A	0.099	2.228	112.8866	0.499475	0.521315	0.034077
555	High pH	Sauvignon Blanc	EC1118	B	0.141	2.493	113.7294	0.503888		
556	High pH	Sauvignon Blanc	EC1118	C	0.154	2.585	126.4663	0.560581		
557	High pH	Sauvignon	VIN 7	A	0.022	0.799	117.7964	0.517748	0.512541	0.006458

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558	High pH	Sauvignon Blanc	VIN 7	B	0.012	0.597	117.1797	0.514559		
559	High pH	Sauvignon Blanc	VIN 7	C	0.034	1.119	114.7997	0.505315		
560	High pH	Sauvignon Blanc	VIN 13	A	0.043	2.26	112.9299	0.499613	0.498221	0.001327
561	High pH	Sauvignon Blanc	VIN 13	B	0.079	1.1157	112.8833	0.49697		
562	High pH	Sauvignon Blanc	VIN 13	C	0.108	1.7157	112.8221	0.49808		
563	High Sugar	Sauvignon Blanc	228	A	16.7868	64.0089	96.42234	0.427017	0.459909	0.02849
564	High Sugar	Sauvignon Blanc	228	B	12.5611	60.6675	111.293	0.476892		
565	High Sugar	Sauvignon Blanc	228	C	12.5238	60.6341	111.0759	0.475818		
566	High Sugar	Sauvignon Blanc	EC1118	A	5.9571	28.3183	130.3858	0.478788	0.465986	0.019269
567	High Sugar	Sauvignon Blanc	EC1118	B	4.5786	24.0742	132.1209	0.475345		
568	High Sugar	Sauvignon Blanc	EC1118	C	5.4903	26.1719	122.0244	0.443826		
569	High Sugar	Sauvignon Blanc	VIN 7	A	3.107	20.4829	135.3094	0.478108	0.45527	0.035874
570	High Sugar	Sauvignon Blanc	VIN 7	B	5.2847	28.0375	113.1155	0.413921		
571	High Sugar	Sauvignon Blanc	VIN 7	C	5.1982	27.2364	129.8943	0.473781		
572	High Sugar	Sauvignon Blanc	VIN 13	A	3.6209	27.5687	129.6689	0.470821	0.457785	0.018356
573	High Sugar	Sauvignon Blanc	VIN 13	B	2.9749	28.1289	128.3098	0.465741		
574	High Sugar	Sauvignon Blanc	VIN 13	C	7.58	36.2609	114.7713	0.436793		
575	Low Temp	Sauvignon Blanc	228	A	0.759	14.6819	104.2244	0.489554	0.498028	0.008423
576	Low Temp	Sauvignon Blanc	228	B	0.731	14.5206	107.9065	0.506399		
577	Low Temp	Sauvignon Blanc	228	C	0.174	8.3528	109.4949	0.498133		
578	Low Temp	Sauvignon Blanc	EC1118	A	0.366	4.1379	112.3233	0.501816	0.500283	0.006467
579	Low Temp	Sauvignon Blanc	EC1118	B	0.273	2.9574	111.0201	0.493188		
580	Low Temp	Sauvignon Blanc	EC1118	C	0.345	3.9084	113.352	0.505846		
581	Low Temp	Sauvignon Blanc	VIN 7	A	0.128	1.0694	115.3059	0.507642	0.526908	0.034193
582	Low Temp	Sauvignon Blanc	VIN 7	B	0.312	3.5215	127.1563	0.566387		
583	Low Temp	Sauvignon Blanc	VIN 7	C	0.175	1.9182	114.6369	0.506695		
584	Low Temp	Sauvignon Blanc	VIN 13	A	0.009	1.524	115.518	0.509328	0.49747	0.021427
585	Low Temp	Sauvignon Blanc	VIN 13	B	0.059	1.3821	115.796	0.510347		
586	Low Temp	Sauvignon Blanc	VIN 13	C	0.097	2.4777	106.7262	0.472736		
587	High Temp	Sauvignon Blanc	228	A	0.002	0.499	113.2824	0.497209	0.487272	0.008609
588	High Temp	Sauvignon Blanc	228	B	0.043	2.427	108.8859	0.482078		
589	High Temp	Sauvignon Blanc	228	C	0.111	1.6394	109.3346	0.482528		
590	High Temp	Sauvignon Blanc	EC1118	A	0.01	0.306	107.7485	0.472536	0.523651	0.045779
591	High Temp	Sauvignon Blanc	EC1118	B	0.013	0.412	122.5126	0.537542		
592	High Temp	Sauvignon Blanc	EC1118	C	0.012	0.407	127.8341	0.560876		
593	High Temp	Sauvignon Blanc	VIN 7	A	0.011	0.162	112.8292	0.494508	0.506361	0.01717

594	High Temp	Sauvignon Blanc	VIN 7	B	0.015	0.308	119.9474	0.526051		
595	High Temp	Sauvignon Blanc	VIN 7	C	0.012	0.246	113.7033	0.498524		
596	High Temp	Sauvignon Blanc	VIN 13	A	0.012	0.646	115.6043	0.50775	0.503915	0.003453
597	High Temp	Sauvignon Blanc	VIN 13	B	0.016	0.873	113.964	0.501054		
598	High Temp	Sauvignon Blanc	VIN 13	C	0.114	2.381	113.5855	0.502941		
599	Control	Chardonnay	228	A	0.004	0.4	116.5762	0.574345	0.545346	0.025137
600	Control	Chardonnay	228	B	0.003	0.446	107.505	0.529771		
601	Control	Chardonnay	228	C	0.002	0.202	108.0717	0.531921		
602	Control	Chardonnay	EC1118	A	0.021	1.227	104.6683	0.517831	0.513823	0.009516
603	Control	Chardonnay	EC1118	B	0.012	0.891	105.4237	0.520679		
604	Control	Chardonnay	EC1118	C	0.022	1.226	101.6624	0.502959		
605	Control	Chardonnay	VIN 7	A	0.027	1.184	105.1205	0.519973	0.509314	0.017488
606	Control	Chardonnay	VIN 7	B	0.049	1.358	104.7894	0.518838		
607	Control	Chardonnay	VIN 7	C	0.001	0.254	99.35288	0.489131		
608	Control	Chardonnay	VIN 13	A	0.024	1.52	107.5798	0.533015	0.52718	0.009256
609	Control	Chardonnay	VIN 13	B	0.021	1.491	107.395	0.532016		
610	Control	Chardonnay	VIN 13	C	0.01	1.064	104.4907	0.516508		
611	Low pH	Chardonnay	228	A	0	0.138	101.9304	0.501531	0.497611	0.010686
612	Low pH	Chardonnay	228	B	0	0.09	102.8186	0.505782		
613	Low pH	Chardonnay	228	C	0	0.051	98.71823	0.485519		
614	Low pH	Chardonnay	EC1118	A	0.008	0.168	103.0084	0.50693	0.505176	0.00169
615	Low pH	Chardonnay	EC1118	B	0.023	0.07	102.6662	0.50504		
616	Low pH	Chardonnay	EC1118	C	0.007	0.267	102.2738	0.503558		
617	Low pH	Chardonnay	VIN 7	A	0.003	0.48	113.2197	0.558026	0.520343	0.033568
618	Low pH	Chardonnay	VIN 7	B	0.003	0.369	100.211	0.49364		
619	Low pH	Chardonnay	VIN 7	C	0.002	0.372	103.4017	0.509362		
620	Low pH	Chardonnay	VIN 13	A	0.007	0.2	97.56714	0.480226	0.497382	0.019405
621	Low pH	Chardonnay	VIN 13	B	0.006	0.285	100.218	0.493477		
622	Low pH	Chardonnay	VIN 13	C	0.004	0.404	105.2272	0.518442		
623	High Nitrogen	Chardonnay	228	A	0.01	1.429	105.9542	0.524688	0.52197	0.023532
624	High Nitrogen	Chardonnay	228	B	0.015	0.75	110.2257	0.544025		
625	High Nitrogen	Chardonnay	228	C	0.014	1.794	100.2192	0.497197		
626	High Nitrogen	Chardonnay	EC1118	A	0.054	1.833	99.25445	0.492604	0.506122	0.011722
627	High Nitrogen	Chardonnay	EC1118	B	0.095	2.322	103.1878	0.513476		
628	High Nitrogen	Chardonnay	EC1118	C	0.07	1.994	103.1298	0.512287		
629	High Nitrogen	Chardonnay	VIN 7	A	0.002	0.329	104.1079	0.512732	0.515678	0.008012
630	High Nitrogen	Chardonn	VIN 7	B	0.009	0.507	106.4502	0.524746		

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631	High Nitrogen	Chardonnay	VIN 7	C	0.003	0.333	103.4604	0.509556		
632	High Nitrogen	Chardonnay	VIN 13	A	0.014	1.294	105.0359	0.519804	0.519484	0.016945
633	High Nitrogen	Chardonnay	VIN 13	B	0.009	1.028	108.5078	0.536266		
634	High Nitrogen	Chardonnay	VIN 13	C	0.009	0.988	101.6716	0.502381		
635	High pH	Chardonnay	228	A	0.001	0.679	103.1074	0.508679	0.496004	0.014788
636	High pH	Chardonnay	228	B	0.002	0.782	101.2101	0.499575		
637	High pH	Chardonnay	228	C	0.019	0.469	97.3372	0.479758		
638	High pH	Chardonnay	EC1118	A	0.011	0.561	102.3492	0.50467	0.502384	0.013511
639	High pH	Chardonnay	EC1118	B	0.024	0.956	98.74445	0.487877		
640	High pH	Chardonnay	EC1118	C	0.018	0.818	104.2286	0.514606		
641	High pH	Chardonnay	VIN 7	A	0.003	0.08	102.1882	0.502664	0.466103	0.077093
642	High pH	Chardonnay	VIN 7	B	0.021	0.101	76.73496	0.377532		
643	High pH	Chardonnay	VIN 7	C	0.003	0.131	105.3026	0.518114		
644	High pH	Chardonnay	VIN 13	A	0.004	0.274	98.48429	0.484909	0.493758	0.018105
645	High pH	Chardonnay	VIN 13	B	0.001	0.158	104.5729	0.514586		
646	High pH	Chardonnay	VIN 13	C	0	0.121	97.92425	0.481779		
647	High Sugar	Chardonnay	228	A	8.4575	50.2801	124.0184	0.512129	0.515517	0.003929
648	High Sugar	Chardonnay	228	B	11.6076	68.7725	113.4792	0.514599		
649	High Sugar	Chardonnay	228	C	12.1828	71.039	113.1542	0.519823		
650	High Sugar	Chardonnay	EC1118	A	9.5678	47.3187	123.3285	0.505417	0.527076	0.02596
651	High Sugar	Chardonnay	EC1118	B	10.6335	48.7644	125.5711	0.519958		
652	High Sugar	Chardonnay	EC1118	C	16.6074	54.1522	127.9241	0.555852		
653	High Sugar	Chardonnay	VIN 7	A	4.6806	34.0155	146.7813	0.559798	0.5491	0.014068
654	High Sugar	Chardonnay	VIN 7	B	6.1517	39.2014	136.2486	0.533165		
655	High Sugar	Chardonnay	VIN 7	C	4.9304	34.8788	144.7322	0.554337		
656	High Sugar	Chardonnay	VIN 13	A	3.737	33.7276	144.9268	0.550142	0.535602	0.012592
657	High Sugar	Chardonnay	VIN 13	B	5.4994	40.7497	134.5103	0.528215		
658	High Sugar	Chardonnay	VIN 13	C	7.8398	45.7752	130.678	0.528451		
659	Low Temp	Chardonnay	228	A	0.011	2.152	109.9898	0.546633	0.555932	0.00828
660	Low Temp	Chardonnay	228	B	0.014	2.257	113.1232	0.562507		
661	Low Temp	Chardonnay	228	C	0.012	2.284	112.3347	0.558656		
662	Low Temp	Chardonnay	EC1118	A	0.02	1.571	115.8669	0.574209	0.567134	0.00616
663	Low Temp	Chardonnay	EC1118	B	0.11	2.1031	113.5023	0.56423		
664	Low Temp	Chardonnay	EC1118	C	0.08	1.4668	113.6227	0.562964		
665	Low Temp	Chardonnay	VIN 7	A	0.002	0.618	115.8691	0.57147	0.56305	0.007981
666	Low Temp	Chardonnay	VIN 7	B	0.024	0.954	112.4518	0.555596		

667	Low Temp	Chardonnay	VIN 7	C	0.008	0.962	113.7696	0.562085		
668	Low Temp	Chardonnay	VIN 13	A	0.008	1.222	111.6435	0.552291	0.55743	0.005289
669	Low Temp	Chardonnay	VIN 13	B	0.009	1.349	113.7072	0.562856		
670	Low Temp	Chardonnay	VIN 13	C	0.024	1.942	112.2141	0.557142		
671	High Temp	Chardonnay	228	A	0.018	1.492	111.5494	0.55259	0.543686	0.01007
672	High Temp	Chardonnay	228	B	0.047	2.441	109.6269	0.545711		
673	High Temp	Chardonnay	228	C	0.117	2.7109	106.8436	0.532757		
674	High Temp	Chardonnay	EC1118	A	0.075	1.622	108.9457	0.540193	0.545845	0.004928
675	High Temp	Chardonnay	EC1118	B	0.072	1.402	110.8921	0.549236		
676	High Temp	Chardonnay	EC1118	C	0.048	1.294	110.7363	0.548106		
677	High Temp	Chardonnay	VIN 7	A	0.002	0.54	108.6334	0.535577	0.551088	0.019754
678	High Temp	Chardonnay	VIN 7	B	0.01	0.588	116.2583	0.573327		
679	High Temp	Chardonnay	VIN 7	C	0.011	0.456	110.4559	0.544361		
680	High Temp	Chardonnay	VIN 13	A	0.006	0.871	111.9903	0.55304	0.538984	0.012619
681	High Temp	Chardonnay	VIN 13	B	0.008	2.401	107.5745	0.535283		
682	High Temp	Chardonnay	VIN 13	C	0.093	0.407	107.2463	0.528629		
683	Control	Shiraz	228	A	1.141	0	116.4165	0.536751	0.547024	0.01094
684	Control	Shiraz	228	B	2.489	0.011	117.6363	0.545795		
685	Control	Shiraz	228	C	1.735	0	120.8078	0.558527		
686	Control	Shiraz	EC1118	A	0.279	0	122.9143	0.564467	0.566794	0.003211
687	Control	Shiraz	EC1118	B	0.211	0	124.2577	0.570458		
688	Control	Shiraz	EC1118	C	0.218	0	123.1646	0.565458		
689	Control	Shiraz	VIN 7	A	0.365	0	123.7532	0.568544	0.565696	0.006516
690	Control	Shiraz	VIN 7	B	0.323	0	124.1601	0.570303		
691	Control	Shiraz	VIN 7	C	0.362	0	121.5124	0.558241		
692	Control	Shiraz	VIN 13	A	0.093	0	120.3948	0.552424	0.55375	0.002932
693	Control	Shiraz	VIN 13	B	0.099	0	121.4129	0.557111		
694	Control	Shiraz	VIN 13	C	0.087	0	120.2437	0.551716		
695	Low pH	Shiraz	228	A	1.151	0	116.729	0.538217	0.530146	0.007001
696	Low pH	Shiraz	228	B	0.802	0	114.3714	0.526499		
697	Low pH	Shiraz	228	C	0.871	0	114.1663	0.525722		
698	Low pH	Shiraz	EC1118	A	0.227	0	114.1928	0.524289	0.535258	0.010169
699	Low pH	Shiraz	EC1118	B	0.268	0	118.5443	0.544371		
700	Low pH	Shiraz	EC1118	C	0.281	0	116.9574	0.537115		
701	Low pH	Shiraz	VIN 7	A	0.557	0	118.7104	0.545858	0.533091	0.011394
702	Low pH	Shiraz	VIN 7	B	0.575	0	113.9377	0.523955		
703	Low pH	Shiraz	VIN 7	C	0.538	0	115.1546	0.529461		
704	Low pH	Shiraz	VIN 13	A	0.167	0	116.2475	0.533576	0.532084	0.008011
705	Low pH	Shiraz	VIN 13	B	0.168	0	117.4819	0.539244		
706	Low pH	Shiraz	VIN 13	C	0.183	0	114.0291	0.523432		
707	High Nitrogen	Shiraz	228	A	0.396	0	117.225	0.538629	0.542415	0.005355

708	High Nitrogen	Shiraz	228	B	0.493	0	118.8202	0.546202		
709	High Nitrogen	Shiraz	EC1118	B	0.121	0	122.8537	0.563779	0.555237	0.012081
710	High Nitrogen	Shiraz	EC1118	C	0.154	0	119.1127	0.546695		
711	High Nitrogen	Shiraz	VIN 7	A	0.227	0	118.0306	0.54191	0.547772	0.012155
712	High Nitrogen	Shiraz	VIN 7	B	0.257	0	122.3344	0.561747		
713	High Nitrogen	Shiraz	VIN 7	C	0.278	0	117.5129	0.539659		
714	High Nitrogen	Shiraz	VIN 13	A	0.088	0	119.243	0.547127	0.543195	0.01328
715	High Nitrogen	Shiraz	VIN 13	B	0.081	0	120.7591	0.554065		
716	High Nitrogen	Shiraz	VIN 13	C	0.094	0	115.1568	0.528393		
717	High pH	Shiraz	228	A	4.4938	0.035	115.2016	0.539578	0.529115	0.012938
718	High pH	Shiraz	228	B	4.7702	0.028	113.6789	0.533119		
719	High pH	Shiraz	228	C	4.7223	0.035	109.7616	0.514649		
720	High pH	Shiraz	EC1118	A	0.339	0	121.3415	0.557397	0.54876	0.008498
721	High pH	Shiraz	EC1118	B	0.371	0	117.6259	0.540409		
722	High pH	Shiraz	EC1118	C	0.337	0	119.4003	0.548475		
723	High pH	Shiraz	VIN 7	A	0.212	0	123.1873	0.565546	0.54765	0.015744
724	High pH	Shiraz	VIN 7	B	0.227	0	116.7287	0.535932		
725	High pH	Shiraz	VIN 7	C	0.214	0	117.9422	0.541471		
726	High pH	Shiraz	VIN 13	A	0.048	0	120.9585	0.554896	0.543766	0.017089
727	High pH	Shiraz	VIN 13	B	0.043	0	114.2458	0.52409		
728	High pH	Shiraz	VIN 13	C	0.066	0	120.3853	0.552312		
729	High Sugar	Shiraz	228	A	40.0952	3.0788	128.9583	0.521908	0.506608	0.014405
730	High Sugar	Shiraz	228	B	41.3059	2.9371	121.3637	0.493306		
731	High Sugar	Shiraz	228	C	38.2749	3.9584	125.1587	0.50461		
732	High Sugar	Shiraz	EC1118	A	1.944	0.091	152.1147	0.527757	0.524906	0.008196
733	High Sugar	Shiraz	EC1118	B	1.757	0.078	148.7331	0.515666		
734	High Sugar	Shiraz	EC1118	C	1.677	0.072	153.2869	0.531296		
735	High Sugar	Shiraz	VIN 7	A	1.368	0.028	154.9097	0.536265	0.525064	0.018382
736	High Sugar	Shiraz	VIN 7	B	1.036	0.011	145.7219	0.50385		
737	High Sugar	Shiraz	VIN 7	C	1.013	0.011	154.766	0.535078		
738	High Sugar	Shiraz	VIN 13	A	0.927	0.016	150.5001	0.520184	0.508413	0.013676
739	High Sugar	Shiraz	VIN 13	B	0.733	0.006	142.8546	0.49341		
740	High Sugar	Shiraz	VIN 13	C	0.934	0.01	148.0288	0.511644		
741	Low Temp	Shiraz	228	A	21.9706	0.94	105.3485	0.539913	0.536429	0.025837
742	Low Temp	Shiraz	228	B	21.2462	0.993	99.66378	0.509027		
743	Low Temp	Shiraz	228	C	33.4856	1.321	102.6698	0.560347		
744	Low Temp	Shiraz	EC1118	A	1.978	0.096	118.0897	0.546818	0.532463	0.023413
745	Low Temp	Shiraz	EC1118	B	2.21	0.116	109.0277	0.505446		
746	Low Temp	Shiraz	EC1118	C	1.611	0.062	117.9426	0.545124		
747	Low Temp	Shiraz	VIN 7	A	0.338	0	122.0962	0.560862	0.550749	0.011582
748	Low Temp	Shiraz	VIN 7	B	0.338	0	117.1441	0.538114		
749	Low Temp	Shiraz	VIN 7	C	0.33	0	120.4482	0.553271		
750	Low Temp	Shiraz	VIN 13	A	0.714	0	121.6906	0.559966	0.534693	0.021993
751	Low Temp	Shiraz	VIN 13	B	0.733	0	112.9723	0.519894		

752	Low Temp	Shiraz	VIN 13	C	0.838	0	113.8578	0.524221		
753	High Temp	Shiraz	228	A	0.329	0	115.5368	0.530708	0.533999	0.004768
754	High Temp	Shiraz	228	B	0.24	0	117.4917	0.539468		
755	High Temp	Shiraz	228	C	0.233	0	115.8302	0.531822		
756	High Temp	Shiraz	EC1118	A	0.068	0	110.278	0.505946	0.526628	0.020298
757	High Temp	Shiraz	EC1118	B	0.072	0	119.1192	0.546519		
758	High Temp	Shiraz	EC1118	C	0.055	0	114.9651	0.527419		
759	High Temp	Shiraz	VIN 7	A	0.21	0	118.8939	0.54583	0.540159	0.004914
760	High Temp	Shiraz	VIN 7	B	0.157	0	117.1065	0.537494		
761	High Temp	Shiraz	VIN 7	C	0.195	0	117.0119	0.537153		
762	High Temp	Shiraz	VIN 13	A	0.11	0	112.8378	0.51779	0.522311	0.004133
763	High Temp	Shiraz	VIN 13	B	0.143	0	114.0098	0.523247		
764	High Temp	Shiraz	VIN 13	C	0.113	0	114.6027	0.525896		
765	Control	Cabernet Sauvignon	228	A	56.6133	13.2691	84.12494	0.495518	0.497704	0.003091
766	Control	Cabernet Sauvignon	228	B	60.7959	14.3552	82.23331	0.49989		
767	Control	Cabernet Sauvignon	228	C	94.8221	21.8553	90.41602	0.735229		
768	Control	Cabernet Sauvignon	EC1118	A	0.473	0.01	126.7142	0.529806	0.522668	0.01565
769	Control	Cabernet Sauvignon	EC1118	B	0.354	0.008	120.776	0.504722		
770	Control	Cabernet Sauvignon	EC1118	C	0.47	0.003	127.5974	0.533476		
771	Control	Cabernet Sauvignon	VIN 7	A	0.083	0	123.7747	0.516652	0.515863	0.011759
772	Control	Cabernet Sauvignon	VIN 7	B	0.072	0	120.6845	0.503729		
773	Control	Cabernet Sauvignon	VIN 7	C	0.079	0	126.3058	0.527208		
774	Control	Cabernet Sauvignon	VIN 13	A	1.461	0.016	125.7422	0.527936	0.526019	0.00493
775	Control	Cabernet Sauvignon	VIN 13	B	1.781	0.025	125.9888	0.529703		
776	Control	Cabernet Sauvignon	VIN 13	C	1.952	0.026	123.691	0.520419		
777	Low pH	Cabernet Sauvignon	228	A	56.4996	13.7992	80.36795	0.474553	0.49293	0.018027
778	Low pH	Cabernet Sauvignon	228	B	58.4264	13.9209	82.59148	0.493653		
779	Low pH	Cabernet Sauvignon	228	C	57.463	24.227	80.65413	0.510586		
780	Low pH	Cabernet Sauvignon	EC1118	A	0.48	0.017	122.6515	0.512849	0.507882	0.004916
781	Low pH	Cabernet Sauvignon	EC1118	B	0.524	0.011	120.2815	0.50302		
782	Low pH	Cabernet Sauvignon	EC1118	C	0.623	0.022	121.3631	0.507776		
783	Low pH	Cabernet Sauvignon	VIN 7	A	0.167	0.001	122.9578	0.513424	0.512396	0.004814
784	Low pH	Cabernet Sauvignon	VIN 7	B	0.152	0.001	121.4632	0.507151		
785	Low pH	Cabernet Sauvignon	VIN 7	C	0.18	0.002	123.7145	0.516613		
786	Low pH	Cabernet Sauvignon	VIN 13	A	0.976	0.013	121.4187	0.508741	0.513899	0.007389
787	Low pH	Cabernet Sauvignon	VIN 13	B	0.851	0.01	121.9259	0.510592		
788	Low pH	Cabernet Sauvignon	VIN 13	C	0.795	0.007	124.7676	0.522364		
789	High Nitrogen	Cabernet Sauvignon	228	A	46.958	7.9949	91.71545	0.496561	0.496386	0.006135
790	High Nitrogen	Cabernet Sauvignon	228	B	48.262	8.1451	89.82132	0.490166		

791	High Nitrogen	Cabernet Sauvignon	228	C	49.3455	8.5906	91.30088	0.502432		
792	High Nitrogen	Cabernet Sauvignon	EC1118	A	0.199	0.001	123.6242	0.516275	0.511753	0.007075
793	High Nitrogen	Cabernet Sauvignon	EC1118	B	0.163	0	120.6076	0.5036		
794	High Nitrogen	Cabernet Sauvignon	EC1118	C	0.156	0	123.4336	0.515384		
795	High Nitrogen	Cabernet Sauvignon	VIN 7	A	0.004	0	114.3561	0.47718	0.502602	0.022443
796	High Nitrogen	Cabernet Sauvignon	VIN 7	B	0.031	0	122.4368	0.510956		
797	High Nitrogen	Cabernet Sauvignon	VIN 7	C	0.035	0	124.5227	0.519669		
798	High Nitrogen	Cabernet Sauvignon	VIN 13	A	0.163	0	124.4183	0.519511	0.514098	0.007225
799	High Nitrogen	Cabernet Sauvignon	VIN 13	B	0.158	0	121.1597	0.505894		
800	High Nitrogen	Cabernet Sauvignon	VIN 13	C	0.168	0	123.7874	0.516888		
801	High pH	Cabernet Sauvignon	228	A	32.1969	3.0094	92.48891	0.452384	0.430248	0.067041
802	High pH	Cabernet Sauvignon	228	B	64.4305	17.8978	76.0545	0.483421		
803	High pH	Cabernet Sauvignon	228	C	2.175	18.5667	77.70038	0.354938		
804	High pH	Cabernet Sauvignon	EC1118	A	0.429	0.003	122.3371	0.511396	0.51671	0.004625
805	High pH	Cabernet Sauvignon	EC1118	B	0.439	0.013	124.3452	0.519833		
806	High pH	Cabernet Sauvignon	EC1118	C	0.387	0.004	124.1535	0.5189		
807	High pH	Cabernet Sauvignon	VIN 7	A	0.028	0	117.7687	0.491469	0.50706	0.014606
808	High pH	Cabernet Sauvignon	VIN 7	B	0.046	0	122.0289	0.509286		
809	High pH	Cabernet Sauvignon	VIN 7	C	0.028	0	124.7077	0.520426		
810	High pH	Cabernet Sauvignon	VIN 13	A	1.991	0.028	124.5023	0.523922	0.514803	0.008667
811	High pH	Cabernet Sauvignon	VIN 13	B	2.39	0.043	120.1935	0.506673		
812	High pH	Cabernet Sauvignon	VIN 13	C	2.449	0.048	121.8547	0.513815		
813	High Sugar	Cabernet Sauvignon	228	A	2.028	35.9828	80.19218	0.304169	0.306326	0.001934
814	High Sugar	Cabernet Sauvignon	228	B	1.962	37.4557	80.48133	0.306904		
815	High Sugar	Cabernet Sauvignon	228	C	1.775	35.9492	81.26531	0.307905		
816	High Sugar	Cabernet Sauvignon	EC1118	A	35.7979	6.7493	127.1778	0.490832	0.497235	0.008303
817	High Sugar	Cabernet Sauvignon	EC1118	B	33.6206	6.01555	132.7426	0.506616		
818	High Sugar	Cabernet Sauvignon	EC1118	C	30.5082	5.2818	131.405	0.494257		
819	High Sugar	Cabernet Sauvignon	VIN 7	A	2.1997	0.175	144.2502	0.481992	0.488974	0.008295
820	High Sugar	Cabernet Sauvignon	VIN 7	B	2.51	0.14	148.9468	0.498143		
821	High Sugar	Cabernet Sauvignon	VIN 7	C	1.839	0.178	145.8595	0.486787		
822	High Sugar	Cabernet Sauvignon	VIN 13	A	46.153	8.8671	118.2611	0.4795	0.488246	0.009187
823	High Sugar	Cabernet Sauvignon	VIN 13	B	46.155	9.0867	122.6685	0.497818		
824	High Sugar	Cabernet Sauvignon	VIN 13	C	44.3829	8.6051	121.2046	0.487419		
825	Low Temp	Cabernet Sauvignon	228	A	2.059	29.8825	64.66314	0.311311	0.32041	0.00794
826	Low Temp	Cabernet Sauvignon	228	B	2.05	30.9731	67.34691	0.325929		
827	Low Temp	Cabernet	228	C	2.029	28.335	67.80807	0.323991		

		Sauvignon								
828	Low Temp	Cabernet Sauvignon	EC1118	A	25.6692	4.6833	105.0547	0.50193	0.51742	0.021094
829	Low Temp	Cabernet Sauvignon	EC1118	B	27.5652	5.3601	111.9319	0.541443		
830	Low Temp	Cabernet Sauvignon	EC1118	C	28.1531	5.508	104.827	0.508887		
831	Low Temp	Cabernet Sauvignon	VIN 7	A	0.677	0.008	121.9392	0.510272	0.523116	0.011514
832	Low Temp	Cabernet Sauvignon	VIN 7	B	0.683	0.008	127.2507	0.532512		
833	Low Temp	Cabernet Sauvignon	VIN 7	C	0.626	0.016	125.8547	0.526562		
834	Low Temp	Cabernet Sauvignon	VIN 13	A	36.6675	8.3717	102.1113	0.524684	0.532729	0.009289
835	Low Temp	Cabernet Sauvignon	VIN 13	B	35.4696	14.679	102.8816	0.542895		
836	Low Temp	Cabernet Sauvignon	VIN 13	C	36.6973	8.782	103.0305	0.530607		
837	High Temp	Cabernet Sauvignon	228	A	37.7179	4.3775	99.53344	0.503817	0.503487	0.006371
838	High Temp	Cabernet Sauvignon	228	B	38.2608	8.873	98.12482	0.509686		
839	High Temp	Cabernet Sauvignon	228	C	41.3234	5.8741	95.64251	0.496957		
840	High Temp	Cabernet Sauvignon	EC1118	A	0.07	0.002	119.1807	0.497452	0.501859	0.011937
841	High Temp	Cabernet Sauvignon	EC1118	B	0.076	0	123.4721	0.515373		
842	High Temp	Cabernet Sauvignon	EC1118	C	0.773	0.007	117.7057	0.492752		
843	High Temp	Cabernet Sauvignon	VIN 7	A	0.055	0	119.1621	0.49734	0.514331	0.017412
844	High Temp	Cabernet Sauvignon	VIN 7	B	0.151	0.006	127.4446	0.532135		
845	High Temp	Cabernet Sauvignon	VIN 7	C	0.142	0.005	122.9912	0.513518		
846	High Temp	Cabernet Sauvignon	VIN 13	A	0.178	0.004	118.6685	0.495542	0.506815	0.009934
847	High Temp	Cabernet Sauvignon	VIN 13	B	0.191	0	122.2737	0.510616		
848	High Temp	Cabernet Sauvignon	VIN 13	C	0.175	0	123.161	0.514287		