Selection and domestication of yeast for improved enological and sensorial properties in wine production

MFS Project Report

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Food Science, Specialization of Enology

by

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Fall 2021

ABSTRACT

Natural yeasts often provide interesting flavor and aroma compounds due to the impact of the environment and terroir. However, their inability to efficiently ferment under harsh conditions has inhibited their potential to be the sole pioneer within the winemaking concert. With technological advancements, these indigenous yeast species are often used as starter cultures to refine these strains with specific characteristics required by the fermentation industry. The domestication of sophisticated commercial *Saccharomyces cerevisiae* yeast strains allows for a more direct approach in selecting strains that possess innovative and useful properties while also guaranteeing the preservation of the genetic biodiversity of the wine producing area. The improvement of wine yeasts permits the process of yeast strain selection to remain an important and fundamental step of the winemaking process. The potential of a particular strain is highlighted in its ability to survive through fermentative conditions as well as successfully maintaining desirable post-fermentative flavors.

BIOGRAPHICAL SKETCH

Gabriela Polanco is from Orange County, California and was inspired to pursue wine through family and friends. In 2018, she earned a B.S. in Plant Sciences from the University of California, Santa Cruz. After graduation, she studied abroad with UC Davis for an 'Introduction to Winemaking' course based in Burgundy, France where she had the opportunity to travel to different winemaking regions in the country and experience the wine and winemaking styles of those areas. After her month abroad, she returned to California and did a harvest internship in Napa Valley with Cakebread Cellars. She learned hands-on the applications and step by step process of making wine. She wanted to take her firsthand experience and apply it towards a more in-depth knowledge of winemaking where she then attended Cornell University to obtain a Masters in Food Science with a specialization in Enology and is a member of the Gibney lab. Her higher education allowed her to take the material she had physically learned during her time in Napa and propel her into a more thorough comprehension of wine through class and lab work. Gabriela hopes to return to Napa Valley and pursue a career in the winemaking industry.

ACKNOWLEDGEMENTS

To my advisor Patrick Gibney, thank you for always being more than willing to lend out advice, support, and encouragement. I am forever grateful for your guidance as well as your kindness and sense of humor. My completion of this degree would not have gone as smoothly without your helping hand especially throughout a pandemic. Thank you for everything.

To my parents, Jackie and Juan Polanco, thank you for always believing in me and pushing me to do my best, especially in moments when I couldn't do that for myself. I am beyond thankful for the sacrifices you have made for educating and preparing me for my future. I would not be where I am today without you.

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PREFACE

My first hands on experience with the winemaking process was during my time in Napa Valley while completing my first harvest. During that time, I remember being fascinated by yeast and being told by my fellow coworkers that they are alive so to speak kindly to them and possibly play them music to flourish. I also remember how fickle and sensitive they were, getting the temperature right during rehydration to bring them to an active state without accidentally killing them. I found it interesting that this singular component in this winemaking process was one of, if not the most crucial aspect, but also possibly the most sensitive. From the memories of this experience, I felt compelled to write this literature review based on the point in which yeast, specifically *Saccharomyces cerevisiae*, acts as the main character of the winemaking process both during and after fermentation.

INTRODUCTION

Most of the yeast strains utilized in the food industry for the production of beer, wine and bread are classified as *Saccharomyces cerevisiae.* This yeast is the most prominently known yeast used within the winemaking process. It exists in diverse niches across the world and can be found in natural habitats associated with fruits, tree soil, and insects. The coexistence of both wild and commercial *S. cerevisiae* indicates its unique ability to be domesticated from temperate forest soil to harsh oenological conditions (Buzzini *et al.* 2017). This adaptation has occurred through mechanisms that contribute to the manipulated evolution of wine yeast genomes. *Saccharomyces cerevisiae* strains naturally possess an ideal combination of traits that make fermentation processes successful, however the refinement of commercial strains have achieved genetic and molecular bounds that greatly improve enological traits (Rainieri and Pretorious, 2000). Genetic improvement of natural yeasts has been fundamental in providing strains with novel and useful characteristics for winemaking.

Saccharomyces cerevisiae is a vital component in carrying out the alcoholic fermentation process, which is essential to wine production. Yeast cells carry out vinification while sensing and responding to stress conditions that challenge their osmotic, ethanol, thermal, and oxidative tolerances. The enological parameters of yeasts are important to conduct a proper fermentation in winemaking. The systems that sense stressors initiate rapid synthesis of protective molecules and the activation of signal transduction pathways that induce secondary events such as the triggering of pre-existing enzyme activities and the transcription of genes encoding factors that have protective functions (Carrasco *et al.* 2000). The selection of a yeast strain that is suitable to endure and thrive based on the conditions presented throughout the winemaking process is instrumental towards production. The interaction between yeast strains and their potential

stressors during alcoholic fermentation can contribute to the aroma, taste and color of the final wine.

The purpose of this report is to discuss the domestication and genetic improvement of natural yeast to commercial yeast and compare the performance of those yeast strains, specifically of *Saccharomyces cerevisiae*. The potential impact of yeast strain variability from their biogeographical origin affected by terroir to their technologically significant characteristics under oenological conditions signifies the contributions in which each aspect of the viticultural and winemaking process enhances an expression of wine quality and style. *Saccharomyces cerevisiae* has evolved to ecological dynamics such as adaptation of vineyards and grape must, as well as enhanced fermentative capacities under specific oenological circumstances. There is value in understanding the genetic and phenotypic variation in these strains and the way their genomic expression ultimately affects the wine. Different strains are capable of changing the wine composition and subsequently its sensorial effects. This study will highlight that different strains show different properties based on their individual physicochemical and ecological properties.

CONTRIBUTION OF YEAST FROM NON-ENOLOGICAL ENVIRONMENTS

It is clear that the involvement of *Saccharomyces cerevisiae* yeast within the winemaking process is vital if not the spotlight of production. Wine yeasts can only grow in wine must for a short period every year, therefore they spend the rest of their lives in and around the vineyard or in insects (Gallone *et al.* 2016). Their contribution to wine composition and quality begins in the vineyard typically associated with the phyllosphere, grapes, and soil. *Saccharomyces cerevisiae* strains isolated from non-enological environments have been found to possess interesting

characteristics. However, these strains typically do not carry out the fermentation process as efficiently because they are not vigorous or competitive in oenological conditions (Rainieri and Pretorious 2000). Selecting a yeast strain for its sensorially elements is important, but if it is unable to initiate or finish fermentation, that is a much greater issue at hand. This portion will discuss the origins of *S. cerevisiae* within their natural environment and the inception of development towards its improvement for a more predictable and efficient process.

Development of Saccharomyces cerevisiae within non-enological environments

Humans have utilized *Saccharomyces cerevisiae* yeast to convert sugars into ethanol and desirable flavor compounds to create food and beverages since prehistoric times. However, we are unsure whether yeast diversity is shaped by selection and niche adaptation to societal needs (domestication) or neutral divergence caused by geographic isolation and limited dispersal (Gallone *et al.* 2016). Studies have used microsatellite-based research to rediscover the origin of *S. cerevisiae*, which has been shown to date back as far as 7000 BC from China where it further traveled towards Europe and later spread to the New World (Marsit and Dequin 2015). The discovery that suggests this is that Chinese isolates harbor almost twice as much genetic variation as isolates from the rest of the world (Liti 2015). Five distinct lineages were discovered based on their technological and geographic origin (Gallone *et al*. 2016). Studies have explored the genetic diversity of *Saccharomyces cerevisiae* strains. An approach often used to establish a relationship between genotypic variation and phenotypes is mapping of quantitative trait loci (QTL). This has allowed us to view the performance of yeast based on factors such as ethanol resistance, acetic acid production, and fermentation (Tofalo *et al.* 2013). Genomic analyses have been utilized to identify the genes impacting volatile formation (Bisson and Karpel 2010). The

two main types of analyses performed are investigations of changes in expression patterns of a single strain where the aroma compounds are produced and the second are comparisons of mRNA profiles of strains with differing levels of aroma compound production under the same growth (Bisson and Karpel 2010). Functional analyses based on the first genome sequence have provided insight into the adaptation of yeast towards the harsh conditions that we now apply towards *S. cerevisiae* within the fermentation process.

Recent studies have investigated *S. cerevisiae* populations to determine their ecological origin by sequencing the genomes of various strains. Wild yeasts show higher rates of spontaneous mutagenesis which can lead to the rapid creation of significant diversity across a population (Bisson 2012). However, strains rapidly lose some phenotypes associated with growth in the wild upon formulated strains (Bisson 2012). A study highlighted that *Saccharomyces cerevisiae* endured hybridization between different domesticated subpopulations, such as wine yeast and beer yeast, which are phenotypically separated from wild stocks due to human selection (Gallone *et al.* 2016). Throughout the year, *Saccharomyces cerevisiae* primarily reside in natural habitats such as insects as well as the vineyard itself (Mardit and Dequin 2015). Insects are important in terms of natural yeast in a vineyard because they are likely the main vectors that spread cultivable yeasts (Mardit and Dequin 2015). During nutrient-poor periods, wine yeasts likely undergo sexual cycles and even hybridize with wild yeasts (Gallone *et al.* 2016). Though *Saccharomyces cerevisiae* strains used in winemaking are genetically more homogenous, they still comprise a great deal of variation.

This variation has been studied based on global-scale results, however, studies have investigated the structure and gene flow based on smaller ecological levels. Analyses of vineyard isolates have provided evidence for region specific subpopulations (Marsit and Dequin 2015).

Between populations within a vineyard, there is evidence of gene flow across small distances, suggesting some degree of connectivity between populations. There is a lot of variability within environmental factors of a vineyard location and the interactions between each organism and its environment influence rearrangement and the evolution of phenotypes (Tofalo *et al.* 2013). Scott Labs has stated that majority of the strains that they sell under the Lallemand brand are isolated from a natural environment in which they are isolated and and commercialized for enological use. The importance of a geographical origin of wine yeast is established at a regional level based on yeast environmental isolates that are useful to tailor strains to meet enological demand and later, consumer demand.

Natural yeast diversity and their effect on wine production

After discussing the basic principles of genetic variation and evolutionary history of *Saccharomyces cerevisiae* yeast, we will now be able to highlight the natural form of this yeast and the diversity that later is utilized for industrial purposes. Originally, fermentation was brought about by the natural yeasts attached to the surface of the ripe harvested berries. The activity of yeast cells on the sugars of the grape juice resulted in the production of alcohol, carbon dioxide, heat, and many other by-products. Natural yeast found on the skin of berries comes in many different strains that will provide different attributes to the wine. The main yeast used in winemaking is *S. cerevisiae,* however, there are many other yeasts that are possibly present at the start of fermentation, they are just strongly inhibited by the high alcohol concentrations found in wine (Linskens and Jackson, 1988). This wine yeast can be isolated from vineyards and is most commonly isolated from heavily damaged grapes whether it be from mechanical harvesting, weather, or rot (Bisson 2012). Upon the crushing that can appear from

this damage, enriched and fermentative conditions are openly presented to this microbe (Bisson 2012). Various factors affect yeast flora present on grapes such as rainfall, temperature, soil type, berry maturity, insects, mechanical damage, and geographical location.

A study in India was conducted where they tested wild *Saccharomyces cerevisiae* yeast flora present on six separate grape cultivars to reveal their significance in wine production. The grapes were picked from different vineyards based in different districts all within Maharashtra, India. The isolation of *S. cerevisiae* yeast strains was carried out by the spread plate method on MGYP agar containing 0.025% tetracycline at 28°C for 48 hours. Nine isolates of the *S. cerevisiae* yeast strain, AM262831, were revealed within the grapes (Chavan *et al.* 2009). Chavan stated that although most studies indicate low concentrations of natural *S. cerevisiae* in grape juice and must, there were reported high counts of the yeast in one of the districts within Maharashtra, Anatolia, due to excessive use of sulfite in the vineyard. This further supports the notion that different factors of viticultural management and environment can affect yeast flora.

The natural availability of yeast strains is unlikely to provide the desirable medley of sensory aromatics and specific compounds one may strive to achieve in wine production. Wild yeast has been found to have a complex and interesting sensorial impact, however they do not ferment as efficiently as commercial yeast strains. Although *Saccharomyces cerevisiae* plays the main role in fermentation, it is not necessarily widespread on grapes as it depends on a vector, such as insects, for its dispersal. As previously stated, there are various other yeasts that grow on grapes, it is mostly that *S. cerevisiae* is one of the main species that is able to survive under the harsh condition of high ethanol, while the other microbial yeast species are inhibited severely as they are not ethanol-tolerant, sensitive to SO_2, and have more potential to produce undesirable compounds (Rainieri and Pretorius 2000). Knowing that *Saccharomyces cerevisiae* was able to

have the most fermentative success as well as it is in low abundance naturally, this began the use of indigenous yeast as starter cultures to produce commercial yeast strains which has improved the reproducibility, efficiency, survival, and possible sensorial effects of the winemaking process and finished product.

COMMERCIAL *Saccharomyces cerevisiae* YEAST STRAINS: ENOLOGICAL USE

We briefly introduced the idea of domestication of *Saccharomyces cerevisiae*, we will now be analyzing the evolutionary divergence of industrial yeasts that developed through industrial application and geographical origin. As previously stated, the concentration of natural yeast in vineyards is relatively low, therefore the domestication of wine yeast was necessary for production. The choice to develop and utilize particular *Saccharomyces cerevisiae* yeast strains for a specific industrial purpose, such as winemaking, has become both historical and scientific (Steensels *et al.* 2014). The likely main purpose was to cultivate yeast strains that will help steer the wine towards an ideal product. Selection of industrial yeast is able to provide certain desirable characteristics whether it be sensory attributes or specific forms of resistance to enological processes (Linskens and Jackson 1988). *Saccharomyces cerevisiae* was the most obvious candidate for yeast starter cultures as it was the most ethanol-tolerant yeast wine species (Rainieri and Pretorius 2000). Once starter cultures were developed, the ability to improve wine yeasts was possible. They could be modified to accommodate the stress factors that are encountered within fermentation. Evidence of this is to detect the differences amongst different *Saccharomyces cerevisiae* strains as they endure different tolerances. A study was done to test the genome of beer yeasts with wine yeasts through these different tolerances to see the difference in their tolerances (Gallone *et al.* 2016*)*. For the beer *S. cerevisiae* strain, it was

evident that it was able to have a significantly higher capacity to metabolize maltotriose while the wine *S. cerevisiae* strain did not (Figure 1). While under sulfite tolerance (Figure 1), wine showed a superior performance as it likely reflects the high-sugar and high-alcohol environment in winemaking while beer strains perform poorly in general stress conditions that are not usually encountered in the brewing environment (Gallone *et al*. 2016). This further highlights that beverage yeasts in general have been molded to improve and adapt under their respective conditions. Methods such as induced mutation and selection, hybridisation, and gene cloning and transformation were among the most widely used techniques for this improvement (Rainieri and Pretorius 2000). Hybridisation was the most common method and occurred because most *S. cerevisiae* strains in nature are homothallic, whereas commercial yeast strains are generally heterothallic, which can provide more direct spore-cell mating to construct better fermentative efficiency when cultivating wine strains (Raineri and Pretorius 2000). Homothallism is defined as self breeding as it produces both types of mating nuclei to form a zygote while heterothallism is defined as outbreeding as it only produces one type of mating nuclei and requires two organisms to form a zygote (Ni et al. 2011). These techniques ultimately are directed towards the construction of strains that will contain useful and innovative properties for winemaking, while guaranteeing the preservation of the genetic biodiversity of wine production.

The exploitation of the existing natural diversity of wine yeasts through the use of techniques to generate artificial diversity or genetic modification could potentially be labeled as the domestication of natural yeast flora. Granted, there still exists wild *S. cerevisiae* independently of domesticated strains, but in general the development of commercial yeast strains originated from the domestication and alteration of wild yeast strains (Steensels 2014). The genetic and phenotypic separation between wild and industrial *Saccharomyces cerevisiae* is

likely due to human selection and manipulation (Gallone *et al.* 2106). The result of this domestication has led to the selection of superior industrial yeast strains that are able to survive under harsh conditions that natural yeast flora may not.

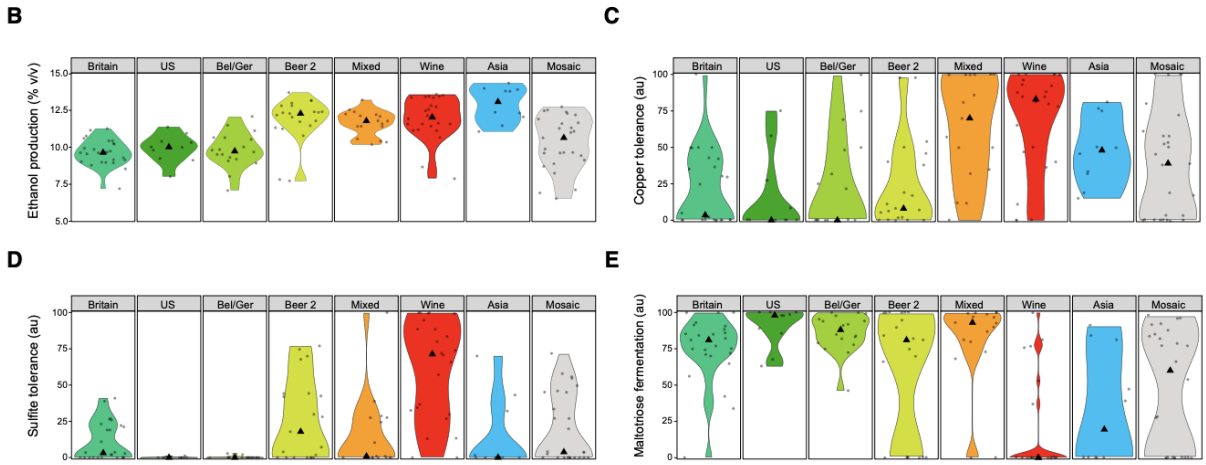

Figure 1: Observes different tolerances of *Saccharomyces cerevisiae* strains which are represented by each point on each plot by measuring the growth of all strains from different subpopulations. Looking specifically at D and E, domestication is evident.

YEAST IN WINEMAKING

The development of more robust strains while still capable of producing a high-quality end product has been the main focus of the exploitation of wild yeast strains. Fermentation conditions are often harsh for yeast cells as they are faced with, and must respond swiftly to, fluctuations in oxygen, pH, osmolarity, ethanol concentration, nutrient supply, and temperature (Steensels *et al.* 2014). Evolved strains showed higher fermentation rates and increased aroma production than those of wild yeast (Steensels *et al.* 2014). This directed evolution was used to fine-tune a specific genetic or phenotypic trait that was already present but not fully optimal. For example, although the majority of the Lallemand brand sold through Scott Labs is isolated from natural environments, many strains are developed through selective breeding. This process is

done to breed out selective qualitative traits like H_2S and SO_2 production to avoid undesirable flavors and aromas in wine. They also sell one strain that was developed through directed evolution that is the first *Saccharomyces cerevisiae* yeast strain to significantly acidify must during fermentation. Innovation like this is what is pursued while modifying yeast strains to perform certain traits. However, the primary goal is for yeast to survive under the harsh conditions of fermentation to be able to apply these characteristics. To further investigate the parameters of fermentation, a study was done where ethanol, osmotic, oxidative, and thermal tolerance were the main stress factors discussed in determining the variation amongst 14 different commercial *Saccharomyces cerevisiae* strains (Carrasco *et al.* 2001). The outcomes of these tolerances are helpful when determining potential strains that could be most optimal for a desired final product. Below I will discuss the results from the paper that conducted this study and highlight some of the differences in wine strains (Carrasco *et al.* 2001).

Ethanol tolerance

Ethanol is the principal stress factor for yeast during alcoholic fermentation. This alcohol primarily targets the cell membrane and is toxic to yeast metabolism and growth. Ethanol stress affects various transport systems, including the general amino acid permease and glucose uptake processes (Aguilera *et al.* 2005). In addition, it inhibits the activity of crucial glycolytic enzymes and has been reported to damage mitochondrial DNA in yeast cells (Aguilera *et al.* 2005). To properly observe the effect of ethanol stress, alcohol was exogenously added to the cells in a single pulse. All 14 strains were significantly tolerant to 10% ethanol. However, solutions containing 12% ethanol significantly affected most of the strains (Figure 2B), the most sensitive being Fermiblanc arom SM102. The authors then used a 13.5% ethanol solution to test 9 of the

14 strains that were least affected by the 12% ethanol solution; only two were unable to grow. Finally, at 15% ethanol, only UCLM S377 was able to grow, indicating that this is the most ethanol-resistant strain analyzed (Carrasco *et al.* 2000) (Table 1).

Osmotic tolerance

Osmotic stress is an adverse condition for yeast cells that occurs at the beginning of vinification. Analyzing the way yeast strains react to this stressor can indicate their ability to start growth and carry out this process. When testing for osmotic tolerance, the adverse situation was introduced before plating and the results showed that the yeast strains were not very tolerant to osmotic stress (Carrasco *et al.* 2000). The least affected were Lalvin T73 and Lalvin M69 (Figure 2A). With the exception of those two strains, the rest showed very similar results towards their osmotic tolerance (Figure 2A).

Oxidative tolerance

Oxidative stress is another adverse effect that primarily affects yeast during biomass production and drying. It was analyzed by a plate assay after the application of H_2O_2. The results indicated that the wine yeasts most resistant to oxidative stress displayed a diameter of growth inhibition of around 3.0 cm, with the exception of three strains that exceeded the others with a value of around 3.5 cm. A diploid laboratory strain, W303, had a significant growth defect; the diameter of the growth inhibition zone was 4.5 cm, meaning that it was most resistant to oxidative stress.

Thermal tolerance

Heat shock affects the yeast at a molecular level and can impact the viability of a yeast cell. The analysis compared both control and heat-shocked cells after an incubation period of two hours at 45°C. Based on the figure, it is difficult to examine the growth, however the author is able to explain rather than show the final results. Two strains, UCLM S235 and Fermiblanc arom SM 102, displayed no growth and were the most affected by this stress condition. Of the strains that did exhibit growth, Fermichamp 67J, Fermivin crio 7303, and UCLM S377 showed the most sensitivity. Vitilevure Pris Mouse, Lalvin T73, Lalvin M69 and CECT 12512 were the most tolerant (Carrasco *et al.* 2000) (Figure 2A). Further experimentation was done when incubating the plates at 50°C, in which there was a significant decrease in survival even for the heat tolerant strains.

Discussion of results

This report presented an analysis of stress responses of 14 commercial wine *Saccharomyces cerevisiae* yeast strains. The results indicated that most strains were tolerant to stress conditions. UCLM S377 was most resistant to ethanol stress surviving at 15% ethanol, however that same yeast strain was also the most sensitive to osmotic stress (Table 2). This suggests the variation amongst yeast strains and their ability to respond to stress at a molecular level. The quality of evidence within the study displayed in-depth analysis of each respective stressor. The evidence dissects individual stress conditions and the ability of *Saccharomyces cerevisiae* yeast strains to display mechanisms for sensing and responding to stress at the molecular level. From this information, a winemaker would be able to select the yeast strain that is most compatible with

the conditions of their wine. This evidence provides insight in the ability of a yeast to survive through these stressors which presents the potential of a particular strain.

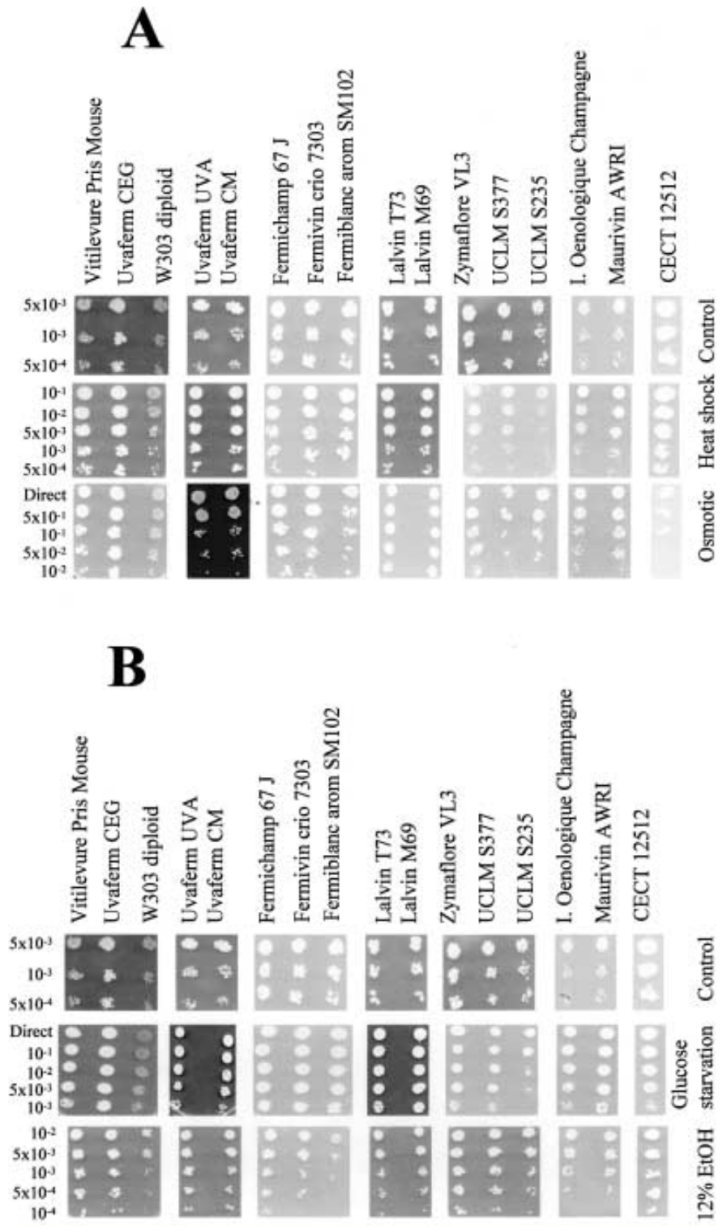

Figure 2: Analysis of tolerance to heat shock and osmotic tolerance (**A**) and 12% ethanol (**B**). Osmotic shock was induced by transferring cells from exponential cultures to YPD liquid medium containing 3 M KCl and the incubation continued for 3.5 hours. Ethanol stress analysis was induced as dilutions of exponential cultures were spotted onto the YPD solid medium containing ethanol concentrations between 10 and 15%. Dilutions indicated on the *left* of the figures were prepared and 5 µl of each were spotted on YPD plates, normally incubated at 30°C until colonies appeared, except for heat shock in which the experimental temperature was used. Figures and data were adapted from Carrasca *et al*. 2001

Strain	Ability to grow on 13.5% ethanol	Ability to grow on 15% ethanol
Vitilevure Pris Mouse	+++	–
Uvaferm CEG	++	–
Uvaferm UVA	–	–
Fermichamp 67 J	++	–
Lalvin T73	+	–
Zymaflore VL3	++	–
UCLM S377	+++	+
W303 diploid	–	–
CECT 12512	–	–

Table 1: The strains that carried out further testing after displaying growth on 10% and 12% ethanol. This table indicates the strains that were able to grow on 13.5% and 15% ethanol. Five µl aliquots of dilutions prepared from cultures of exponentially growing cells were spotted on YPD plates containing these percentages of ethanol. The + and - indicates the ability to grow or not on the plates. Figures were adapted from Carrasca *et al*. 2001.

Stress condition	Most resistant strains	Most sensitive strains
Oxidative stress		Fermiblanc arom SM102 UCLM S235 CECT 12512
Heat shock	Vitilevure Pris Mouse	UCLM S235
	Lalvin T73 Lalvin M69 CECT 12512	Fermiblanc arom SM102
Osmotic stress	Lalvin T73	UCLM S377
	Lalvin M69	UCLM S235 CECT 12512
Ethanol stress	UCLM S377	Fermiblanc arom SM102 Fermivin crio 7303 I. Oenologique Champagne Maurivin AWRI

Table 2: Summary of the results reported on stress resistance of the wine yeast strains under the study cited. Shows which of the *Saccharomyces cerevisiae* strains tested had shown the most resistant and the most sensitive results towards the stressors experimented under. Figures were adapted from Carrasca *et al*. 2001.

YEAST STRAIN CONTRIBUTION TO CHEMICAL COMPOSITION DIVERSITY IN WINE

The need for *Saccharomyces cerevisiae* strains to better adapt to the fermentation process is an ever-growing demand for new and improved wine yeast strains. In addition to survival and tolerance for yeast to achieve their most primary role of fermentation, the desire to complete this conversion without the development of off-chemical and sensory properties is also a main objective within the winemaking process. The ability to obtain satisfactory enological properties within various strains of *Saccharomyces cerevisiae* varies. However, with commercial *S. cerevisiae* wine strains, their ability to successfully complete fermentation is higher than indigenous strains due to their competitive nature. As previously stated, *S. cerevisiae* is one of the main species that is able to survive under the harsh condition of high ethanol, while the other microbial yeast species are inhibited severely as they are not ethanol-tolerant, sensitive to SO_2, and have more potential to produce undesirable compounds (Rainieri and Pretorius 2000). Because of this, *Saccharomyces cerevisiae* strains are often selected based on the fermentative conditions of the wine. A certain strain, based on their physicochemical properties, may produce a more suitable chemical composition depending on what is desired for the final product. This portion will be evaluating and comparing the fermented chemical composition of four different strains of *S. cerevisiae* on Chardonnay grapes. The compounds analyzed were alcohol, reduced sugar, total acidity, volatile acidity, and pH (Herjavec *et al.* 2003).

The harvested Chardonnay grapes were divided into five lots, each destemmed and crushed. Free-run juice of each lot was sulfured with SO_2 (100 mg/L) and settled for 24 hours. The must of each lot was racked and divided into 15 L glass bottles for the following commercial *S. cerevisiae* fermentation treatments: Uvaferm-CM, Uvaferm-CS2, Lalvin-71 B and

Lalvin-2056 (Herjavec *et al.* 2003). Fermentation with commercial strains began one day after inoculation. The chemical analysis occurred after the first racking in November. Ethanol, total, and volatile acidity were determined using methods such as analysis through a gas chromatograph (Herjavec *et al.* 2003). Results (Table 3) show no significant differences among the physicochemical properties found. The samples fermented by Lalvin-71 B had a significantly lower content of alcohol and total acidity as tartaric acid. According to the study, Lalvin-71 B can cause a decrease in malic acid of up to 30%, this could potentially also be linked to the malic acid degradation by malolactic fermentation of the Chardonnay (Herjavec *et al.* 2013). Although the chemical composition of these strains displayed similar results, they differ in the concentration of volatile compounds. In the next section, we will be comparing the volatile compounds present amongst the *Saccharomyces cerevisiae* strains and the effect they have on the flavor and aroma chemistry of the finished wine.

Compounds	Treatments				
	Uvaferm CM	Uvaferm CS2	Lalvin 71 B	Lalvin 2056	LSD***
φ (Alcohol) / %	12.90	12.90	12.10	12.50	1 % =0.5
γ (Reduc. sugar) /g L^{-1}	3.0	2.0	2.0	2.0	n.s.
γ (Total acidity) / g L^{-1}*	8.30	8.40	7.00	8.90	1 % =0.31
γ (Volatile acidity) / g L^{-1}**	0.62	0.68	0.63	0.61	n.s.****
pH	3.03	3.02	3.08	3.02	n.s.****

* as tartaric acid ** as acetic acid ***Least Significant Difference **** not significant

Table 3: Chemical composition of wines based on different commercial *Saccharomyces cerevisiae* wine yeast treatments. The chart exhibits that there were no essential differences among the strains in respect to reducing sugar and volatile acidity. As for alcohol and total acidity, Lalvin-81 B strain had significantly lower content. Figures were adapted from Herjavec *et al*. 2013.

FLAVOR AND AROMA CHEMISTRY : SENSORIAL EFFECTS ON WINE

The flavor and aromas that are experienced in wine stem from a complex system of interactions among various compounds. The impression of both aroma and taste components derive from volatile compounds which evolve as a result of fermentation. *Saccharomyces*

cerevisiae is responsible for primary alcoholic fermentation of sugar, but producing ethanol can also lead to a number of by-products. Yeast-derived metabolic products such as higher alcohols, acetates, fatty acid ethyl esters all contribute to the fermentative bouquet of wines (Lambrechts and Pretorius, 2000). These aroma compounds are linked to the metabolism of yeast cells. The formation of metabolites can contribute to specific flavors. Many of these metabolites derive from nitrogen, sulfur, and sugar metabolism (Bisson and Karpel 2010). They act as precursors and result in flavor active components such as ethanol, ester, fusel alcohols/acids/aldehydes, and other volatiles (Bisson and Karpel 2010). The principal flavor characteristics detected are variable and yeast strain specific. Winemakers are able to intentionally select a yeast strain that is able to unveil their desired chemical and flavor complexity. Employing a specific wine yeast strain for certain advantageous and particular characteristics ensures a reproducible product and allows for a predictable control of fermentation and quality. This portion will discuss the effects of commercial *Saccharomyces cerevisiae* yeast strains on volatile composition and the sensory characteristics that are identified from the produced compounds.

Higher alcohols

The most important flavor and aroma compounds formed from amino acids are higher alcohols and their produced esters and volatile acids. The process by which these amino acids are catabolized into higher alcohols derived via the Ehrlich pathway which impacts the synthesis of other aroma compounds both directly and indirectly (Styger *et al.* 2011). The important factor of higher alcohols is that they are substrates for acetate ester production. Small amounts of higher alcohols have a positive effect on wine quality, however excessive amounts, typically above 300 mg/L, can cause an undesirable aroma in wine (Herjavec *et al.* 2003). Below 300 mg/L, higher

alcohols contribute to the desired complexity of a wine. As previously mentioned in the last section, we will further discuss the analysis of the four *Saccharomyces cerevisiae* yeast strains in the Chardonnay and their volatile compound production after fermentation.

The volatile compound evaluation of the Chardonnay took place in April after the wines were bottled and stored under cellar conditions. The concentrations of individual higher alcohols differed depending on the yeast strain utilized to carry out fermentation. Lalvin-71 B had a considerably lower higher alcohol concentration than the others (Table 4) with a result of 264 mg/L (Herjavec *et al.* 2003). It was also the only strain used that was below 300 mg/L. Both the Uvaferm strains had significantly higher content of isoamyl alcohol, while Lalvin-71 B had significantly lower of this alcohol as well as isobutanol (Herjavec *et al.* 2003). 2-Phenyl ethanol has a solvent characteristic but acts as a substrate that can be esterified into 2-phenylethyl acetate which has rose, fruity, and honey characteristics (Cordente *et al.* 2021). Lalvin-2056 had a significantly lower concentration of 2-PE in comparison to its counterparts. Based on the overall higher alcohol concentrations of these yeast strains, it is likely that Lalvin-71 B would have the best results as it is within a desirable range to avoid any off aromas (Table 4).

Acetates

As previously stated, higher alcohols are substrates for acetate ester production. This is a reaction catalysed by yeast alcohol acetyltransferases (Cordente *et al.* 2021). Acetate esters are typically associated with the desirable “fruity” and “floral” aromas in wine. An example of this conversion is 2-Phenyl ethanol to 2-phenylethyl acetate which leads to rose, fruity, and honey characteristics. Another popular conversion is isoamyl alcohol to isoamyl acetate which influences the wine to have banana and general fruity aromas and flavors. Acetate esters are

important contributors to the aroma of young wines and decrease throughout the length of storage time.

Looking at the results (Table 4), the most influential acetate in terms of concentration is ethyl acetate. It is the main ester occurring in wine and has a significant influence on quality at very low concentrations of 50 mg/L (Herjavec *et al.* 2003). Each yeast strain fell below 50 mg/L for ethyl acetate. This is likely to have a positive effect on the wine and will provide those fruity and floral notes. In terms of 2-phenyl ethyl acetate and 2-phenyl ethanol, there seems to be an established positive correlation between their resulting concentrations (Herjavec *et al.* 2003). As for isoamyl acetate, the results showed some varying correlations in accordance with isoamyl alcohol concentrations. One would assume that higher concentrations of the alcohol would lead to higher concentrations of the acetate, similar to what is displayed for phenyl ethyl acetate. However, this does not seem to be the case.

Ethyl esters of fatty acids and fatty acids

Fatty acids produced by yeasts are intermediates in the biosynthesis of long-chain fatty acid metabolism. These, along with their ethyl esters are natural components of wine and can act as potential inhibitors of alcoholic fermentation. This inhibition of yeast growth is directly proportional to their solubility which is dependent on ethanol concentrations during fermentation (Lambrechts and Pretorius 2000). Thus the content of fatty acids and their ethyl esters depend on the wine strain used, highlighting the importance of ethanol tolerance within a strain as previously mentioned.

Based on the concentrations (Table 4), there were no significant differences amongst the fatty acids ethyl esters in the different fermentation treatments. However, Lalvin-71 B did have a

slightly higher concentration of butyric and caproic acids, ethyl butyrate, ethyl caproate, and ethyl caprate (Herjavec *et al.* 2003). This is likely due to growing conditions such as pH, temperature, and nutrients.

Sensory characteristics of the wines

The study had a panel of nine judges that performed a sensory evaluation of the wines by ranking them as well as applying a statistical method for a second ranking. Panelists did not decipher any significant differences in terms of quality but were able to detect differences of aroma and flavor. The results of both rankings indicated that the fermentation performed by the Lalvin-71 B strain resulted in wines of better aroma and flavor (Herjavec *et al.* 2003). These wines were described as having a clear and stronger bouquet, likely due to the considerably lower total concentrations of higher alcohols and the slightly higher concentrations of fatty acids ethyl esters (Herjavec *et al.* 2003). This balance between alcohol and acidity led to a more balanced product that was received more positively. The yeast strain that was ranked the lowest was Lalvin-A-2506. Panelists described it as having no prominent qualities or characteristics. Through this experiment we are able to highlight the varying influence that different *Saccharomyces cerevisiae* strains have on the composition and sensory properties of a wine.

Compounds	Treatments					
	Uvaferm-CM	Uvaferm-CS2	Lalvin-71 B	Lalvin-2056	LSD*	
Higher alcohols						
Propanol-1	7.1	7.3	16.3	9.2	1 % = 2.8	5 % = 2.0
Isobutanol	25.9	35.9	14.3	22.0	1 % = 8.2	5 % = 5.9
Isoamyl alcohol	278	275	166	232	1 % = 41.5	5 % =30.0
Hexanol	0.5	0.5	0.7	0.6		
2-Phenyl ethanol	54	55	50	38		
Σ Higher alcohols	365	374	264	301		
Esters						
Ethyl acetate	28.8	49.2	27.8	28.8	1 % = 13.9	5 % = 10.0
Isoamyl acetate	0.76	0.58	0.59	0.79		
Isobutyl acetate	n.d.	n.d.	n.d.	n.d.		
Hexyl acetate	0.05	0.05	0.05	0.03		
Isobutyl acetate	n.d.	n.d.	n.d.	n.d.		
Phenyl ethyl acetate	0.09	0.26	0.16	0.06		
Σ Acetates	0.90	0.89	0.80	0.88		
Ethyl butyrate	0.09	0.08	0.23	0.10		
Ethyl caprate	0.07	0.08	1.15	0.70		
Ethyl caproate	0.31	0.43	0.47	0.33		
Ethyl caprylate	0.25	0.27	0.29	0.23		
Σ Ethyl esters of fatty acids	0.72	0.86	1.15	0.70		
Ethyl lactate	1.45	0.90	1.15	1.05		
Diethyl succinate	0.35	0.30	0.35	0.20		
Butyric acid	0.78	0.75	1.24	0.95		
Caproic acid	3.4	4.9	5.6	3.1		
Caprylic acid	3.4	4.7	4.6	3.1		
Capric acid	1.9	2.5	2.4	1.6		
Σ Fatty acids	9.48	12.85	13.84	8.75		

* Least Significant Difference

Table 4: Concentration of volatile compounds in Chardonnay wines (mg/L) and the differing compositions depending on the *Saccharomyces cerevisiae* yeast strain used. Analyses of many of the compounds were made on gas chromatography. Figures were adapted from Herjavec *et al*. 2013.

CONCLUSION

Proper selection of *Saccharomyces cerevisiae* yeast strains remains a crucial component of both the technical and sensorial elements of the winemaking process. The natural availability of yeast strains possessing an ideal combination of enological characteristics was highly improbable. These indigenous strains lacked competitiveness and tolerance to fermentative conditions. The domestication of this wine yeast allowed for the refinement of strains that were utilized to properly and efficiently execute fermentation while also limiting the potential for off-aroma. The recent developments of expanding traditional genetic techniques have empowered *S. cerevisiae* strains to be more stable, vigorous, and efficient in their chemical and

sensorial compositions. Further research into yeast strains will only contribute to the diversification of yeast strains that can contribute to the wine industry. Ultimately, the genetic analysis is crucial to better understand and improve the genetic and phenotypic basis of yeast evolution towards winemaking that will be useful to tailor strains to meet enological and consumer demand.

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