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Barrel Sanitation & Solid Phase Extraction of Phenolic Compounds in Columns

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Barrel Sanitation & Solid Phase Extraction of Phenolic Compounds in Columns

By

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Abstract

Because of laboratory constraints posed by the pandemic, two projects are included as part of this thesis, a theoretical prediction of barrel sanitization and an experimental exploration of phenolic extraction in columns.

Oak barrels are often used to give wine, beer, and distilled beverages flavors and colors. However, microorganisms can penetrate into the wood, making cleaning and sterilization difficult. This can lead to undesired microbial growth in the contents of the barrel during subsequent use. Information on heat penetration into barrel staves during steam treatment and associated thermal inactivation rates is scarce. Therefore, we incorporated heat transfer and Arrhenius-type death kinetics to build a mathematical model to predict the killing rate of the microorganisms when steam sterilizing a wooden barrel. First, we use this model to predict temperature profiles in barrel staves as a function of distance from the steamed surface and time of steaming. Next, we evaluated the thermal inactivation of microorganisms at 0.8 cm into the stave (the maximum wine penetration depth into a stave) to calculate the time needed to achieve 5-log reduction in live cells. Using this approach, we found that the required sterilization times for *Brettanomyces bruxellensis, Saccharomyces cerevisiae,* and *Leuconostoc mesenteroides* are 9 minutes, 12 minutes, and 200 minutes, respectively. This result is useful for winemakers to determine how long they desire to steam a barrel to prevent growth and contamination of key microbes in their wine.

Phenolic extraction is essential for red winemaking. Fermentation along with phenolic extraction usually takes 7 to 14 days, although the tannin extraction can continue for weeks until an equilibrium is reached. Yet process intensification would allow winery equipment to be used multiple times during harvest, thus decreasing capital investment. While previous work showed

the potential to speed up the conversion of sugar to alcohol considerably, this methodology will only be truly useful for red winemaking if phenolic extraction can be completed more rapidly and with more control. In this work, we performed phenolic extraction from grape solids in a column setup and let juice or wine flow through the column to model a continuous extraction. The effect of four different factors was tested: column temperature, liquid ethanol concentration, flow rate through the column, and column diameter using a design of experiments approach. We found that liquid flow rate is the most significant factor, and the temperature and ethanol concentration had minor effects but not statistically significant. With all optimized factors, we were able to reach the same concentration as the bucket control for the final extraction in 86 hours on the bench scale compared to 144 hours for the control process. Both the pigment concentration and tannin concentration were similar. This work shows the potential to achieve process intensification and more control of the phenolic values in the finished wine.

Chapter 1. Introduction

Because of the timing of the COVID pandemic, this thesis is really a combination of two projects, a theoretical/computational study of oak barrel sterilization and an experimental investigation of phenolic extraction from grape skins and seeds in a column.

Using oak barrels for wine and beer for storage can have the added benefit of extracting desirable flavor into the beverage. Reusing barrels has economic and environmental benefits, as barrels can be expensive, even though extraction of flavor will decrease with use. However, the spoilage microorganisms that can be present in wine can penetrate the staves of the barrel and contaminate the wine during subsequent use and storage. Using ambient pressure steam to sterilize the barrels between uses can alleviate this problem, but information on the necessary length of steam treatment is not readily available. Therefore, by combining the Arrhenius-type death kinetics coefficients of wine spoilage organisms and a model of a semi-infinite medium heat transfer system, the steaming time to reach 5-log reduction of various organisms was predicted.

While we were working on the barrel project, the COVID pandemic came. Then we decided that we did not want to continue with the experimental section of the barrel project, and we published the theoretical work as a research note. When we could return to the lab and winery, we decided to extend a phenolic extraction project initiated by previous students, and this project was the bulk section for this thesis.

Fermentation during red winemaking usually takes 7 to 14 days. Previous studies showed the possibility of speeding up the conversion of sugar to ethanol by yeasts, making the limiting step the extraction of phenolic compounds that provide red wine mouthfeel and color. A controlled and faster extraction of phenolics, without loss of quality, could be critical to the wine

industry, as a faster process has the potential to significantly reduce equipment utilization and thus capital investment. One possibility for making the process faster would be to flow wine or juice through a column of grape solids external to the fermentor. A factorial experimental design was used to study this innovative way of extracting phenolics while reducing the amount of material required and the experiment time. Four factors that were focused on were temperature, ethanol concentration of the liquid phase, flow rate, and column diameter, all using frozen grapes. Then different molecules in the column effluent were analyzed using reverse-phase HPLC. More confirmational experiments were performed based on the results from the factorial experiments, as well as a pilot-scale experiment in the winery.

This thesis contains a chapter reviewing the different factors of phenolic extractions and potential reactions of phenolics, a chapter on barrel sterilization, a chapter on solid-phase extractions of wine phenolics, and a chapter on the overall conclusion of the thesis.

Chapter 2. Literature Review of Phenolic Extraction

Phenolic extraction is essential for red winemaking because phenolics provide the red wine with color, mouthfeel, and aging potential. Different winemaking practices can have various effects on the final wine quality. Understanding different practices and different factors can help winemakers to make decisions. In this review, we will focus on four distinct factors: temperature, solvent composition, contact area, and contact time. On top of the extraction, reactions also have a minor effect on the phenolic extraction during the fermentation. We will also look at how researchers consider the different effects and build the factors into mathematical models of the extraction process.

There are two phenolic classes that are mainly associated with wine quality: anthocyanins and tannins. Anthocyanins provide the color for red wine, and they can react with other molecules, usually tannins, to form polymeric pigments. There are five primary anthocyanins in grapes: malvidin, delphinidin, peonidin, petunidin, and cyanidin (Boulton, Singleton, Bisson, & Kunkee, 1998; Waterhouse, Sacks, & Jeffery, 2016). Anthocyanins are most abundant in grape skins. Tannins are polymers of flavon-3-ols, which provides the wine mouthfeel. They can be found in both grape skins and seeds (Adams, 2006; Singleton & Noble, 1976).

Temperature

The higher temperature usually has a positive influence on phenolic extraction. From Cissé et al.'s research, anthocyanins extraction from *Hibiscus sabdariffa* increases as temperature increases. They studied the diffusion coefficient between 25°C and 90°C and showed that the extraction time could be decreased by increasing the temperature. At a high temperature above 60°C, the extraction reaches the highest concentration at around 20 minutes, and then the concentration decreases due to the degradation (Cissé et al., 2012). In grape

phenolic extractions, increasing temperature also increases the diffusion coefficient. Medina-Plaza et al. tested temperatures 15°C and 30°C and studied the desorption of phenolics from the grape solids. Higher temperature results in more anthocyanins in the liquid phase due to solubility increase (Medina-Plaza et al., 2020). For skin phenolics, as temperature increases, the rate of extraction increases, but the final values are not affected. For seed phenolics, as the temperature increases, both the extraction rate and the final values increase (Lerno et al., 2015). Other studies showed a similar result. At a higher fermentation temperature, both the extraction rate and final level of tannins are higher (Beaver et al., 2020; Miller et al., 2019). The monomeric flavan-3-ol and anthocyanin concentrations are negatively correlated with fermentation temperature due to conversion to polymers (Ntuli et al., 2022) or degradation (Lerno et al., 2015).

Temperature is the main factor that can increase overall phenolic extraction during wine processing (Lerno et al., 2015). Flash détente is a pretreatment in winemaking practice that heats the must to a high temperature (85°C). This treatment can significantly increase caftaric acid and quercetin glycosides. However, it can lower the color stability compared to conventional must heating due to the degradation of phenolics (60°C) (Ntuli et al., 2020; Ntuli et al., 2022). It should be noted that flash détente can change the sensory profile of the resulting wine in addition to the phenolic profile, so winemakers should keep that in mind if they decide to use this pretreatment (Ntuli et al., 2020; Ntuli et al., 2022).

While higher temperatures during fermentation lead to better phenolic extraction, some winemakers use freezing as pretreatment of the grapes. This treatment is controversial. Some researchers believe that frozen grapes do not have a significant influence on the final wine product. In a study done in 2007, Schmid et al. found that the freezing process has a minor effect

on the phenolic concentration of the final wine. The spectrophotometric color analysis along with the HPLC analysis on individual anthocyanins molecules on wines made from frozen grapes and fresh grapes were similar (Jiranek & Schmid, 2011; Schmid et al., 2007). However, another study showed that using frozen grapes, dry ice, and cold maceration had effects on phenolic extraction. For both Cabernet Sauvignon and Syrah grapes, dry-ice treatment gave significantly higher anthocyanins content, and frozen grapes and maceration had some positive impact depending on the grape variety. All low temperature treatments increased the total phenolics extracted at some level (Gil-Muñoz et al., 2009).

Solvent Composition

Ethanol and sulfur dioxide are known to affect the extraction of phenolics. Cacace and Mazza found that there is higher diffusivity of phenolics in milled berries at a higher SO_2 concentration in the juice. As ethanol concentration increases, diffusivity increases, but it will reach a maximum and then decrease (Cacace & Mazza, 2003). However, for wine grapes, a SO_2 concentration below 150 mg/L has minor or even no significant impact on the extraction of anthocyanins. For total phenolics, Bakker et al. showed increasing SO_2 concentration slightly increases total phenolics at the early stage of extraction. But, Watson et al. showed at a higher SO_2 concentration, the polymeric pigment and caftaric acid were the lowest (Bakker et al., 1998; Watson, Price, & Valladao, 1995). Phenolic extraction has a linear relationship with the ethanol concentration at a lower temperature. However, at a higher temperature, the relationship is not linear anymore; the effect of the ethanol change is minor and can lead to a decrease in diffusivity. One group found the optimized condition for extraction is 54% ethanol in water by volume at 83.6°C (Karacabey & Mazza, 2008), though these conditions would not happen naturally during winemaking where maximum ethanol concentration is typically 14-15% alcohol

by volume. González-Manzano et al. tested different ethanol concentrations during maceration, and the skin flavan-3-ols were more affected by ethanol concentration than the seed flavan-3-ols. In an extended maceration, the seed flavan-3-ols continoued to be extracted and dominated the total flavan-3-ols in the wines (González-Manzano, Rivas-Gonzalo, & Santos-Buelga, 2004). Setford et al. found that under both natural convection and forced convection conditions, malvidin-3-glucoside, the major anthocyanin present in red wines, in the solid phase depleted fastest at the highest ethanol concentration, and in the liquid phase it increased the fastest (Setford, Jeffery, Grbin, & Muhlack, 2018).

Medina-Plaza et al. found that ethanol concentration impacts the adsorption and desorption of phenolics onto the cell wall material. Higher ethanol concentration results in more anthocyanins in the liquid phase due to higher pigment solubility. Additionally, more polar anthocyanins adsorb onto the cell wall material (Medina-Plaza et al., 2020). Beaver et al. studied ethanol ranges from 0% to 15%, and found that the adsorption is negatively correlated with ethanol concentration, and they were able to incorporate these observations into a Langmuir model (Beaver et al., 2020).

Contact Area

Pump-over, punch-down, and baffled rotation are three common ways that winemakers use to increase solid and liquid contact. Fisher et al. studied the extraction of different contact methods. They found that extraction of different phenolic molecules depends on the grape variety, and there was no method that is better than all the other methods (Fischer, Strasser, & Gutzler, 2000). In Setford et al.'s publication, forced convection in the fermentation tank was studied. The publication showed that by 10 hours, the malvidin-3-glucoside reaches maximum

concentration, and with more ethanol, there is more malvidin-3-glucoside that is extracted. By natural convection, the concentration reaches the maximum around 30 hours.

Another study found that chemical gradients form in the fermentor during fermentation. Lerno et al. showed that in a 2000 liter working volume pilot-scale fermentor, around 8 hours after the pump-over, the skin phenolics reached saturation, but the seed phenolics did not reach saturation (Lerno et al., 2017). Pump-over as one of the ways of cap management is essential to the final wine. It was predicted that lack of cap management could lead to heat accumulation in the cap and cause a higher seed tannins ratio. The pump-over was necessary for large scale tall fermentors because the liquid and solid surface to the fermentor volume ratio is small (Miller, Oberholster, & Block, 2019b, 2019a). For a smaller fermentor, such as a research-scale pilot fermentor (around 150 L), pump-over volume and frequency do not influence phenolic extraction significantly. Using these smaller fermentors, for Cabernet Sauvignon grapes, very low pump-over frequency and volume, and even no pump-overs did not change phenolic extraction. Five months after the treatment of the bottled wine also showed no significant difference in phenolic content (Lerno et al., 2018). Setford et al. used their experimental results to create a mass transfer model and predict that with forced convection, the extraction of malvidin-3-glucoside was initially faster than neutral convection. But later on, due to the limitation of the diffusion process within the grape solids, the trends of extraction are the same. With hindered convection, the extraction was slow the entire time, and the concentration only achieved the level close to the other treatments at around 300 hrs.

Contact Time

The longer the maceration, the more phenolic compounds were extracted (Gómez-Plaza, Gil-Muñoz, López-Roca, Martínez-Cutillas, & Fernández-Fernández, 2001; Miller et al., 2019).

Gómez-Plaza et al. studied three different maceration lengths, 4, 5, and 10 days. They found that with longer skin maceration, they had greater anthocyanins and polymeric compounds and also higher color density before bottling. The longer maceration time also led to higher sensory rating and better preservation of the wine characteristics during storage (Gómez-Plaza et al., 2001). Researchers found that as the fermentation proceeds, more anthocyanins and tannins are extracted into the wine. One exception is that at a high temperature trial (35^{o}C), anthocyanins start to decrease at around 75 hours. Anthocyanin decrease is due to the degradation. Anthocyanins can reach a plateau or maximum at the first six days, while tannins increase as long as the wine is in contact with the grape solids (Miller et al., 2019).

Reactions

Polymeric pigmentation can be described as a condensation reaction between anthocyanins and other wine components such as tannins or flavan-3-ols. The formation of polymeric pigments could affect both the color and astringency of red wine. During aging, monomeric anthocyanins and co-pigmentation will deplete (Singleton & Trousdale, 1992). In Nagel and Wulf (1979), the researchers found that polymeric pigment content was not detectable until day 26. On day 26, the percentage of polymeric pigment was only 4%. This indicated that the polymerization reactions of anthocyanins are limited during fermentation and will increase during aging and reach around 70% at day 240 regardless of grape variety (Nagel & Wulf, 1979). Most polymeric pigments form during aging. Plenty of studies showed the concentration of the polymeric molecules increases as a function of time during aging (Monagas, Núñez, Bartolomé, & Gómez-Cordovés, 2003; Sims & Bates, 1994). Even though not many polymeric pigments were formed during maceration, the length of maceration was strongly correlated with pigments after aging (Sims & Bates, 1994).

While polymeric pigment formation is positive to wine aging, some reactions can be harmful to wine quality. Oxidation is a complex problem for winemaking, especially during aging. Iron can reduce oxygen to the hydroperoxyl radical. Phenolics have good hydrogen-donating ability so that they can react with hydroperoxyl radicals. There are secondary oxidation reactions as well, such as quinones reacting with phenolics (Waterhouse et al., 2016). Adding oxygen to the must prior to fermentation led to a significant decrease in free anthocyanins while the fermentation rate stayed the same. The total anthocyanins were lower in the oxygenated trials even after six-months of storage (Castellar, Arfell, Riponi, & Amati, 1998). It was mentioned in some literature that anthocyanins could degrade during fermentation. Tseng et al. (2006) conducted a degradation study on malvidin-3-glucoside because it is the most abundant anthocyanin in wine. The disappearance of malvidin-3-glucoside exhibits first-order kinetics. The disappearance accelerates as ethanol concentration increases (Tseng, Chang, & Wu, 2006).

The reaction of the red pigment becoming colorless is called bleaching, which is reversible. Potassium bisulfite can be sprayed on grapes, added to the must or the wine before bottling to prevent microbial growth. When anthocyanins are in a cation form, known as the flavylium ion, they act as electrophiles and react with the nucleophile bisulfite. Wine pH is between the two pK_a values, so the sulfur dioxide molecule has a negative one charge. When the sulfo group (SO_3H) attaches at the C4 position, the molecule becomes colorless (Timberlake & Bridle, 1967).

Mechanistic Models

Somers and Evans first found that pigment extraction can be described in two steps: an initial faster extraction and a slower depletion. The depletion of color is due to the destructive effect of ethanol but is mostly reversible. Other hydrogen bonding forces can also cause pigment

loss, such as dimethyl formamide and dimethyl sulphoxide, which are much more effective than ethanol in observation (Somers & Evans, 1979). In the book Principles and Practices, these two steps can be described in the rate of extraction. Both steps are in first-order and based on the anthocyanin concentration (Boulton et al., 1998).

Zanoni et al. described the extraction process as diffusion. They hypothesized that the solutes on the grape surface instantaneously dissolve, followed by slow leaching from the grape interior. They used Fick's second law and considered that the phenolics are evenly distributed in the grape solids. The total phenolics as a function of time was modeled by pseudo first-order kinetics. For anthocyanins, they added a pseudo zero-order kinetics term to describe the degradation phenomena (Zanoni, Siliani, Canuti, Rosi, & Bertuccioli, 2010).

Setford et al. also described the extraction of anthocyanins as diffusion phenomenon and found a mathematical model to find the mass transfer coefficients. They studied both natural and forced convection and the effect of ethanol concentration. Their simulation shows that during the first 10 hours, the forced convection has the fastest extraction rate, but then slows down and follows the same trend as the natural convection. The hindered convection has the slowest extraction rate and also the final value of malvidin is lower than both convection trials (Setford et al., 2018).

Miller et al. combined the different phenomena and created a mechanistic model. In this model, they described phenolic extraction in three steps: first, the release of the phenolic compounds from grape skin and seed; second, the adsorption and desorption of the phenolic compounds onto the grape solids; and third, the reaction of the phenolic compounds, usually due to reactions such as oxidation and polymerization. This mechanistic model considers the temperature and ethanol concentration effects on these phenomena, and it also describes the

adsorption and desorption process, which are relatively new. This model was combined with a computational fluid dynamics (CFD) model for a red wine fermentation to explore the impact of fermentor design, fermentor scale, and fermentation processing choices such as temperature and cap management (Miller et al., 2019).

Prediction of Effective Steam Sterilization Times for Wine Barrels Using a Mathematical Modeling Approach

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Abstract

Oak barrels are often used to give wine, beer, and distilled beverages flavors and colors. However, microorganisms can penetrate into the wood, making cleaning and sterilization difficult. This can lead to undesired microbial growth in the contents of the barrel during subsequent use. Information on heat penetration into barrel staves during steam treatment and associated thermal inactivation rates is scarce. Therefore, we incorporated heat transfer and Arrhenius-type death kinetics to build a mathematical model to predict the killing rate of the microorganisms when steam sterilizing a wooden barrel. First, we use this model to predict temperature profiles in barrel staves as a function of distance from the steamed surface and time of steaming. Next, we evaluated the thermal inactivation of microorganisms at 0.8 cm into the stave (the maximum wine penetration depth into a stave) to calculate the time needed to achieve 5-log reduction in live cells. Using this approach, we found that the required sterilization times for *Brettanomyces bruxellensis, Saccharomyces cerevisiae,* and *Leuconostoc mesenteroides* are 9 minutes, 12 minutes, and 200 minutes, respectively. This result is useful for winemakers to determine how long they desire to steam a barrel to prevent growth and contamination of key microbes in their wine.

Keywords: oak barrel, steam sterilization, heat transfer, *Brettanomyces, Saccharomyces cerevisiae,* and lactic acid bacteria

Introduction

Oak barrels are commonly used to add flavors and colors to alcoholic beverages. Flavor impact molecules, such as oak lactones, guaiacols, furfurals, and tannins, are extracted from the wood and into the wine, beer, and distilled beverages during aging (Spillman et al. 1998, Puech et al. 1999, Pérez-Coello et al. 1999). However, without proper cleaning and sterilization of the barrel, the beverages can be contaminated from microbes on the surface and some distance into the wood, thereby creating undesired flavors. Some yeasts like *Saccharomyces cerevisiae* are common in winemaking and, though not harmful, still need to be controlled. However, other yeast strains can lead to spoilage; for example, *Brettanomyces (Dekkera) bruxellensis, Pichia, and Candida* (Guzzon et al. 2011). *Brettanomyces* is a common yeast found naturally on the surfaces of wineries and even in fermentation tanks or presses (Woolfit et al. 2007). It can produce unpleasant aromas and tastes described as “barnyard”, “band aid”, “old”, and “after bitterness” (Gilliland 1961, Licker et al. 1998). Aside from yeasts, some bacteria can also cause off-characters of wine. Examples include *Acetobacter aceti* and *Leuconostoc mesenteroides* (Sponholz 1993). In order to avoid these issues, it is crucial to have effective sterilization methods, such as steam treatment, for oak barrels that achieve inactivation of all microbes to at least 0.8 cm depth into the barrel staves. Unfortunately, information on the necessary timing of this treatment is scarce.

To date, limited studies have been published defining effective barrel sterilization techniques. Experimental studies are not straightforward, as these microbes are not only found on the inner surface of the barrel, but also inside the layers of the wood. This is because oak wood contains tyloses, and a lack of tyloses results in a more porous structure that is more susceptible to microbes (Singleton 1974, Wilker et al. 1997). This, combined with the fact that yeast can

penetrate up to 0.8 cm into wood, creates a problem (Barata et al. 2013), as the wood shields the microbes from the heat. One related study took barrels contaminated with *D. bruxellensis* and treated them with various sanitation and sterilization methods including rinsing with cold and various temperatures of hot water, treating with sulfur dioxide, and steaming. Then, results were observed by scraping 0.2 cm layers of wood off until no wine was observed (Barata et al. 2013). While important, this study only used one steaming treatment for a specific time and did not include data on stave temperature, making generalization difficult. Another study used water, UV, steam, and ozone to treat the barrels and then studied the microorganisms in the water used to wash the barrel at the end. They determined that steam was the most effective sterilization method with the ability to reduce more than 3 log-units of the yeast sample in the 30-minute treatment (Guzzan et al. 2011). Again, the temperature of the stave was not measured, so a general steam sterilization procedure for specific organisms is difficult to predict quantitatively. Alejandra et al. (2018) did measure temperatures in barrel staves during steaming, though their study was limited to enumerating yeast from wash water post treatment. While this study is an important advancement over previous studies in the quantification of thermal inactivation, it would be valuable to understand the thermal inactivation of the cells inside of the wood structure, though this would be difficult to achieve with a purely experimental approach.

Because the experimental investigation of thermal inactivation in barrels is difficult, an alternative approach would be to simulate treatment using physical and mathematical modeling. This would require modeling of both heat transfer and the thermal inactivation. While modeling heat transfer through oak is difficult to find in the literature, some researchers have modeled the thermal inactivation of relevant organisms, though not usually in the context of wine barrel sterilization. However, these simulations require kinetic parameters for thermal inactivation for

each specific organism, usually in the form of a death constant with an Arrhenius dependence. Very few relevant organisms have been characterized in this way. Those that have include: *Brettanomyces* (Couto et al. 2005), *S. cerevisiae* (Alejandra et al. 2018), and *L. mesenteroides* (Yao et al. 2008).

Therefore, it is our goal to combine a model for heat transfer into oak staves with a standard model for thermal inactivation of wine-relevant microbes in order to predict effective sterilization processes for wine barrels. To do this, we first simulate heat transfer into a semi-infinite oak slab to calculate temperature profiles over space and time. We then combine this analysis with a model for thermal inactivation to calculate the time necessary to achieve 5-log reduction in live cells for various common wine microbes. The result should provide guidance for winemakers to establish their own protocols.

Methods

Heat transfer in oak. The model of temperature change during steaming inside of the wood is described as a semi-infinite medium heat transfer system (Welty et al. 2008). The steam circulates only on the inside of the barrel, and the barrel stave is usually 3 cm thick. This situation can be assumed to be a semi-infinite medium and can be described using the heat transfer expression in Equation 1:

$$\frac{T - T_o}{T_s - T_o} = 1 - \mathrm{erf}\left(\frac{x}{2\sqrt{\alpha t}}\right), \qquad \alpha = \frac{K}{\rho * Cp} \qquad Eq.1$$

where T is the temperature at a certain position and time. T_o is the initial temperature, which is room temperature (25°C) and T_s is the temperature of the steam (100°C). The position (x) is the depth in the wood from the inside surface at any time (t). K is the thermal conductivity, ρ is the density, and Cp is the specific heat.

Modeling death kinetics. To model microbial death kinetics (thermal inactivation), we started with the equation

$$\frac{dN}{dt} = -kN \qquad Eq.2$$

where N is the number of the live cells and k is the death constant of the form:

$$k = Ae^{\frac{E_a}{RT}} \qquad Eq.3$$

In this equation, A is the pre-exponential factor, and E_a is activation energy of death for a particular organism, both of which can be determined through experimental curve fitting. R is the ideal gas constant, and T is the absolute temperature.

To find the fraction of the live cells remaining, Equation 2 needs to be integrated over time. However, since the temperature is changing over time at any given depth, we need to numerically integrate Equation 2. This was accomplished by choosing a constant time step of 1 second, assuming that the temperature remains constant during the time step and summing all time steps during the treatment.

Combining heat transfer and death kinetic model. Combining the heat transfer Equation 1 for semi-infinite medium and integrated death kinetics (Equation 2), we were able to build a model to predict the total log kill of target microbes within the wood. We chose our goal for thermal inactivation to be a 5-log kill, which corresponds to $N/N_o = 10^{-5}$, where N_o is the initial number of the live cells. Other inactivation goals could be chosen by the winemaker and would change the calculated inactivation times accordingly.

Simulation parameters. The physical properties for the barrel were found on the engineering toolbox website (https://www.engineeringtoolbox.com) for (white) oak: the density (ρ) is 756 kg/m^3, thermal conductivity (K) is 0.17 W/(m*K) at 25°C, and specific heat (Cp) is

2400 J/(Kg*°C) at 25°C. The death kinetics equation parameters are derived from other studies shown in Table 3.1 (Couto et al. 2005, Yao et al. 2008, Alejandra et al. 2018).

Modeling the heat transfer and death kinetics was accomplished using MATLAB (ver. R2019b). Simulations were performed on a MacBook running macOS Mojave Version 10.14.6.

Results

Modeling the heat transfer into a barrel stave. Winemakers usually only consider the sanitation and sterilization of the inside surface of a barrel. However, the spoilage organisms can penetrate up to 0.8 cm into the wood. The heat from steaming cannot reach 0.8 cm depth instantaneously. Therefore, in order to see how the temperature changes during steaming, a heat transfer model was built based on Eq. 1 to predict the temperature profile. 85°C is commonly used as a goal temperature for sanitization and sterilization. The time to reach 85°C at different depths of the barrel was simulated and is shown in Table 3.2. It takes 89 minutes to reach 85°C at 0.8 cm depth. Our results show that it takes one hour longer than the current recommended steaming time used by industry. Figure 3.1a shows the temperature profile in the barrel with four time periods chosen, and it follows an expected pattern, where the temperature gradient between the surface and the inner part of the stave decreases over time. Figure 3.1b illustrates how the temperature changes over time at 0.8 cm depth during steam treatment. The temperature increases rapidly up to 65°C and then slows down as it approaches the surface temperature. The result suggests a possibility that steaming 20 to 30 minutes may not achieve the temperature standard for sanitization in the wine industry at 0.8 cm depth. Therefore, it is important to combine the thermal inactivation kinetics with the heat transfer modeling in order to evaluate the thermal inactivation potential of steaming protocols.

Simulating microbial kill. In order to simulate the required steaming time for complete thermal inactivation of wine microorganisms, we chose three model organisms to analyze: *Brettanomyces*, *S. cerevisiae*, and *L. mesenteroides*. For each of these, we used the death kinetics described by Equation 2, along with the predicted stave temperature profiles from above, to calculate the log reduction of viable organisms as a function of time and depth (Figure 3.2).

Figure 3.2a shows the predicted thermal inactivation for *Brettanomyces*. There is no significant killing at the beginning of heating at any depth, but once the temperature reaches around 55°C in the stave at each depth, thermal inactivation proceeds quickly and achieves the goal of 5-log kill within a minute. At 0.8 cm depth, it only takes 9 minutes to get a 5-log kill for *Brettanomyces*, with an additional 3-log kill in the subsequent half minute. Figure 3.2b illustrates the death curve for the species *S. cerevisiae*. It shows a steep curve similar to that of *Brettanomyces*. It takes 12 minutes to get a 5-log kill for *S. cerevisiae* at 0.8 cm depth, and the inactivation increases dramatically once the temperature reaches approximately 60°C. This difference corresponds to *S. cerevisiae* being slightly more heat tolerant than *Brettanomyces*. Figure 3.2c shows the death curves for the species *L. mesenteroides* at different depths. It takes 198 minutes to achieve a 5-log kill at 0.8 cm depth for this bacteria. Therefore, it will take over an order of magnitude longer steaming time to achieve the same kill for this heat stable bacteria. From these predictions, it is clear that the steaming time chosen by the winemaker will have to be driven by their knowledge of likely barrel contaminants in their production environment.

Discussion

Even though barrel use in wine production is common, data on the efficacy of steam sterilization procedures is scarce in the literature. To supply useful information to winemakers,

we successfully combined mathematical models for heat transfer and thermal inactivation of microorganisms to predict the length of time necessary to achieve kill levels common in the food and beverage industry. This combination is important, as the temperature is not uniform in the stave during steaming, and yet yeast and bacteria can penetrate into the wood at least 0.8 cm of depth.

In comparing our predictions with literature, our heat transfer model seems to be trustworthy. According to Alejandra et al. 2018, the highest temperature that could be reached using steam for 10 minutes is 57.5°C at 0.8 cm depth. Our heat transfer model predicts the temperature to be 58.8°C which matches the experimental result quite well. They also found that the highest temperature that could be reached at 1.4 cm depth is 42.5°C, where our model prediction is 39.0°C, again demonstrating good agreement between theory and experiment. Based on the heat transfer model, we found that the oak wood does not heat up instantaneously when steaming the inside of the barrel. We predicted that it will take an hour and half to reach 85°C at 0.8 cm depth, which is a common sanitization temperature used in wineries. This time is much longer than what is suggested by some manufacturers of barrel steaming equipment. However, our results demonstrate that this timing should still be sufficient to achieve the desired thermal inactivation for some key organisms because significant kill is observed at lower temperatures for organisms like *Brettanomyces* and *Saccharomyces*.

Our simulations demonstrate that the time to reach a 5-log kill at 0.8 cm depth varies substantially depending on the microorganism. For instance, we found that it takes more than three hours to kill *L. mesenteroides* to this level, while it takes less than 10 minutes for *Brettanomyces* and only a minute longer to achieve 10-log reduction. Winemakers can determine how long they need to sterilize a barrel based on the potential microorganisms. In Alejandra et al.

(2018), the inactivation for different yeast strains for 3-log at 52.5°C /55°C is less than one minute which also matches our result for yeast. In Couto et al. (2005), it takes 72 s to get 4-log reduction for *Brettanomyces* at 55°C, and it takes 3 s to get 6-log reduction at 45.1°C or 45.9°C of the same organism in a wine matrix. This emphasizes the need for the heat transfer part of the analysis, as heating the stave will take longer than the thermal inactivation. Our analysis also demonstrates the importance of understanding the key microbes in the winery setting, as the choice of microbes for this analysis greatly affects the calculated time for thorough thermal inactivation. For instance, *L. mesenteroides* is a Gram-positive bacteria, and is substantially more heat stable. Additionally, the bacteria is able to build up heat tolerance if it is pretreated with mild heat (Osorio et al. 2016). Therefore, if the heating process is relatively slow, *L. mesenteroides* may be able to adapt, and have an even better survival at a high temperature. For example, one study shows that it takes three minutes to achieve a 6-log kill at 80°C for this organism (Kosin et al. 2010). While these data are derived from a strain of *L. mesenteroides* different from the strain used to calculate death kinetic parameter in our work (Yao et al. 2008), they suggest that *L. mesenteroides* is harder to kill compared to yeasts, which is also corroborated by our results.

One of the limitations of this modeling approach is that the death parameters, activation energies (E_a), and pre-exponential factors (A) are required to run the simulation for each potential spoilage organism. For example, data are not available for *Acetobacter*. Wilker et al. (1997) have performed studies on treatment of acetic acid bacteria on wood barrels, but not enough information was generated at various temperatures and time to calculate the Arrhenius constant and activation energy of death. This issue would also be present if a winery were to have a different strain of *Brettanomyces* (or *S. cerevisiae* or *L. mesenteroides*) that was

considerably more difficult to kill using steam. In any of these cases, a winemaker would need to conduct an experiment incubating the organism at, for instance, three different temperatures and then measure viability over time. The resulting data could be used to calculate the activation energies and pre-exponential factors for specific organisms (Blanch et al. 1997, Atkins et al. 2010). After obtaining these parameters, it is very straightforward to calculate new suggested steam treatment times. Additionally, it would be good to validate the model predictions experimentally, but it is beyond the scope of this particular project.

With this modeling approach, winemakers will have a tool to determine how long they need to steam a barrel either before or between uses to minimize contamination that could lead to a negative impact on product quality. This model is easily adaptable to any organisms that winemakers expect to find in their production environment.

Conclusion

We used a combined heat transfer and thermal inactivation model to predict the death kinetics of wine relevant microbes over time as a function of distance into barrel staves. Based on this model, we predicted that it takes 9 min, 12 min, and 200 min to get a 5-log reduction for *Brettanomyces, Saccharomyces cerevisiae,* and *L. mesenteroides* respectively at 0.8 cm depth. This model can serve as a straightforward tool for winemakers to determine barrel steaming protocols based on their knowledge of the microbial ecology of their barrels and winery.

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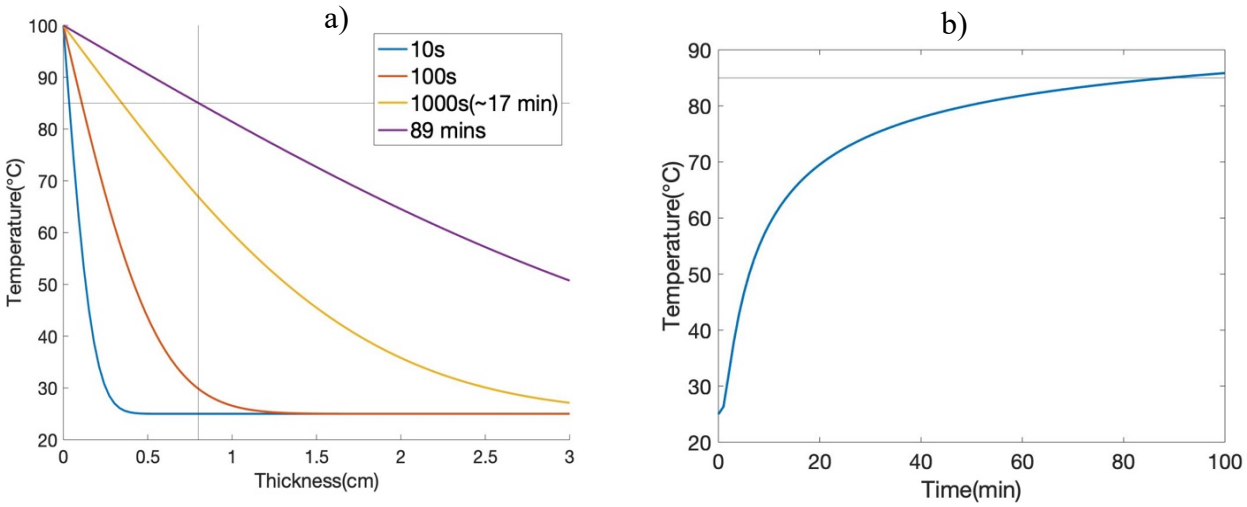
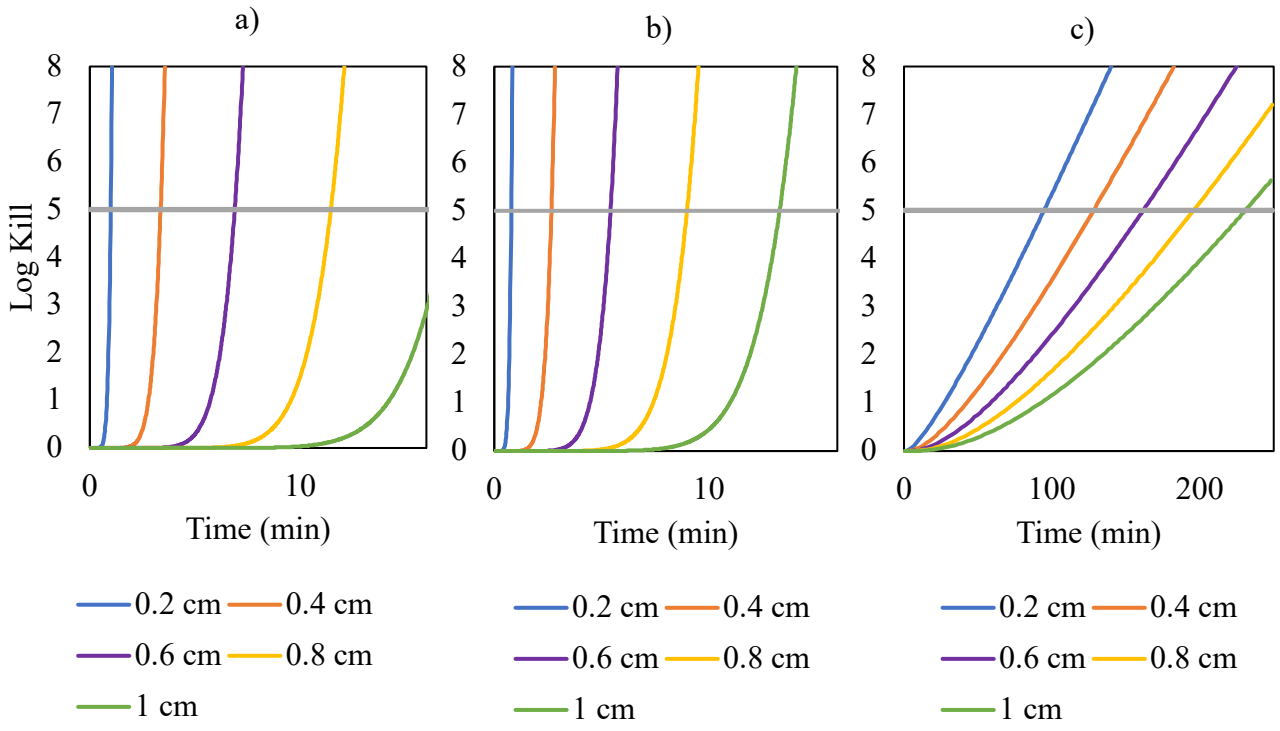
Figure Captions

Figure 3.1. Prediction of heat transfer into an oak stave. The temperature profiles as a function of depth into a stave are plotted for various steaming times (a). The vertical line at 0.8 cm represents the maximum depth known for penetration of microbes in oak. The temperature as a function of time at 0.8 cm depth is shown in (b). The horizontal line in both (a) and (b) is the target temperature 85 °C often used for sterilization in a winery setting.

Figure 3.2. Thermal inactivation of wine-relevant microorganisms at various depths in a barrel stave. Predicted kill curves are shown for (a) *Brettanomyces*, (b) *S. cerevisiae*, and (c) *L. mesenteroides*. By estimating when these kill curves cross the 5-log reduction line (a typical goal for thermal sanitization and sterilization), the time necessary for steaming can be determined. Here, it can be seen that these times correspond to 9 min, 12 min, and 200 min for the three organisms, respectively.





Tables

Table 3.1. The death kinetics parameters of certain species.

Species	A (1/h)	E_a (J/mol)
Brettanomyces	$2.558*10^{60}$	364100
S. cerevisiae	$1.680*10^{68}$	419100
L. mesenteroides	$7.163*10^{12}$	84220

Table 3.2. The time to reach to the target temperature 85 °C based on the depth.

Distance to the inner barrel surface (cm)	Time to reach 85 °C (min)
0.1	1.39
0.2	5.54
0.3	12.5
0.4	22.2
0.5	34.7
0.6	49.9
0.7	67.9
0.8	88.7
0.9	112
1	139

Chapter 4. Continuous Solid Phase Extraction of Grape Solids in a Column with Wine and Juice

Abstract

Phenolic extraction is essential for red winemaking. Fermentation along with phenolic extraction usually takes 7 to 14 days, although the tannin extraction can continue for weeks until an equilibrium is reached. Yet process intensification would allow winery equipment to be used multiple times during harvest, thus decreasing capital investment. While previous work showed the potential to speed up the conversion of sugar to alcohol considerably, this methodology will only be truly useful for red winemaking if phenolic extraction can be completed more rapidly and with more control. In this work, we performed phenolic extraction from grape solids in a column setup and let juice or wine flow through the column to model a continuous extraction. The effect of four different factors was tested: column temperature, liquid ethanol concentration, flow rate through the column, and column diameter using a design of experiments approach. We found that liquid flow rate is the most significant factor, and the temperature and ethanol concentration had minor effects but not statistically significant. With all optimized factors, we were able to reach the concentration as the bucket control for the final extraction in 86 hours on the bench scale compared to 144 hours for the control process. Both the pigment concentration and tannin concentration were similar. This work shows the potential to achieve process intensification and more control of the phenolic values in the finished wine.

Introduction

Fermentation of red wine contains two concurrent steps: fermentation of yeast transforming sugar to ethanol and extraction of phenolic compounds from grape skins and seeds. Fermentation provides the wine with the alcohol content, and the phenolic extraction provides the red wine with color, mouthfeel, and aging potential (Boulton et al., 1998). The fermentation process usually takes 7 to 14 days in a fermentor. A previous study done in our laboratory showed the potential to speed up sugar conversion to ethanol by programed nitrogen feeding (Miller et al. in preparation). Therefore, if it is possible to speed up the extraction of phenolics as well, red winemaking can be completed in a shorter amount of time, thus intensifying the process. Assuming that process intensification can be accomplished without loss of quality, this will benefit the wine industry as equipment needs and capital investment will be reduced.

During red wine fermentation, grape skins and seeds float to the top and form a cap, while the bulk juice is at the bottom. The solids in the cap accumulate heat created by the yeast and the heat is difficult to remove by conduction. The liquid below has natural mixing by carbon dioxide made by the yeast and by density difference from a temperature gradient caused by the coolant in the tank jacket. Extraction happens in the cap when the liquid and solid phases come in contact with each other. To do this, winemakers either pump the liquid from the bottom of the tank over the cap (“pump-over”) or by pushing down on the cap every couple of hours to mix the two layers (“punch-down”). Pump-overs and punch-downs can help to control the temperature in the cap and facilitate extraction.

Phenolic extraction has been shown to be impacted by various factors. The temperature effect has been investigated the most. Higher temperature and higher ethanol concentration result in more anthocyanins in the liquid phase (Medina-Plaza et al., 2020). For skin phenolics, as temperature increases, the rate of extraction increases but not the final values. For seed

phenolics, as the temperature increases, both the extraction rate and the final values increase (Lerno et al., 2015). A similar result was shown in other studies. At a higher temperature, both extraction rate and final level of tannins are higher (Beaver et al., 2020; Miller et al., 2019). Temperature improves the extraction, but it can also induce reactions that lead to a decrease in concentration in anthocyanins. The monomeric flavan-3-ol and anthocyanin concentrations are negatively correlated with fermentation temperature due to degradation (Lerno et al., 2015) and conversion to polymers (Ntuli et al., 2022). Similar effects of temperature on extraction are seen with other systems as well. For *Hibiscus sabdariffa*, Cissé et al.'s group showed that at a high temperature above 60°C, the extraction of anthocyanins reached the highest concentration around 20 minutes. After 20 minutes, the concentration decreased due to degradation (Cissé et al., 2012).

Chemical gradients form during fermentation in between cap management events. Lerno et al. showed that in the pilot-scale 2000 L, around 8 hours after the pump-over, the skin phenolics reached saturation in the cap and well above the concentration in the liquid below, but the seed phenolics did not reach saturation in the cap (Lerno et al., 2017). Pump-overs or punch-downs will eliminate these gradients and distribute the extracted phenolics into the liquid below the cap. It was predicted that lack of cap management could lead to heat accumulation in the cap and cause a higher seed tannins ratio. The pump-over is necessary for a large scale fermentor because the ratio of the jacket surface to the fermentor volume is small (Miller et al., 2019b, 2019a). For a smaller fermentor, such as a research-scale fermentor (around 150 L), pump-over volume and frequency do not influence phenolic extraction because of the greater control of temperature in the cap, as well as shorter distance between the bottom of the cap and the bottom of the liquid (Lerno et al., 2018).

Temperature is the main factor that can accelerate extraction. Flash détente as a thermovinification method is a pretreatment in winemaking practice that heats the must to a high temperature (85°C). In Ntuli et al.'s study, they found that flash détente significantly increased caftaric acid and quercetin glucosides. However, it lowered the color stability compared to conventional must heating due to degradation (60°C). It also resulted in lower monomeric flavan-3-ol (seed extraction). Proanthocyanidin and total phenolic concentrations after flash détente were relatively similar to extraction in liquid phase fermentation. Flash détente can change the sensory profile as well, so the winemakers should keep that in mind if they decide to use this pretreatment (Ntuli et al., 2020, Ntuli et al. 2022).

Using a physical understanding of phenolic extraction, Miller et al. were able to build a mathematical mechanistic model for the underlying phenomena. This model accounts for the release of phenolics from grape solids, the adsorption and desorption onto grape skins and seeds, and the different reactions of phenolics (Miller et al., 2019; Miller et al., 2019b). Adsorption and desorption of anthocyanins happen relatively fast compared to the fermentation process; they can reach equilibrium within an hour. The interactions with the solids affect the final concentration, and both temperature and ethanol concentration have positive correlations to the extraction (Medina-Plaza et al., 2020). Two common reactions phenolics undergo are polymerization and degradation. Although those reactions are more involved during aging, anthocyanins' disappearance during fermentation was noticeable in high-temperature fermentation. Setford et al. used a slightly different mathematical model approach to describe the extraction of anthocyanins as diffusion phenomena. They studied both natural and forced convection and the effect of ethanol concentration and found the diffusion parameters. Their simulation shows the forced convection has the fastest extraction rate at the beginning but then slows down and

follows the same trend as the natural convection. The hindered convection has the slowest extraction rate, and the final value of malvidin is lower than both convection trials (Setford et al., 2018).

Fermentation usually takes place inside a fermentor which is considered a batch reactor. Even though industrial extraction is commonly performed in packed beds, sometimes called plug flow reactors (PFR), there has not yet been a study that performed phenolic extraction in a PFR for winemaking purposes. In a fermentor, in between the pump-overs, the extraction occurs only in the cap and the surface between the cap and the liquid. Even though the concentration never quite reaches saturation before pump-over, the chemical gradient is relatively small compared to a PFR. For a PFR, the liquid concentration at the entrance is the lowest and the solids have a relatively high phenolic concentration. In Miller et al.'s model, the release of the anthocyanins and tannins from grape skins depends on the concentration difference between the available in the skin and the free in the solution (Miller et al., 2019). The potential of a larger concentration gradient in a PFR reactor may lead to a faster phenolic extraction.

Therefore, we wanted to use solid-phase extraction in a column to determine if a rapid extraction can be performed and more readily control the ratio of different phenolic compounds extracted. We used one of the most common red grape varieties, Cabernet Sauvignon, to study four factors: liquid temperature, ethanol concentration in the liquid, flow rate through the column, and column diameter. We used a factorial experimental design to quantify the impact of these factors. Then we used the experimentally determined optimal conditions to perform an extended run to confirm that these conditions can extract the phenolics faster than a similar scale normal fermentation.

Material and method

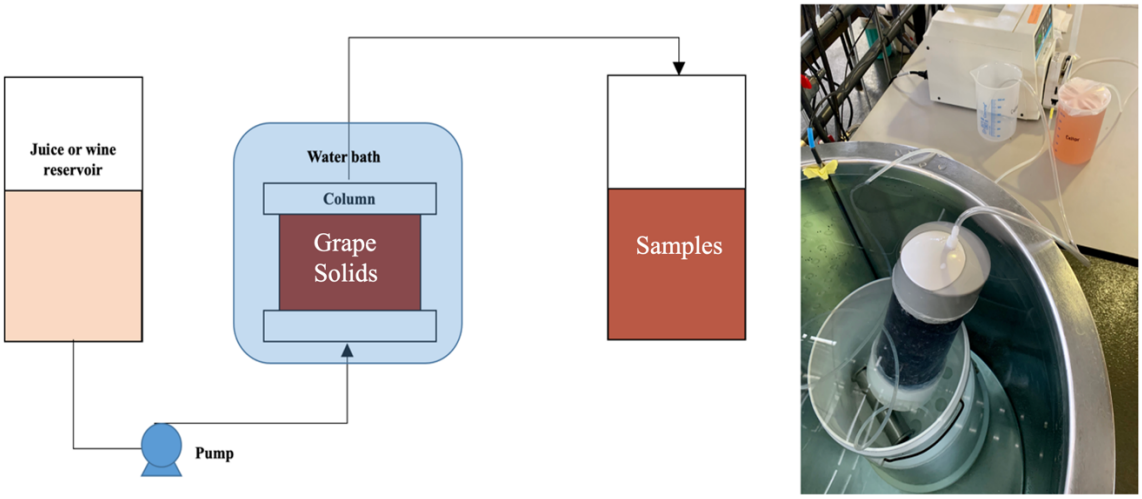


Figure 4.1. A schematic of the experimental apparatus and a photo of the actual setup.

Chemicals. HPLC. Acetonitrile (HPLC grade), (+)-catechin, (-) -epicatechin, p-coumaric acid, caffeic acid, quercetin, gallic acid were purchased from Sigma Aldrich (St. Louis, MO, USA). Malvidin-3-O-glucoside and quercetin-rhamnoside were purchased from Extrasynthese (Genay, France). Deionized water was prepared in-house to a final purity of 18.2 MΩ. All solvents prior to injection were filtered through a 0.22 µm filter. Winemaking additions, Go-Ferm and Fermaid K, were purchased from Scott Labs (Petaluma, CA). Tartaric acid was purchased from ATPGroup (Larchmont, NY). Diammonium phosphate and potassium metabisulfite were purchased from Laffort (Petaluma, CA).

Winemaking/juice preparation. Cabernet Sauvignon grapes were harvested from Davis, CA, for the 2020 harvest. Grapes were destemmed and crushed using a Bucher Vaslin Delta E2. Research fermentor tanks were used as fermentation vessels, filled successively in 20 L intervals to a final volume of 120 L to minimize vineyard variations. The tanks were used for different purposes. For the preparation of the column experiments, the juice was drained from the must, and then grape solids were packed in 1 gal bags, and the juice was stored in 1 and 0.5 gal jars. Both solids and juice were stored under -18°C. For the control fermentation process, prior to

fermentation, initial juice panels were performed to prepare for juice adjustment. Additions were made to each vessel to adjust yeast assimilable nitrogen to 250 mg/L using diammonium phosphate and Fermaid K, 50:50. 2.5 g/L Tartaric acid was added to increase titratable acidity, and sulfur dioxide was added to 50 mg/L using potassium metabisulfite. The red wine fermentations were controlled at 25°C with a programmed water jacket and inoculated with *Saccharomyces cerevisiae* strain EC1118 (Scott Labs, Petaluma, CA). All fermentations were sampled every day to check the brix and temperature to prevent stuck fermentation. A three volumes pump-over was performed four times a day. Once the red wine was dry, the must was pressed, and 15% potassium metabisulfite was added to achieve 60 mg/L SO_2 to stop the microbial activity and to preserve the wine. Rosé wine fermentation was prepared with identical additions but without grape solids, and then was fermented in a temperature-controlled room at 22°C without an active temperature control system. Once the rosé wine was dry, a 15% potassium metabisulfite solution was added to achieve 60 mg/L SO_2 to preserve the wine. A duplicate bucket fermentor treatment used frozen grapes solids and juice (3:1 ratio) and was adjusted to the same level of nutrient. The trial was fermented in a temperature-controlled room at 22°C and was punched down twice a day.

Large scale experiment. Cabernet Sauvignon grapes were harvested from Davis, CA, for the 2021 harvest. We used the same process and additions as the previous year to prepare for six 120L must fermentors. We duplicated three different treatments. The first treatment was control, which pumped over four times a day with three volumes. The second treatment (continuous flow) was pumped over once an hour, and the total volume for 24 hours was one volume. The third treatment (extraction column) first separated the juice from the solid, which was put into another fermentor. Then the juice was fed from the seed fermentor bottom and recycled back to

the juice fermentor from the top. One volume total was passed per day, and the pump turned on at the beginning of every hour for 2.8 minutes.

Column experiments. Four different factors were examined in a factorial experimental design, with all experiments performed in triplicate. The factorial design was assigned by Design-Expert version 13.0.8.0, and the design is shown in table 4.1. The temperatures were chosen to be 25°C and 35°C with a midpoint of 30°C because the control wine was fermented at 25°C, and this range is common in winemaking practice. Ethanol concentrations were 0% and 15%, which were grape juice and rosé wine made from the Cabernet Sauvignon grapes, respectively. The midpoint was a 50:50 volume ratio of the two liquids. The flow rates were chosen as 1 ml/min and 4 ml/min with a midpoint of 2.5 ml/min. The flow rates were chosen, given the column diameters, to assess if fluid velocity played a role in phenolic extraction. Lastly, the diameters of the columns were 2 in (5 cm) and 4 in (10 cm). The setup of the experiment is shown in Figure 4.1. For each run, the grape solids and the liquid were defrosted and warmed up to the desired temperature to prepare for the trial. Then, the column was filled with drained grape solids and sealed with adhesive. While waiting for the adhesive to dry, the flow rate of the pump was verified with the liquid used for that run. The column was then backfilled with the liquid, and the timer started, as well as the pump to feed the liquid from the bottom of the column. Samples were collected every hour. At the end of the trial, both the total mixture of effluent and the top of the inside of the column were collected for analysis. One volume of liquid was 1000 ml. For a short run (4 ml/min), 1200 ml liquid was passed through. For a long run (1 ml/min), 540 ml was passed through.

Sample Analysis. High-performance liquid chromatography (HPLC) was used to analyze the samples for each trial. Monomeric phenolics were identified and quantified by reverse-phase

HPLC. Gallic acid, (-)-epicatechin, epigallocatechin, polymeric phenols (280 nm), caftaric acid (320 nm), quercetin-glycosides (quercetin-3-O-glucoside, quercetin-3-O-galactoside, and quercetin-3-O-glucuronide) (360 nm), malvidin-3-O-glucoside, and polymeric pigments (520 nm) were quantified using a diode array detector. Calibration curves were created by authentic standards. An Agilent 1260 Infinity HPLC (Agilent Technologies) was used for the analysis and was equipped with an autosampler, a binary pump, and a diode array detector. Agilent CDS ChemStation software (version D.04) was used to perform instrument control and data analysis. The HPLC column was an Agilent PLRP-S 100A (150 × 4.6 mm, 3 μm) controlled at 35°C. Mobile phase A was water with 1.5% phosphoric acid (v/v), and phase B was 80% acetonitrile with 20% mobile phase A. The pump was set up for a flow rate of 1.0 ml/min (Girardello et al., 2020; Peng, Iland, Oberholster, Sefton, & Waters, 2002).

Data analysis. All data were analyzed with Design-Expert version 13.0.8.0. The significance test was set to a 95% confidence interval. The factorial experiment was a resolution 4 design, so the labeled factor interactions are chosen not the alias because they contained more significant primary factors.

Results

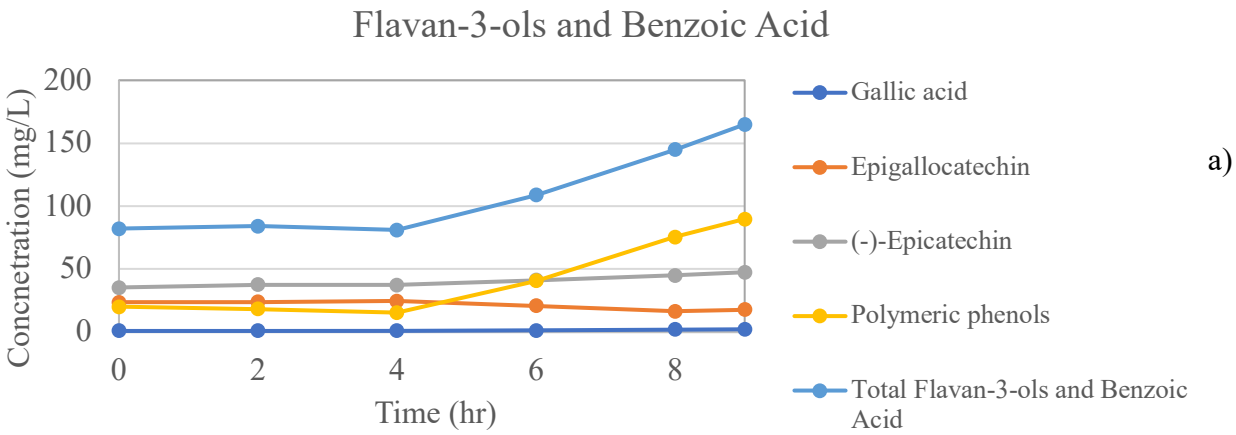
Kinetics of phenolic extraction in a column. Phenolic extraction in a flow-through column was investigated over time to understand the kinetics of the appearance of phenolics in the column effluent. Figure 4.2 demonstrates the phenolic profile from a run with the conditions of high temperature (35°C), higher ethanol concentration (15% v/v), low flow rate (1 ml/min), and small column diameter (2 in/ 5 cm). RP-HPLC was used to determine the different phenolic molecules extracted and quantify the amounts (Peng et al., 2002). The different molecules in the flavonoid fraction are tannin-related molecules. For example, epicatechin is a signal for seed tannins, and it

is extracted slower than the polymeric phenols which are found in the skins and the seeds. In the data analysis, we considered polymeric phenols at 280 nm wavelength as tannins. We also grouped all measured anthocyanins monomers and polymeric pigment measured at 560 nm wavelength as pigments. The quercetin glycoside peaks were not separated enough to have individual monomer concentrations, so quercetin-3-O-glucoside, quercetin-3-O-galactoside, and quercetin-3-O-glucuronide were treated as a group, quercetin-glycosides. In this set of experiments, the only hydroxycinnamates observed was caftaric acid, but limited extraction of this compound was observed. Thus, quantification of this class of compounds was not included subsequently in this study.

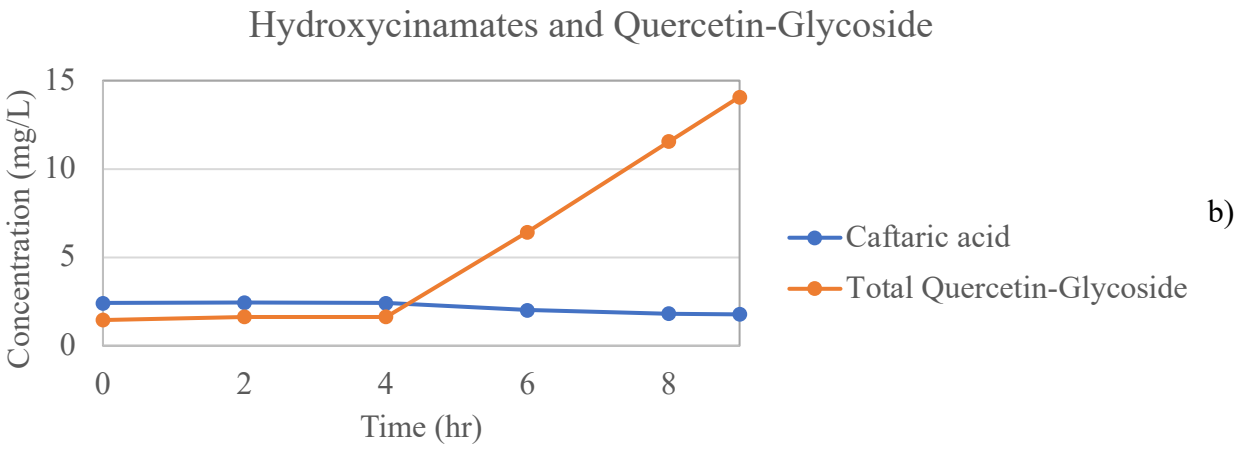
In figure 4.2, it can be observed that there was not much extraction in the first four hours, as all the liquid leaving the column up until this point has not contacted with the grape solids. The column was filled up all the way to the top, and there was an empty space without grape solids between the wire mesh and reducer that could not be further eliminated. Therefore, when the experiment started, there was no extraction on the top, and only limited diffusion was observed for the low flow rate trial. We observed that as soon as the liquid was in contact with the solid, there was color change. We were able to find that for the 5 cm diameter column, there was 120 ml empty space; for the 10 cm diameter column, there was 240 ml empty space. In the later data analysis section, the empty space volumes were taken out to have a better description of the extraction.

Flavan-3-ols, quercetin glycoside, and pigments increased during the course of the experiment. Polymeric phenols, malvidin-3-glucoside and its derivatives increased more significantly than the other molecules. Plot d contains two different trials with the same conditions; one collected samples for the first 9 hours and the other part collected samples from

10 hours to 22 hours. Due to the time constrain, it was not possible to collect samples every hour for a volume slow run (1 ml/min) in a day. For this experiment, we chose to collect samples during the first half for the slow flow rate runs because there was more extraction of a variety of molecules. Pigments were extracted most rapidly during the first 10 hours, and then plateaued. Toward the end of the run, pigments showed a trend of decreasing. Quercetin glycosides reached the maximum around the same time as anthocyanins. Tannin concentration increased slowly but steadily.



a)



b)

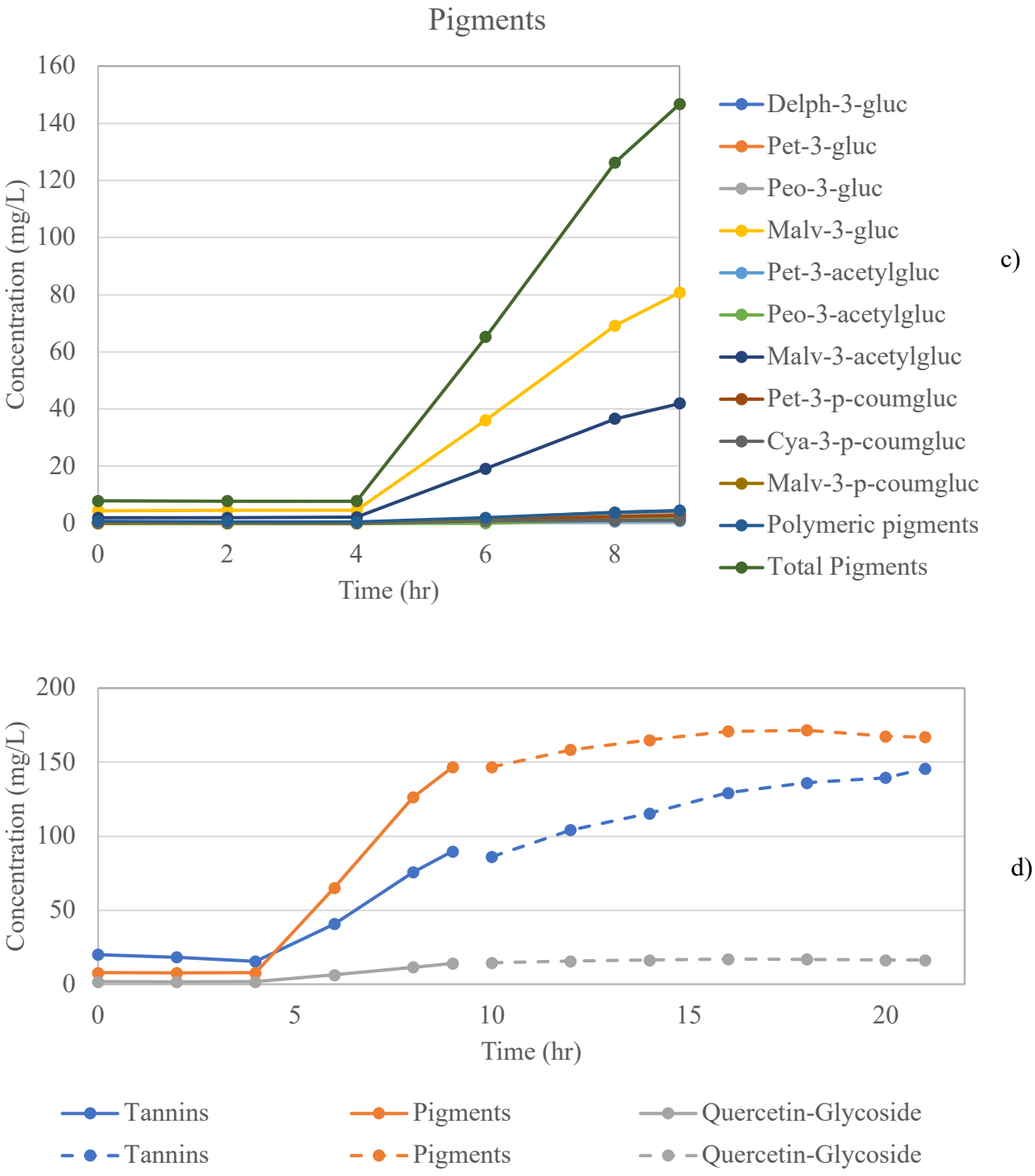


Figure 4.2. The result of one of the trials with high temperature (35°C), higher ethanol concentration (15% v/v), low flow rate (1 ml/min), and small diameter (5 cm).

The influence of process parameters. Given these data for one set of process conditions, we decided to examine the factors that were likely to affect extraction kinetics and extent. Four factors were chosen and studied using the fractional factorial design shown in table 4.1. To study the influence of temperature, ethanol concentration in the liquid, flow rate, and column diameter, we chose to compare the phenolic composition at the same effluent volume of 240 ml across all the runs. The same volume means that the same amount of liquid has passed through the column. This method was chosen because, for typical winemaking, the liquid to the solid ratio depends on the grape, and usually, winemakers would not vary this ratio much. In this case, the flow rate factor is equivalent to space-time, which is how long the grape solids, and the liquid are in contact. Figure 4.3 shows an overall concentration profile of each set of conditions at this volume. This volume is an early sample point, and the whole run passed 1200 ml liquid through the column. Pigments represented the largest values as they are typically extracted first, and tannins represented the next most abundant group of molecules. For the entire extraction run, tannins continued increasing and pigments reached a maximum during the run. The first four combinations of conditions exhibited more extraction and represented the experiments with the lower (1 ml/min) flow rates.

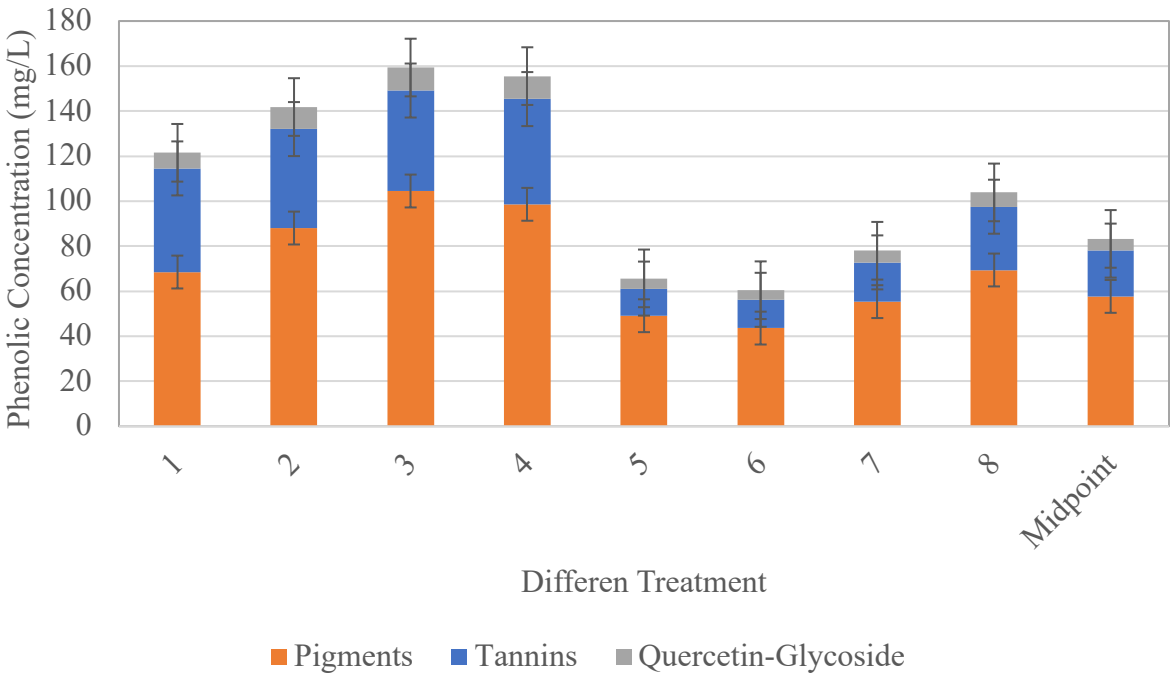


Figure 4.3. Overall concentration profile of each condition combination. The different treatment condition combination is defined in table 4.1.

To evaluate which factors were statistically significant, we performed a t-test analysis. The result of this analysis is shown in figure 4.4. The flow rate factor is the most significant for all three classes of phenolic compounds on which we have focused. For tannins, ethanol concentration and temperature are also important, and diameter is the least impactful of the individual factors. For pigments, ethanol concentration is significant for the t-value limit, followed by column diameter. Quercetin-glycosides have the same trend as pigments, but the ethanol concentration is insignificant. The factors seem not to interact with each other, and all the cross factors have low effects. The flow rate has a negative effect, while temperature, ethanol concentration, and diameter all have a positive effect on the extraction. In the next steps, the most critical factor, flow rate, was investigated.

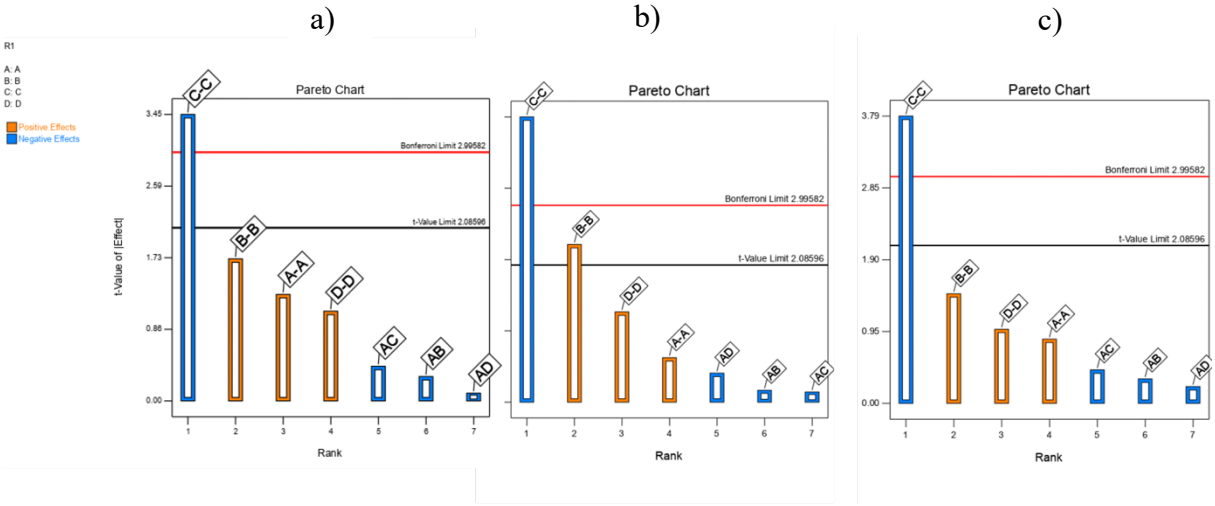


Figure 4.4. Pareto chart for different factors on different phenolics. Temperature, ethanol concentration, flow rate and column diameter are factor A, B, C and D respectively. Blue is negative effect and orange is positive effect. a. Tannins, b. Pigments, c. Quercetin-glycosides

The influence of the velocity of the flow on extraction. Both the flow rate and column diameter will affect the velocity of the fluid past the solids in the column. We compare the four different combinations of the flow rate and the column diameter shown in figure 4.5. For the 5 cm diameter column, 1 ml/min and 10 cm diameter column, 4 ml/min, the velocity of the fluid is the same. However, we did not observe a similar trend in phenolic extraction, which indicates that the velocity of the fluid is not as important as the flow rate. The trend for the different diameters with the same flow rate is similar. This is consistent with the results in the previous section that indicates the flow rate is the major factor while the diameter is less important. The difference between the starting increasing time point is due to the space void of solids on the top of the column. As mentioned earlier, the 5 cm column had less headspace. We then also compared the trend with the time variable on the x-axis. Even though the fast flow rate has a higher concentration through the run, at 5 hours, the two lines merge, and the slow run continues increasing after that point.

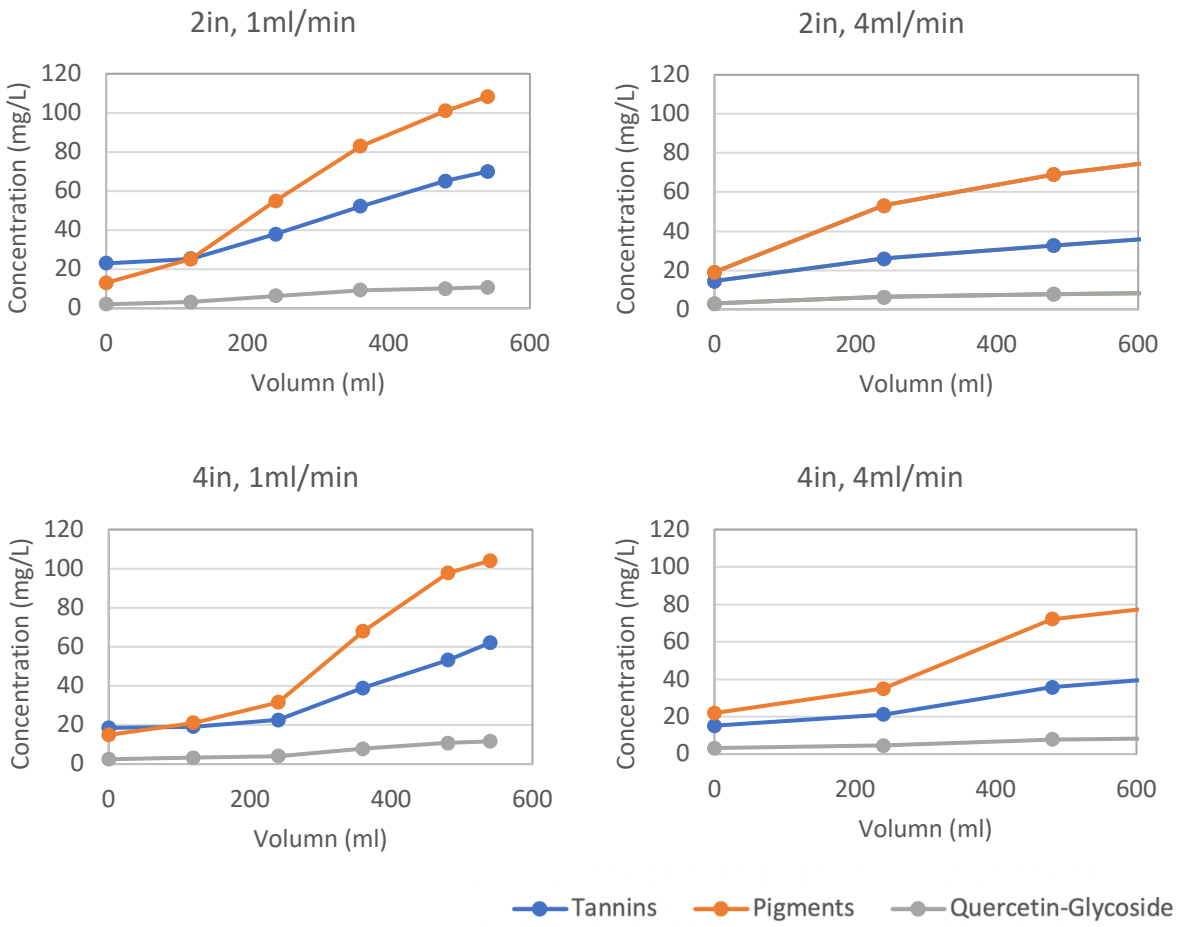


Figure 4.5. Outlet concentration based on the total outlet volume.

Focusing on high temperature and low flow rate operation. From the study of the factors influencing phenolic extraction in a column, the data indicate that the most crucial factors are flow rate and temperature. Even though ethanol concentration has some impact, it is challenging to vary during the winemaking process independent of the yeast fermentation. Therefore, we decided to examine the impact of an even higher temperature (45°C), along with the faster flow rate (4 ml/min). Higher temperatures were not possible with the current experimental apparatus. We chose a fast flow rate for this experiment, in order to limit the extent of anthocyanin reactions, especially degradation. Figure 4.6 illustrates the results of this study, which shows a

similar trend as other fast flow rate runs. However, we observed that it reached a maximum at an earlier time, and then there was slow extraction after three hours. The final values were not nearly as high as the control winemaking process.

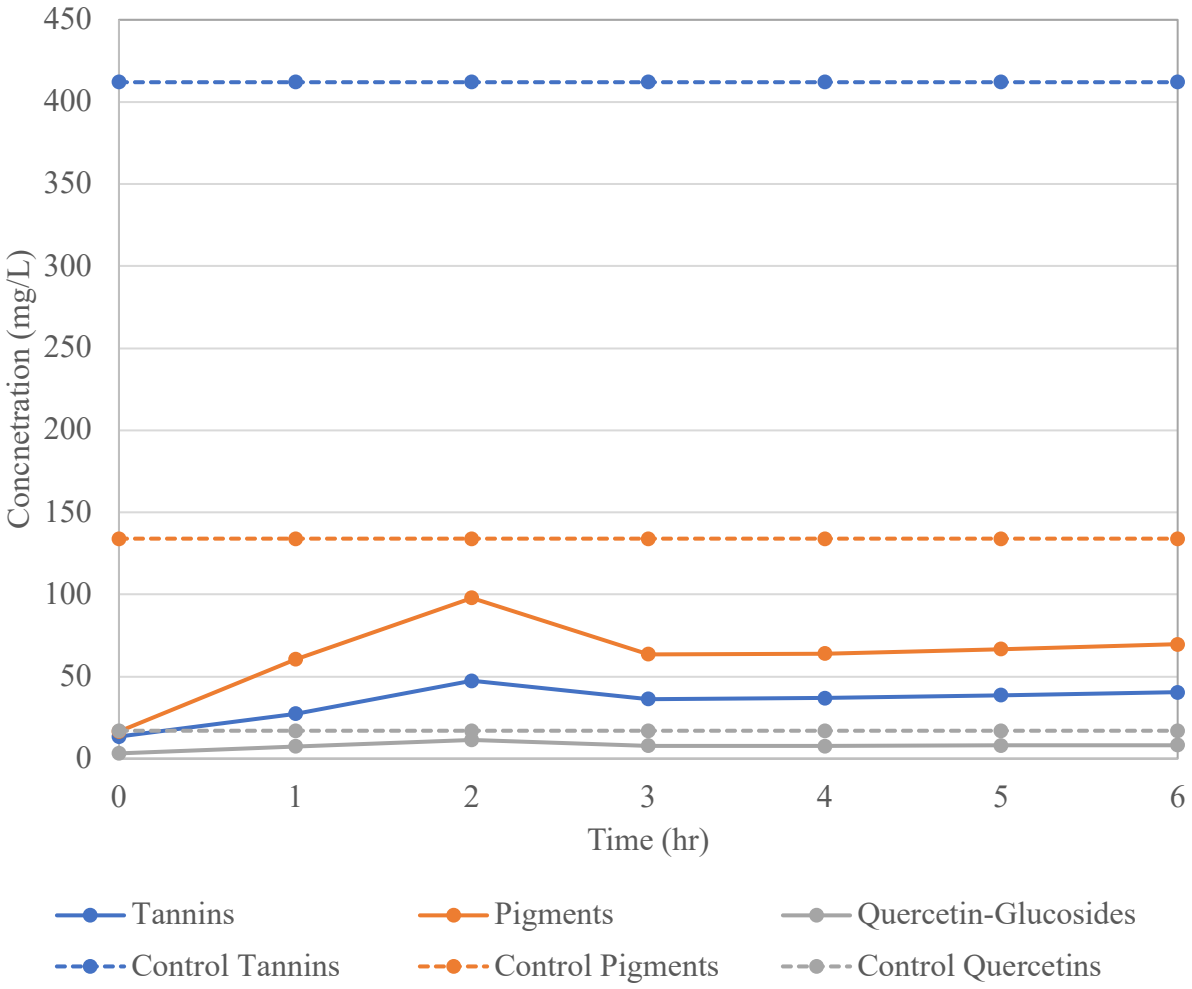


Figure 4.6. A high temperature with fast flow rate attempt.

Next, we examined whether an even lower flow rate would enable larger phenolic extraction. To do this, we chose a flow rate of 0.5 ml/min, along with 35°C, 15% ethanol (typical of finished wine), and 10 cm diameter column. Again, we did not further increase the temperature because of the increased degradation of anthocyanins. After flowing for 40 hours (which allowed for one volume of liquid to pass through the column), we drained the column and

fed the column with the total outlet of the first 40 hours. The total outlet for the first 40 hours had a lower average concentration compared to the later outlet samples (after 16 hours). That is, the sample at 42 hours is lower than the samples at 36 hours. Then we provided the column with the liquid drained from the column with a flow rate of 1 ml/min, and we recycled the outlet. We stopped the experiment at 86 hours which allowed the volume to pass through the column three times total. The outlet concentration is shown in figure 4.7. Note here that the mixture of the whole system had a higher concentration than the outlet sample at the last time point. The mixture of the outlet and liquid inside the column was examined and showed an even higher concentration, shown in Table 4.2.

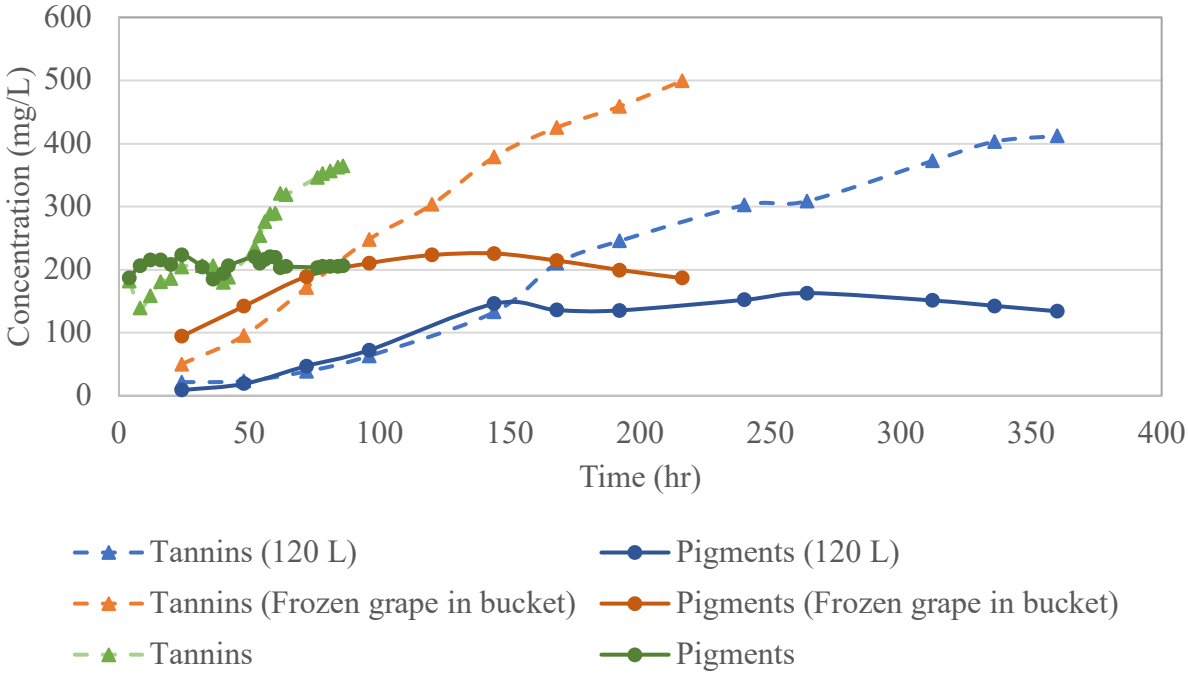


Figure 4.7. Concentration of the experiment and the different control. Blues are control fermentation with a total of 120 L grape must. Oranges are frozen grape fermentation in buckets, with 9 L must. Greens are the recycling run with a total of around 3 L frozen grape solids and juice.

Table 4.2. Different phenolic concentration of the baseline and different extraction practices in mg/L.

	JUICE	ROSÉ	CONTROL (360 HR)	BUCKET (216 HR)	EXPERIMENT (86 HR)
TANNINS	16	13	412	499	375
PIGMENTS	16	9	134	187	206
QUERCETIN GLYCOSIDE	3	2	17	26	20

The traditional extraction happens in a batch reactor that is a closed system. In this research, there were three different trials for traditional winemaking. The control red wine was performed in research fermentors which had must volumes of 120 L each. The rosé wine was made with the same Cabernet Sauvignon grapes in a stainless-steel fermentor that had 50 L juice. The frozen grapes fermentation was performed in a plastic bucket with 9 L must from the thawed grapes solid and juice. The phenolic profile of the red wine during fermentation is shown in figure 4.7. The last time point was collected when the yeast fermentation was done, and there was less than 2 g/L sugar left. The final phenolic compound profile is shown in table 4.2. The juice was drained from crushed grapes, and half of the juice was fermented to rosé wine to use as a high concentration of ethanol feed. The juice and the rosé were used as a baseline for the extraction experiment analysis. Compared to the bucket, the volume of the experiment was similar, and both experiments used frozen grapes solids and frozen grape juice. The result is promising because it reaches the same level of phenolic extraction in 40% less time.

Larger scale experiment. In the 2021 harvest season, we tried to set up a larger scale column in a research fermentor (120 L must). We had three different trials, which were described in the experimental section. The control and the continuous flow had very similar concentrations in

final tannins, quercetin glycosides, and pigments, but not in the caftaric acid concentration. The column extraction was different. The first time we tried the setup, there were lots of unexpected problems that occurred. After we fixed the issues, we reran the experiment. However, the result in the first trial was better than the second trial. The first trial had higher final phenol concentrations than the control, but the difference was not significant. The second trial had a final concentration similar to the control at the end of the fermentation and after press. The press was done on the 12th day, which was 288 hours of extraction.

Discussion

Extracting phenolics in columns outside of a wine fermentor represents an innovative means of extracting phenolics from grape solids. This is the first time that the extraction has been performed in columns instead of a batch reactor, which is commonly used in industry and research. We were hoping to find a faster overall extraction of phenolics and potentially more control over the composition. We found that the flow rate is the most significant factor followed by temperature, and ethanol concentration depending on the specific compound. With a slow flow rate, high temperature, higher ethanol, and with recycling after one volume passed through and a total of three volumes passed through the column, phenolic extraction can be done in 3.6 days on a bench scale, which was shorter than 7 to 14 days of standard winemaking. Therefore, this new way of extraction has the potential to achieve process intensification.

In this experiment, we can get a much faster extraction in the column scale, and we found that the flow rate is the main factor and had a significant impact on pigments, tannins, and quercetin-glycosides. For some studies done in other extraction matrices, the flow rate is also important. Researchers found that if the limiting step in extraction is desorption/kinetic, then the extraction rate is negatively related to the flow rate. If the limiting step is the solubility/elution

process, the extraction rate is positively related to the flow rate (Hawthorne, Galy, Schmitt, & Miller, 1995). Another study also found that with a slower flow rate, they had increased extraction (Westerman, Santos, Bosley, Rogers, & Al-Duri, 2006). They believed that a slower flow rate favors the diffusive behaviors through the Amaranth seed matrix. This is likely because it requires a longer contact time to diffuse into the micropores (Westerman et al., 2006). The negative correlation between the flow rate and the extraction may indicate a special physical structure of the grape solids. It requires a longer contact time to extract, and the relative speed between the liquid and the seed should be small. As previous studies showed, the longer the contact time between the liquid and the solid, the more phenolics can be extracted (Lerno et al., 2017; Miller et al., 2019b). Based on the result of the four different factors studied, we saw that the higher flow rate experiments reach higher phenolic concentrations more rapidly but then drop. In the temperature study, we observed that after the concentration drop in the outlet sample, there was a very slow increase of all phenolics. We can hypothesize that the extraction process can start with an initial fast dissolving of the chemicals on the surface, followed by extraction from the grape solid cells. One other study explained the extraction in a similar manner (Zanoni et al., 2010).

The next most important factors were temperature and ethanol concentration, which is consistent with past studies performed in batch systems as well. For tannins, as temperature increases, the rate of extraction increases for both seed and skin phenolics (Lerno et al., 2015). Similar to temperature, as ethanol concentration increases, the desorption of the phenolics from grape solids increases, which leads to a higher concentration in the solution (Beaver et al., 2020; Medina-Plaza et al., 2020). With the low temperature, there was minimal extraction. Therefore, we wanted to investigate higher temperatures, but equipment limitations only allowed us to

investigate as high as 45°C. In our experiment of high temperature and higher flow rates, we did not see significant extraction, indicating that the temperature and ethanol concentration are not nearly as important as flow rate.

As we tried to perform the solid-phase extraction on a larger scale, we ran into issues that might result in a slower extraction rate. The first obstacle was enlarging the column to the industrial scale. The existing apparatus, such as large scale fermentor, is difficult to convert into a PFR. We were able to use tubing and pumps to mimic a column situation in a pilot-scale fermentor (120 L), but we were not able to have a continuous flow due to the limitation of the pump. To accomplish a similar flow, we used the built-in pump on the pilot-scale fermentor, which ran at too high a rate, forcing us to use a discontinuous flow rate to simulate the desired flow rate. This intermittent flow could have caused changes in the local chemical equilibria that hindered further extraction. Also, for this trial, the flow rate needed to be 100 times faster to finish three complete cycles in the first 80 hours. We knew that the flow rate is the most important factor. By increasing it 100 times, it is likely that the extraction is slower than the experiment. In the future, it might be a good idea to test a slower continuous flow rate at a higher temperature in the initial three days of fermentation and otherwise better mimic the experimental conditions in the column extractions.

We also suspect that the temperature may be another explanation of the slow extraction. We used 25°C as fermentor temperature because the yeast is well adapted to this temperature, making stuck fermentation less likely. Higher temperatures can cause heat accumulation in the cap. We know that in a short run, the temperature is not as important as the flow rate. But with previous studies, the temperature is an important factor when the run is long (Lerno et al., 2015; Miller et al., 2019). In the future, it might be a good idea to test a slower continuous flow rate at

a higher temperature in the initial three days of a fermentation and otherwise better mimic the experimental conditions in the column extractions. Alternatively, we could try a high temperature and slow flow rate for the first two days and then lower the temperature to one that is more desirable for yeast growth and sugar conversion. This way, we can achieve high temperatures (35°C or 45°C) without inhibiting the yeast or affecting wine quality. These conditions would be more similar to the experimental column setup, which may help us to achieve a faster extraction than standard winemaking.

Another possible explanation of the 120 L larger scale extraction column having slower extraction compared to the bench scale experiment is the impact of the scale. Both Miller et al.'s papers found that the larger the fermentor is, the worse temperature management within the cap. The higher temperature in the cap can lead to better seed tannins extraction (Miller, Oberholster, & Block, 2020; Miller et al., 2019b). However, all the fermentors in the studies are larger than the 120 L fermentor we used and the bench scale extraction column. In the small-scale range, the effect of the size on the extraction is not clear. In the control (120 L) and bucket (9 L) trials, we found that the bucket extraction was faster. This may indicate why our pilot-scale column (120 L) had a slower extraction.

In table 4.2, we can see that the control, bucket, and experiment column run have different final tannin to pigment ratios. The experiment with the 0.5 ml/min flow rate and recycling the flow has the lowest ratio, and the control wine has the highest. One possible explanation is that the total contact time between the juice and the solids was significantly shorter than other runs. The extraction of tannins takes a longer time, while the anthocyanins can participate in reactions as the time proceeds. The result of the short contact time for the extraction is that the tannins were slightly lower while the anthocyanins were higher, so the ratio

was smaller than the other treatments. However, at an earlier stage (240 ml) for the 0.5 ml/min flow run at 240 ml, the ratio of tannins to pigments is 0.99, which is much higher than all other factor combinations with an average of 0.50. It seems that with a slower flow rate, the ratio is higher. This is also consistent with the previous studies, in which the extraction of the tannins is slower than the extraction of anthocyanins.

One of the limitations of this work is that we used frozen grapes to accommodate the length of experiments—it would be impossible to keep the clusters of grapes fresh for months otherwise. However, from previous studies, the frozen process has a minor effect on the phenolic concentration of the final wine (Jiranek & Schmid, 2011; Schmid et al., 2007). Also, because all the experiments used frozen grapes, it should not impact the comparative effects observed.

Despite these limitations, the result of this research is promising. We found that the flow rate is the most important factor in continuous column-based extraction of grape solids, and we were able to finish the extraction in less than four days at the bench scale, which was 40% less time than in the traditional red wine fermentation using frozen grape in a bucket. This is the first attempt to extract grape phenolics in columns, and there are many directions in which we can continue to work to achieve process intensification.

Conclusion

This work found that in a plug flow reactor setting, the flow rate is of primary importance for phenolic extraction. The choice of 0.5 ml/min combined with the optimal characteristics of high temperature and high ethanol, can obtain the highest extract amount in a short amount of time. However, it was necessary to recycle the wine through the column multiple times in order to achieve extraction similar to that found in the conventional red wine fermentation. In the

future, it would be good to develop a mechanistic model to predict the extraction in the column, as this type of model would greatly facilitate process optimization and scaleup.

Table 4.1. The arrangement of the factorial experimental design. Runs with the same experimental condition are given the same condition code.

Std	Run	Factor A: Temperature	Factor B: Ethanol Concentration	Factor C: Flow Rate	Factor D: Diameter	Condition Code
1	16	-1	-1	-1	-1	1
2	18	-1	-1	-1	-1	
3	6	-1	-1	-1	-1	
4	12	1	-1	-1	1	2
5	20	1	-1	-1	1	
6	2	1	-1	-1	1	
7	11	-1	1	-1	1	3
8	5	-1	1	-1	1	
9	27	-1	1	-1	1	
10	26	1	1	-1	-1	4
11	1	1	1	-1	-1	
12	29	1	1	-1	-1	
13	3	-1	-1	1	1	5
14	21	-1	-1	1	1	
15	4	-1	-1	1	1	
16	24	1	-1	1	-1	6
17	23	1	-1	1	-1	
18	13	1	-1	1	-1	

19	7	-1	1	1	-1	
20	14	-1	1	1	-1	7
21	19	-1	1	1	-1	
22	15	1	1	1	1	
23	22	1	1	1	1	8
24	28	1	1	1	1	
25	10	0	0	0	-1	
26	9	0	0	0	-1	
27	17	0	0	0	1	Center
28	8	0	0	0	1	Point
29	25	0	0	0	1	
30	30	0	0	0	-1	

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Chapter 5. Conclusion and future work

We were able to apply chemical engineering methodology to the winemaking field. We first were able to combine mathematical expressions for heat transfer and thermal inactivation of microbes to predict the death kinetics of unwanted microbes in barrel staves. We used this model to calculate how long to steam a barrel to achieve 5-log kill in a used barrel. This work is a tool for winemakers to determine steaming protocols for their used barrels based on the microbial ecology and desired contamination risk.

We then studied four different factors affecting phenolic extraction from grape solids in a column. The four different factors were temperature, ethanol concentration, flow rate, and column diameter. We found that flow rate was, by far, the most significant factor for all the different phenolics. With this information, we tested a recycle system where the column effluent was continuously returned to the column inlet multiple times. This system allowed extraction to occur in four days to the same degree as a parallel traditional red wine fermentation. This work shows the potential of an innovative means of winemaking, which can achieve process intensification and more control over the extraction.

In the future for our barrel sterilization work, it would be good to test the death of three different microbes experimentally and to see if our model can accurately predict the time needed to sanitize the barrels. It would also be important to incorporate the thermal inactivation values of other potential wine contaminants into the model and find how long it would take to reach 5-log kill.

For the phenolic extraction research, we can further improve the setup in the winery setting, and then to see if we can achieve the extraction in a faster way. This will help us to see if we can scale up our result and apply it at a commercial scale. We could facilitate this work by

using COMSOL or other software to model the column of the grape solids, and then predict the extraction based on the temperature, ethanol concentration and flow rate. Since the column diameter seems not important, the model can be set up as a series of continuous stirred tank reactors. We can then apply the equations determined previously in our lab and then compare the results to this study. If the model can give us a reasonable result, we can use it to simulate more experiments and find the most ideal situations for phenolic extraction.

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