# VOLATILE AND COLORIMETRIC COMPOSITION COMPARISON OF PRE-FERMENTATION PROCESSING METHODS OF CABERNET SAUVIGNON WINE GRAPES

By

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VOLATILE AND COLORIMETRIC

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ABSTRACT:
This research project was designed to evaluate flash détente and thermovinification relationship to color development and aroma composition. Parameters investigated in this study include standard analyses of wine, colorimetric analyses, and volatile composition characterization. The methods of pre-fermentation include a flash vacuum expansion process, termed flash détente, involving high heat and high vacuum and another technique involving heat only, termed thermovinification. The standard analyses include pH, titratable acidity, and alcohol by volume. The process of flash détente involves a combination of heating wine grape berries from 80 to 90 ºC for a brief period of 2 to 6 minutes. After heating, the solid mass is introduced into a low pressure vacuum chamber below 10 kPa, where, hypothetically, the cellular matrix of the grape skins burst from the inside, giving audible popping sounds, allowing for better extraction of anthocyanins, skin tannins, and other grape skin phenolic compounds in addition to modulating flavor and aroma compounds. Thermovinification involves heat over a longer period, generally 1 to 24 hours, minus the vacuum step. Flash détente and thermovinification resulted in an increase in the wine color density, a measure of the color intensity of red plus yellow/brown pigments. Furthermore, yeast assimilable nitrogen, a measure of nitrogen available to yeast and an indirect measure of volatile concentration formed, increased in both flash détente and thermovinification trials. Volatiles were impacted by processing, resulting in a decrease of undesirable aromas. Results demonstrate ability of the flash détente process to improve the coloration of red wines as well as accentuate fruity characteristics.

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## INTRODUCTION

The grape is the most valuable horticultural crop in the world; while the berry is mostly processed into wine, some is destined for fresh consumption as table grapes, dried into raisins, processed into non-alcoholic juice, and distilled into spirits (Myles et al. 2011). Wine is an alcoholic beverage produced with the fermented juice of grapes. Wine has received particular interest from consumers in recent times due to its valuable nutritional content as well as its bioactive components, including many classes of phenolic compounds, responsible for the taste and coloration of red wines. Phenolic compounds are synthesized in grapes as a plant defense against ultraviolet radiation, infection by pathogens, parasites, or predators (Stalikas 2007). Phenolic compounds are important to red wine quality because they impart color, mouthfeel, and ageability (Sacchi et al. 2005). Although phenolics are present in grape juice, the amount is generally much lower than in red wine, because of higher solubility in ethanol than in purely aqueous solutions and thus are better extracted during fermentation (Sacchi et al. 2005).

Color is easily the most recognized aspect of red wine quality for consumers. Two classes of phenolics, called anthocyanins and polymeric pigments, are responsible for the red-purple coloration of red wines (Sacchi et al. 2005). Once grapes are crushed, polymeric pigments begin to form, and over time, their amounts tend to increase (Somers 1971). Researchers

estimate the total phenolic distribution in red wine grapes to be: pulp, 1%; juice, 5%; skin, 50%; and seeds, 44% (Kramling and Singleton 1969). Each class of phenolic compounds is compartmentalized within specific areas within the grape berries. Anthocyanins, the color component responsible for red grapes characteristic color, are found in the skins, specifically the hypodermal cells (Sacchi et al. 2005). Cinnamic acids and their esters are found in the juice and their skins, which account for flavor and aroma (Sacchi et al. 2005).

Due to the major influence on phenolics on red wine quality, many winemaking techniques have been developed to influence extraction of specific compounds during the vinification process. Compartmentalization factors limit the extraction of different classes of these phenolic compounds. Since anthocyanins are located in the vacuoles of the hypodermal cells, successful breakdown of the physical barrier is necessary to release these specific compounds. Thus, it is important to rupture the membranes of the grape skins rather than merely collapse the cell, as a collapsed membrane can trap internal compounds and components (Sacchi et al. 2005).

Fermentation has a profound effect on the concentration and quality of the compounds that promote health benefits and consumer desirability. Traditional fermentation factors such as alcohol, carbon dioxide, and sulfur dioxide, combined with heat increases permeability of the cells and specific membranes (Sacchi et al. 2005). Even with these factors, the extraction of desirable compounds is not optimal. The fermentation process for grapes relies on the ability of yeast to convert sugars to alcohol, esters, and other volatile and non-volatile compounds (Duarte et al. 2010). Interestingly, the fermentation process increases the level of antioxidant activity by facilitating the extraction of anthocyanins and other phenolic compounds. However, many

volatiles are tightly bound to the cellular wall, necessitating rupture of the membranes for fermentation methods to capture and catalyze new volatile reaction products.

Wine flavor and aroma comprises a complex chemistry in both qualitative and quantitative terms. Several classes of compounds, such as hydrocarbons, alcohols, terpenes, esters, aldehydes, ketones, acids, ethers, lactones, and sulfur and nitrogen compounds define wine aroma (Etievant 1991). Particular attention has been devoted recently to the analytical characterization and the quality improvement of the varietal aroma of wine (García-Carpintero et al. 2011). Furthermore, there is an increasing interest in the world wine market to supply a red wine with fruity characteristics (Geffroy et al. 2015). Aroma production is influenced by several factors: environment (soil, climate), grape variety, ripeness, fermentation conditions, and biological factors, winemaking processes and aging (Rapp 1998). Most of the volatile compounds may play a role in the aromatic profile of a wine depending on two factors: concentration and their sensory perception factor. A better understanding of wine volatiles may guide winemaking quality control and may have an impact on the viticultural and wine technological processes (Bonino et al. 2003).

While there are clear sensory differences in the aromas of different grape varieties, the overall volatile composition of most varieties is similar (Herszage and Ebeler 2008). Since these specific volatile compounds are present in the range of mg/L to ng/L concentrations, their sensory thresholds may have an enormous impact on the overall wine aromatic profile. Gas chromatography is an important technique for the analysis of these volatile components responsible for the aroma of a particular wine.

This research project was designed to evaluate color extraction and volatile composition of two grape pre-fermentation processing techniques, flash détente and thermovinification, using grape berries grown in Oklahoma. To accomplish this goal, standard measurements of the must and wine were taken over time including: pH, titratable acidity, alcohol concentration, colorimetric analysis, and other factors. These factors are important to the quality of the finished wine, as most producers set quality parameters to control the overall product consistency that is sold to consumers and buyers. Furthermore, the concentration of aromatics present in the wines were quantitatively determined using Gas chromatography with a flame ionization detector to investigate if the different processes had an impact on certain compounds.

The following specific study objectives were tested:

1. Flash détente pre-fermentation processing extracts higher concentrations of color parameters, including color density and anthocyanin pigments.
2. Flash détente removes undesirable aromas from wine through use of a condenser in the flash process
3. Pectinase further breaks down the grape skins and releases higher concentrations of pigmentation than treatment alone

# Chapter II

## LITERATURE REVIEW

Because grapes readily ferment, wine has an archeological record dating back more than 75,000 years ago (Jackson 2008). Some researchers would argue that this does not constitute the modern definition of wine, and thus, the first incidence of winemaking techniques appears in the representation of a winemaking press from the reign of Udimu (Egypt), around five thousand years ago (Petrie 1923). Wines took their modern expression in the 17$^{th}$ century, where sulfur additions became common, greatly increasing the likelihood that higher quality wines were produced and extended their aging potential (Jackson 2008).

The basic overall process of the production of wine occurs in many discrete steps. Red wine grapes are picked by hand or less commonly, mechanically, at physiological maturity after determination of an adequate brix value by refractometer measurements. Other determination factors for grape picking are color, pH, acidity level, and taste. The grapes may be stored in refrigerated storage until processing to remove field heat and preserve the quality of the finished wine. When the grapes are ready to be processed, whole bunches of grapes are weighed and then processed through crusher/destemmer equipment to partially release juice and remove stems from the process. After this step, the grapes are collected into bins and transferred into fermentation vessels and fermentation can proceed.

Yeast is added at specific levels and the type of yeast is dependent on the winemaker's desires. Fermentation generally occurs over seven to ten days in contact with the skins and seeds depending on the requirements of the winemaking process. Fermentation with the skins allows the tannins, anthocyanins, and other compounds to be extracted to enhance flavor and astringency on the tongue. After the initial fermentation, the skins are pressed from the juice after the yeast has nearly metabolized all free sugars into alcohol and carbon dioxide. At this point, the winemaker has a decision to make, either bottle the wine or continue through secondary fermentation involving the use of lactic bacteria. Currently, the process is standard for winemakers in red wines to convert the tart tasting malic acid to the softer tasting lactic acid (Jackson 2008). Furthermore, malolactic fermentation removes residual sediment containing dead yeast cells and precipitated compounds in the solid phase. An addition of a sulfur dioxide solution is used to kill off any native yeast or bacteria that might be present after processing of the must, which could cause spoilage of the juice and subsequent wine. Generally, wine is allowed to age in oak barrels to add complexity and the addition of oak lactones before bottling, although it is not necessary (Jackson 2008).

Innovation in the wine business helped pave the way for new wine processing techniques to be developed. Thermovinification is one process developed in the 1950s involving heat treatment of must in California (Berg 1950). In the early 1990s, researchers at The National Institute of Agricultural Research, INRA, in France, developed a unique vacuum processing method for extracting phytochemicals from grape skins and seeds, termed flash détente. While vacuum processing may be relatively new to the wine industry, other similar processes have been utilized for many decades in other sectors of the food industry.

Vacuum cooling is one such technology used in the US for over 50 years on specific horticultural products, including leafy greens, fruits, and vegetables (McDonald and Sun 2000). When leafy greens or other horticultural products are sent through the vacuum system, an immediate drop in temperature occurs, similar to flash détente processing. The vacuum cooling process is used to remove field heat from the products, and to extend the shelf life for both fruits and vegetables, as well as leafy greens (McDonald and Sun 2000).

Deaeration is also a process similar to flash détente that resolves certain issues surrounding unwanted odors and color underdevelopment. The goal of deaeration is to reduce levels of dissolved oxygen that affect overall flavor deterioration. Deaerators are commonly utilized in the food industry for applications of milk, juices, fruit fillings and preserves, sauces, in addition to other food products.

Jordan, Goodner, and Laencina (2003) analyzed the effects of deaeration and pasteurization of orange juice aromatic fraction, finding that deaeration had the largest effect on the volatile composition. Another benefit of the deaeration process is its ability to extract color compounds in juices and fruit purees. Flash détente grape processing, like deaeration, strives to resolve issues related to color development and undesirable aromas. Flash détente may then be considered a natural evolution utilizing aspects and principles of these similar processes, in order to create high quality wine for the industry.

The flash détente process is not a supplementation of steps in the winemaking process, rather it is an addition of steps to increase the likelihood of producing a high quality wine. After grape picking, grapes are crushed and destemmed to partially breakdown the grapes and remove stems. Since heat is applied in the flash process, field heat removal is not necessary. Following crushing, twenty to thirty percent of the total mass in the form of liquid is removed before

heating. Next, the flash detente process involves heating grapes rapidly in an oxygen-reduced atmosphere. Once a temperature of 80-90°C is reached in the center of the berry skins with residual pulp, they are transferred a vat under a low-pressure, high vacuum (less than 10 kPa), where an abrupt drop in pressure causes immediate cooling and release of steam (Paranjpe 2008). This is where the term détente comes from; it is the French term for instant relaxation, as in relaxation of the grapes to release water vapors. The flash détente technology causes mechanical de-structuring of the cellular matrix of the grapes, releasing targeted volatile compounds, mainly from the skins of the grapes. What is important to keep in mind is that flash détente enables optimization of other steps in winemaking, and produces wines with greater typicality, or more consistency year over year. This physical pre-treatment of the grapes is the starting point for numerous winemaking methods, and is intended to result in the production of richly colored grape juice containing a higher concentration of polyphenols (INRA 2013).

Vapor that is released during the flash vacuum process is condensed using a condenser and collected in a container for disposal. The condensate, known as flash water, is generally high in aromatic volatiles known as methoxypyrazines and other volatiles that impart grassy or earthy characteristics to the wine. After the flash détente process, the must is then either pressed off into the fermentation tank or it is pumped to the tank for fermentation with the skins. Juice that is pressed from the flash détente process rapidly cools and separates from the skins. During the heating process, destructive enzymes (oxidases like tyrosinase and laccase produced by botrytis) and microbes are inactivated, improving the chances that high quality wine can result after fermentation, bottling, and aging (Miguel and Valero 2014).

In the flash détente and thermovinification process, to distinguish it from a traditional fermentation, fermentation of the wine can occur once the juice decreases temperature to around

$24^0$C. The justification for $24^0$C is dependent on the yeast, as most wine yeast ferments optimally at $24^0$C (Jackson 2008). The resultant product is filtered to remove the free run juice from the crushed berries. After the free run juice is filtered, the grapes are then heated with a heat exchanger until they reach the desired temperature of 85-$95^0$ C. Upon reaching the temperature range, grapes are placed into the sample chamber and subjected to low vacuum pressures, at which point they theoretically imploded and released phytonutrients into the juice phase. Condensate can be isolated from the juice containing higher amounts of anthocyanins, polyphenols, and overall color, a benefit of the process.

The flash technology is PERA® patented worldwide since 2002 but as of 2013, only a few systems for Flash Détente have been produced to date: 75 units in France, 10 in South Africa, 10 in Spain and 5 in the US (Favarel 2013). Moreover, commercial Flash détente systems are able to handle anywhere from 1 ton per hour to more than 50 tons of grapes per hour (Paranje 2009). The flash détente process is known to enhance tannin, color, and polyphenol extraction as well as resolves issues with underripe grapes. Furthermore, because no research focuses on the specifics of flavor and aroma compounds produced during the flash détente process, there is an opportunity to explore the possibility for the wine industry to utilize the technology increasing the likelihood of producing quality enriched red wines.

Due mainly to its midcontinental climate, Oklahoma has common issues surrounding the quality of specific varieties of grapes produced in the state, specifically color development and aroma composition. Understanding of grape varieties that will thrive in Oklahoma and command high market prices may help build a foundation that could improve the wine business in Oklahoma.

Hybrids generally fare better in the Oklahoma environment, though at market they command a lower price less per ton of grapes, with the average per ton of hybrid grapes at 400-700 dollars and the average price paid for *vinifera* grapes is 1000-1500 dollars per ton (Stafne 2014). V. *vinifera* the preferred grape variety of consumers all around the world, for the reason that it makes a high quality wine, likes hot and dry conditions in summer, and is prized for its productivity.

The reason that *vinifera* does not fare well in parts of Oklahoma is due the lack of cold hardiness, reduced disease resistance, and its need to be intensively managed (Stafne 2014). Even in spite of the issues surrounding vinifera, it is the preferred grape variety to grow for commercial production. For these reasons, a processing method that addresses low brix accumulation in the grape berry as well as weak coloration and off flavors for red wine production is essential. Currently, there exists a multitude of solutions to assist in the processing of grapes in wine but still overall quality issues still endure.

The traditional method of color and flavor extraction from the skins of red grape berries is to macerate at a temperature between 24-32$^0$C, requiring contact between grape skins and juice from the berries (Jackson 2008). Thermovinification is a winemaking technique used by certain wineries, where heat is used to extract color and flavor from the grape skins, an advance over classic maceration techniques. After the crushing and destemming step, the crushed mass is heated to 60-75$^0$C for 30 minutes up to 24 hours (Geffroy et al. 2015). After this heating step, the must is cooled down to yeast pitching temperatures. One downside to this process is that the wine can result in a wine having a "cooked" flavor (Jackson 2008).

From thermovinification, the concept of flash détente using heat and pressure was developed by the INRA to ease problems extracting color and flavors from the grape berries.

According to the official blog of the Society of Wine Educators, “In Europe during the early years of flash technology, it was mainly used for lower quality grapes or difficult vintages that had problems needing fixed. Now the use of this technology is expanding its application to all quality levels of the wine industry” (Audino 2015).

One reason that the process became successful initially is that it eliminates enzymatic activities, particularly laccase enzymes (Escudier et al. 2008). Since producers seek to eliminate waste, which can increase operational costs, the process has major implications for waste reduction. Rotting grapes that would otherwise be wasted can possibly be utilized by flash détente processing. The other potential major benefit is the decreased need for fermentation vats by elimination of the entire solid mass after flashing of the grape berries, though later studies demonstrated the importance of fermenting in the solid phase as it better extracts all free and loosely bound polyphenols and polysaccharides in general. Work done in the early 2000’s provided some insight into so called “Appellation wine,” which was macerated in the solid phase, demonstrating that wines produced were structured, tannic, while keeping their terroir and some ripe sweetness (Desseigne et al. 1998).

Flash détente is a logical progression in the development of new processing techniques to satisfy consumer’s desires for new wine products. Flash produced wines allow the winemaker to develop a wider range of wines from grapes with controlled maturity. According to Escudier et. al., “The objective is not, through technology, to develop industrial wines but, instead, to use it as more directive way to diversify and better express the qualities, being vinification, the potential composition of the harvest with a reverse engineering approach” (2005). Escudier stated that there are two options available to winemakers in practice: one is respect for the grape

berries to trigger anaerobic metabolism naturally, and the other is cellular disorganization in order to increase the diffusion phenomena (Escudier et al. 2005).

## Grape Physiology

To understand the impact of flash détente and thermovinification, a brief physiology of grape berries is necessary. The grape skin contains many layers: the cuticle, which is the outermost layer, is composed of hydroxylated fatty acids and is covered in hydrophobic waxes, the intermediate epidermis, the inner layer, and the hypodermis, which is composed of several cell layers that contain most of the phenolics in grape skins (Bell 2007). Figure 2 illustrates the concept of grape physiology and the spatial location of certain compounds.

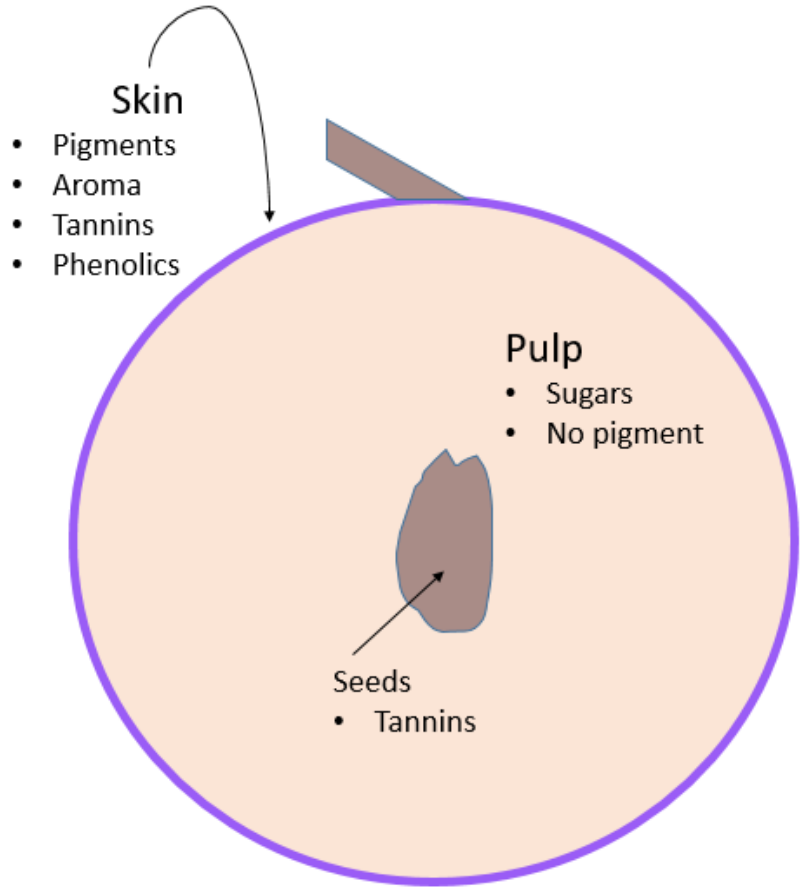

*Figure 1. Grape physiology*

The skins of grape berries form a barrier to the diffusion of components including aromas and phenols and acts as protection against external factors (Pinelo et al. 2006). Furthermore, physical traits of the cell wall, such as surface topography, porosity, and chemical composition, can also influence the association of cell wall polysaccharides and phenolic substances (Pinelo et al. 2006). Differential analysis of skin walls of red wine grapes may provide some further understanding of the application of flash détente. What is known now is that fermentation temperature is one of the most crucial variables affecting the extraction of phenols from grape skin, and increases flavor extraction by enhancing the solubility of solute.

## Macromolecular Composition

According to the Institute of Vine and Wine in France, the hot juice from that comes out from heat treatment contains strongly laden solids and often have a higher turbidity close to 3000 NTU (Vingevin 2016). NTU, or Nephelometric Turbidity Units, is a unit of measurement that measures light scattering. Thus, it is desirable before setting alcoholic fermentation to clarify the hot or warm musts. According to a skilled winemaker at Carneros Vintners, since the must from the flash process comes out virtually sterilized, little sulfur dioxide is needed, though exogenous tannin is added to prevent oxidation and to stabilize the must (Intardonato 2013). The addition of pectinolytic enzymes is theorized to remove off flavors including mold, smoke, and vegetative aromas. Exogenous enzymes can be added before the vacuum step or after the vacuum step, though most commonly the enzyme blend is added before any vacuum step is initiated. It has been theorized that the enzymatic process improves the organoleptic quality of grape juice by degradation of pectic substances to create a clear juice (Paranjpe et al. 2012).

The macromolecular fraction of wines includes polyphenols and polysaccharides, which have a major influence on the quality of red wines, mainly color and taste (Doco et al. 2007). Doco and his research group (2007) studied the effect of flash release on wine polysaccharide composition, and found that the flash release treatment increased extraction of both polysaccharides and polyphenols. Because extracted tannins help stabilize color, loss of this color, in part, is a consequence of low quality and quantity of extracted tannins, common in traditionally macerated wines. The benefit of flash détente is that the process is highly effective in separating color compounds, but extracts only the most soluble tannins, generally low molecular weight tannins (Doco et al. 2007). To obtain extraction of higher molecular weight and more complex tannins, alcohol is necessary. Furthermore, to accomplish extraction of higher molecular weight and complex tannins, maceration with the skins is highly recommended after the flash process. Extension of contact between the solid phase and the liquid phase during the winemaking process gives more aromatic complexities and better taste. In 2007, Doco et. al. provided empirical evidence that substantiated claims that the wine macerated with skins had a statistically significant higher concentration of polysaccharides compared to the wine macerated in the liquid phase only (Doco et al. 2007). Additionally, the wine macerated in the solid phase also was higher in polysaccharides arabinose and galactose, demonstrating the ability of flash détente to produce enriched wines. Temperature also plays a large factor in the extraction of polysaccharides, though increasing the temperature too much can lead to cooked flavors in the finished wines.

Some of the effects of excessive heat during fruit growth and ripening include: reduced color development (anthocyanins) in red berries and decreased sugar concentration in fruits (Williams, 1994). These are two important factors that the flash détente process seeks to resolve.

In industry, a popular method for increasing the brix value is to add sugar directly into the fermentation vats, a process known as chaptalization, though the process results in a wine that generally does not have the same characteristics of a wine produced using only natural sugars present in the grape berry (Martin 1990).

## Benefit and Importance of Anthocyanins

Anthocyanins are a class of flavonoids that are water soluble, responsible for the familiar hue of wines. They function as pigments and comprise the orange, red, purple, and blue colors of many fruits, vegetables, flowers and roots. Spatially, anthocyanins are located within the vacuoles of the epidermal cells and upon crushing of the grape berries can release into the liquid phase during winemaking. When present in water, they are more stable at a low pH, a unique factor for wine, as it contains high amounts of organic acids that lower the pH naturally during vinification (Somers 1971). Meanwhile, the concentration of anthocyanin is dependent on different factors, including geographical origin, cultivar, or variety (Makris 2006).

Anthocyanin extraction occurs early in fermentation, generally at its maximum concentration by day 4 into primary fermentation (Mansfield 2015). Wine is prized for its health-promoting qualities, particularly due to the anthocyanidins. Not only do anthocyanins provide color to wine, but also many studies have correlated these health-promoting compounds with antioxidant, anti-inflammatory, and anticarcinogenic properties (Pojer 2013). Additionally, these specific compounds provide protection against specific types of heart disease and cancer, as well as reduce risk of diabetes and cognitive function disorders (Pojer 2013). Thus, moderate consumption of red wine on a regular basis may improve the health of the consumer, while providing an enjoyable experience.

## Wine Quality

Unlike most other modern processed foods, people's fascination with wine is difficult to define due to its reliance on subtle perceptions, not on its consistency or strong flavor profile (Conde et al. 2007). Wine is mostly water, accounting for between 75 and 95 percent of the total content by volume. The 15 percent variation is explained by the amount of phenolics, organic acids, mineral salts, and pectins which form the wine extract, and can differ from wine to wine (Conde et al. 2007).

Certain features of grapes make it the perfect medium for wine production. These features include the high concentration of sugars and other nutrients that provide excellent growing conditions for the yeast, natural acidity that controls microorganism growth during and after fermentation, a high concentration of ethanol to also provide protection against microorganisms, and lastly, a unique manifestation of aromas and flavors (Kunkee 1996). Quality largely depends on many complex factors including soil composition, growing temperature, growing season, maturity at harvest, light, microorganisms on the grape surface, and many other similar factors (Kunkee 1996). These factors can also change from growing season to growing season even for the same area of land, keeping all human intervention in the growing cycle the exact same.

However, scientific analysis of wine only provides a small but significant part of the bigger picture of wine consumer preference. More importantly, scientific research in wine quality guides decision making by master winemakers. Thus, sensory analysis is of paramount importance to understand variable's interaction with consumer's tastes, which ultimately may provide information for winemakers to help improve the quality of wines.

## Quality Yields and Impedance Analysis

A study carried out on the quality and yield of juice from grapes treated by flash détente vacuum extraction, using combinations of heat, vacuum, and enzymes provides further information about the processes effect on grapes for wine (Paranjpe 2008). The objective of this research was to analyze the effects of temperature and pressure on flash détente processing of red grape juices. Thompson seedless grapes were used, a common table grape, in a full factorial design for the flash expansion experiments with two factors (temperature and pressure) at three level for each. Temperature was varied at 60, 75, and $90^0$C, while the initial pressure was varied at 1, 5, and 10-kPa absolute pressure. These conditions were chosen since results from Thompson seedless grapes indicated that they were the most efficient in extracting the highest concentration of juice and polyphenols from grapes.

At 1 kPa absolute pressure, the boiling point of water is $7^0$ C, thus when the fruit enters the vacuum at the desired temperature of 90-$95^0$C, the grape skins expand violently, releasing the cellular components into the liquid phase of the flash juice. The flash water that is produced is generally discarded as it has a high concentration of aromatics that are highly disagreeable (Flash Détente: Making Red Wine Redder 2015). Lower chamber pressures and higher fruit temperatures were found to improve juice yield and phytochemical composition. Quantity yields from the cold pressed and heat-treated were at 71.0 g per 100g and 75.8g per 100g of starting grapes, respectively. Yields for enzyme processed and flash vacuum expansion treatment were 84.8 and 84.3 g per 100 grams of starting grape berries, though the two treatments were not statistically significant from one another (Paranjpe et al. 2012). Anthocyanin extraction and total phenol content was statistically higher for the flash vacuum expansion treatment than other treatments with an extraction efficiency of 87.0 g and 2.6 g/L total phenol content, respectively.

The study then compared impedances obtained from grape skins using the different treatments in his published paper; results indicate that the flash vacuum expansion ruptures more cells in the grape skin than enzyme processing and heat treatment, therefore extracting more solutes from the grape skin (Paranjpe et al. 2012). Since this method of processing grapes is intended to be applicable to industry, Paranje observed the process using a batch method and a continuous process method. While Paranje was able to achieve a method that allowed for continuous processing of grapes, only a negligible amount of grape berries were ran through the system before any kind of reliable trends could be observed and documented.

Meredith Bell's research study (2007) on flash détente provides a great overview of the effect of ethanol, press temperature, and flash détente on the extraction and partitioning of condensed tannins, iron reactive phenolics, and anthocyanins during fermentation. To quantify these compounds, free and bound tannins were measured in the grape berry skins during each day of fermentation, in order to investigate how and when condensed tannin molecules become free or bound to the insoluble cell wall matrix of grape berry skins (Bell 2007). Bell demonstrates in her research study that flash détente grape processing effectively extracts anthocyanins, iron reactive phenolics, and tannins, though adding the enzyme after the heat and pressure step appears to have a noticeable effect on all response variances by decreasing these variables in the vast majority of all samples.

The most interesting discovery in Bell's study is the majority of flash détente produced wines were higher statistically than the control wines in iron reactive phenolics, tannins, and anthocyanins, which were all over 1000mg/L catechin equivalents. Average tannin levels were reported at 544 mg/L catechin equivalents for a survey that included over 1300 wines (Harbertson 2008). Overall, while all three variables increased with the flash détente wine

compared to the control, the tannins and iron reactive phenolics showed the most drastic changes, while anthocyanins were only slightly higher in concentration for the flash détente treatment.

Ageron et. al. (1995) compared flash détente processed skins to a standard vinification, or control, using electron microscopy. The flash détente processed skins displayed a large network of cracks all along the skin, while the control demonstrated virtually no cracking. This demonstrates that flash détente is effective in lysing cells to ease diffusion of cellular components into the liquid phase, increasing quality attributes of the wines. Rheological measurements show that the resistance of grape skins to piercing after flash-release and vinification is less than from a control vinification (Ageron et al. 1995).

Organoleptically, flash détente produced wines were judged to be round and fruity (Escudier et al. 2008). Consumer panels have reported this as well, which may provide a desirable product to consumers (Miguel 2014). Altogether, these research studies provided solid background of sensory and chemical compositional differences of flash détente, namely that flash détente gave the winemaker useful options in processing raw materials.

A study that investigated the effect of the flash release treatment on phenolic extraction and wine composition found that the flash process efficiently extracted all phenolic compounds (hydroxycinnamic acids, flavanols, anthocyanins, catechins, and proanthocyanidins) resulting in polyphenol-enriched grape juices (Morel-Salmi et. al. 2006). Phenolic compounds are of importance as they are responsible for major wine sensory characteristics such as color and astringency. In the four types of wine varieties studied, flash release extraction resulted in higher levels of anthocyanins, flavanols, catechins, and proanthocyanidins, though hydroxycinnamic

acids were lower in 3 of the 4 varieties of wines by an average of 27 percent (Morel-Salmi 2006).

While traditional methods of extraction, such as thermovinification, often pull harsh tannins from seeds and skins, flash détente extracts desirable anthocyanins from the grape skins without simultaneously over extracting abrasive seed tannins. European-type grapes grown in Oklahoma for wine production typically encounter specific problems that result in lower quality wine grapes than more established wine terroirs. Furthermore, grapes grown in Oklahoma encounter less than ideal Brix values because of the high temperatures and the imperfect red clay soil (Stafne 2014). Thus, flash détente may be a viable option to reduce quality-related issues through the process's ability to extract phytochemicals using a heating and vacuum system.

## Wine Volatiles and Aroma

Wine aroma is attributable to large range of molecules coming from different chemical families such as aldehydes, ketones, esters, terpenes, norisoprenoides, acids, alcohols, and sulfur compounds (Torrens et al. 2004). Some of the compounds formed have desirable aromas, while compounds such as methoxypyrazines and sulfur containing compounds generally have a strong degree of disagreeableness to wine drinkers. Since there is increasing interest in the wine buying community in red wines with fruity characteristics, Researchers have attempted to gain more insight into the compounds present that are responsible for a vegetal or herbaceous character in certain wines.

Chiefly, the persistence of vegetal aromas in wines is due mainly in part by a class of compounds called methoxypyrazines. Pyrazines are nitrogen containing heterocyclic compounds distributed widely in nature in both animal and plant life forms. Some of the volatile compounds in wines are from the grapes themselves and some volatile compounds are formed during

fermentation and aging. Methoxypyrazines are a class of extremely potent odorous molecules present in certain vegetables in nature, including most notably, bell peppers and green peas. In certain wines, "persistence of the methoxypyrazines causes a defect that gives off green, grassy, and vegetative aromas.

Specifically, the presence of 3-isobutyl-2-methoxypyrazine (IBMP), 3-sec-butyl-2-methoxypyrazine (SBMP) and 3-isopropyl-2-methoxypyrazine (IPMP) in wines has been related with the green and vegetative aromas characteristic of some wines made with Cabernet Sauvignon, Sauvignon Blanc, Merlot or Cabernet Franc grapes" (López et. al. 2010). The concentrations of these compounds are extremely low (ng/L), but they can influence wine aroma due to their extremely low sensory threshold. Reports show that the sensory threshold in redwines is about 10-16ng/L (Roujou de Boubee 2003).

In a study conducted in 2002, researchers in France were able to demonstrate that methoxypyrazines in grapes are highly extractable in a traditional winemaking process (Roujou de Boubee 2002). Furthermore, the researchers were able to show that all methoxypyrazines separated from the grapes into the juice even before any alcoholic fermentation was able to start.

Thus, it may be advantageous to pick wine grapes off the vine once they are adequately ripened to decrease the incidence of methoxypyrazine-related aromas. In some areas and within some wineries this process may not be possible due to the extremely short period of time that grapes are picked for processing, generally three weeks out of the year. For this reason, deeper research into the flash détente potential effect on wine grape's methoxypyrazine compounds reduction is necessary for winemakers to make informed decisions about developing quality wines.

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# CHAPTER III

# MATERIALS AND METHODS

## Grape collection and storage

Approximately 110 kg (250 lbs) of Cabernet Sauvignon grapes were picked from Jones, Oklahoma on August 30, 2016 from Duncan Family Farms as shown in figure 2 below.

*Figure 2. Grape collection from Jones, Oklahoma*

Grape clusters were sprayed with a 300 ppm sulfur dioxide solution and placed in a $4^0$C refrigerator for 48 hours before crushing and destemming. Initial processing of the grapes consisted of crushing, destemming, and addition of 30 ppm $SO_2$ and exogenous sugar to ensure the viability of samples. An Enoitalia crusher (Rome, Italy) partially crushed the grapes,

releasing some of the juice from the berries. The lot of grape must was stirred in a Vulcan model VDLT-40 until roughly homogenous. Samples were split into homogeneous lots of around 8 liters (2 gallons) storage buckets, twelve in total, before sample treatment as shown in figure 3 below.

*Figure 3. Homogenous lots of grape must*

All samples were stored at -15 °C with sealed lids until further processing or analysis.

**Preparation of juice samples**

Frozen, crushed and destemmed Cabernet Sauvignon grapes were placed at room temperature for 48 hours to thaw. After thawing, samples were separated and experimental treatments were applied as determined randomly.

**Soluble solids**

Wine grape sugar concentration was estimated as percent soluble solids using an Anton Paar Abbemat 200 refractometer (Ashland, VA), and recorded as Brix value (in g/100g). Samples were taken in duplicate for reliability of measurements.

**Yeast Assimilable Nitrogen**

Samples of juice were analyzed for the concentration of yeast assimilable nitrogen, a measure of the nitrogen sources available to the yeast for fermentation vigor. The formol titration technique was employed to estimate the nutritional status of the grape must in terms of mg/L of yeast assimilable nitrogen. Samples were measured after treatment or no treatment depending on the batch type randomly assigned. Ten milliliters of sample was filtered, pipetted into a 25 ml volumetric flask, and then topped to the 25 ml fill line with deionized water. The 25 ml sample was transferred to a beaker and a magnetic stir bar was added. Sodium hydroxide, with a concentration of 0.05 N, was added to the solution of juice and water until the pH reached 8.0, measured with an Accumet AB 15 pH meter (Buffalo, NY). At this point, 2 ml of pH 8.0 of a 37% formaldehyde solution was added. Next, the sample was titrated with the same 0.05 N sodium hydroxide until the pH reached an endpoint of 8.0. The estimate for yeast assimilable nitrogen was calculated according to:

$$\text{YAN (mg N/L)} = (\text{ml of 0.05 N NaOH titrant}) \times 175$$

**pH**

The pH of the Cabernet Sauvignon wine was measured using an Accumet AB 15 pH meter (Buffalo, NY). The pH meter was calibrated using pH standards before measuring samples. pH measurement was collected after 38 days after the beginning of fermentation by stirring samples in a beaker with a magnetic stir bar. Samples were tested in duplicate at room temperature to ensure homogeneity of sample measurements.

**Titratable acidity**

The titratable acidity of the Cabernet Sauvignon wine samples were measured manually at 38 days after the beginning of fermentation. Five mL of room temperature wine was mixed with 125 mL of deionized water in a 250 mL beaker. The sample was stirred using a magnetic stir bar and the initial pH was measured using an Accumet AB 15 pH meter (Buffalo, NY). The sample was titrated with 0.1 N sodium hydroxide (Arcos Organics, Fair Lawn, NJ) until it reached pH 8.2 and the volume of titrant used was recorded. The titratable acidity was then calculated as % tartaric acid using the following formula: % tartaric acid = [(mL NaOH x N NaOH x mili-equivalent wt. of tartaric acid)/ mL sample added] x 100 = [(mL NaOH x 0.1 x 0.067)/5] x 100. Two duplicate readings were taken from each wine sample and averaged.

**Percent alcohol**

The percent alcohol (w/w) of wine samples was measured using an Alcoholizer Wine M (Anton Paar, Ashland, VA). Samples of aged wines, after 30 days following pressing of wine from grape skins (day 38 total), were collected into 60 mL beakers. Approximately 30mL of wine per sample were used in the analysis. Two duplicate readings were taken from each wine sample and averaged. Alcohol readings were repeatable to 0.01% (v/v).

**Colorimetric Analysis**

Spectral analysis of wine samples was done using a Beckman DU520 UV/VIS (Brea, CA) single cell module spectrophotometer. Wine samples were tested at day one and day eight following the start of fermentation and day thirty after pressing of the wine from grape skins for differences between treatments and with the treatment effect of pectinase.

To accomplish color analysis, the method by The Napa Valley College Department of Viticulture and Enology was utilized for all samples (Elridge and Liles 1997). Wines were

filtered through .45-micron filters to remove incidence of turbidity that could lead to inaccurate data. Measurements of 1:10 diluted wine samples (at pH 3.6) were taken at 420 nm and 520 nm in a 10 mm quartz cuvette on day one and day eight, while the full color analysis was used for day thirty after pressing for analysis. Blanks were ran using de-ionized water before samples were analyzed.

On day thirty after pressing, measurements were taken as previously described, in addition to more specific color analysis than at day 1 or 8 after fermentation started. For the sulfur dioxide test, two milliliters of wine were combined with 160 microliters of a 5.0% solution of $SO_2$ and then absorbance was measured at 520nm for each wine sample in triplicate.

A buffer solution was made using 24 ml of pure ethanol and added to 174 ml of deionized water. To the solution, 0.5 grams of potassium bitartrate was dissolved and adjusted to pH 3.6. 100 microliters of wine was mixed with 1900 microliters of this buffer solution and the absorbance was measured at 365 nm. The absorbance values determined by this metric determine the flavone-phenol measurement, a measure of certain phenolics and flavones in the wine. To determine free anthocyanins in the wine, the 365 nm absorbance measurements were subtracted by the absorbance measurement of sulfur dioxide at 520 nm. Lastly, color hue was determined by the ratio of absorbance values at 420 nm to the absorbance values at 520 nm.

**Trial Preparation**

Containers of frozen, crushed and destemmed Cabernet Sauvignon grapes were placed at room temperature for 48 hours to raise the temperature of the juice to accomplish treatment, when appropriate, and yeast addition. Samples were randomly assigned to treatment groups and treatment was applied when selected for the batch.

**Control Trial Preparation**

Four liters of grape must was added to an 8-liter container and RC 212 yeast (Lalvin) was added when the temperature of the must reached above 20$^{o}$C for the control trial. If pectinase treatment was selected for random treatment, it was applied to the mixture using 5 g per 4 liter batch before yeast addition.

**Thermovinification Trial Preparation**

After thawing, samples of 4 liters of must were placed into Ziploc® bags (1.5-gallon) and sealed as shown in figure 4 below. If pectinase treatment was selected for random treatment, it was applied to the mixture using 5 g per 4 liter batch before yeast addition.

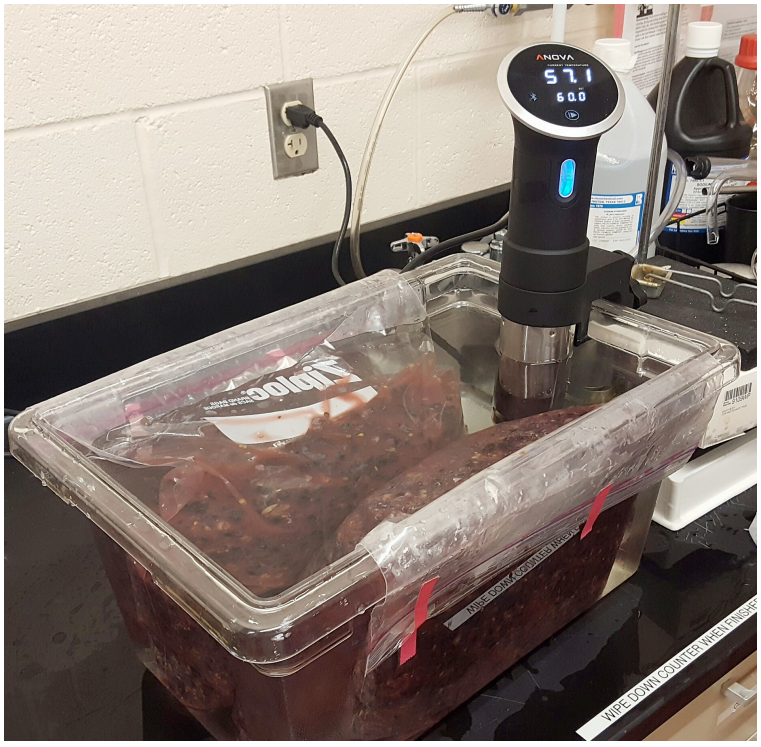


*Figure 4. Thermovinification processing*

The sealed samples were transferred to a heat circulating bath with an immersion circulator, specifically the Anova Culinary PCB-120US-K1 Bluetooth Precision heater (San

Francisco, CA), at 60$^0$C for 3 hours. After 3 hours, the samples were then cooled to room temperature with an ice bath and yeast was introduced using the method described in a following section.

**Flash Détente Trial Preparation**

After thawing, 4-liter samples were strained to collect free run juice before heating began, shown in figure 5 below.


*Figure 5. Filtering of free run juice from solid mass*

Samples of grape solids minus free run juice were quickly heated in a 40-liter DC-6 Vulcan-Hart® steam-jacketed kettle (Louisville, KY) for 2-3 minutes, until they reached the desired temperature of 90-91$^0$C. Grape skin material was stirred with a rubber spatula to ensure even heating. The resultant batch of heated grapes was then filtered from the hot juice using a Home Basics® metal mesh strainer over a metal bowl (manufacturer unknown) to collect juice

before the sample was injected into the flash détente vacuum system. The flash détente vacuum consisted of a 3.5-liter stainless steel sample container connected to a 95-liter stainless steel flash détente vessel. The flash vacuum equipment is depicted in figure 6 below.

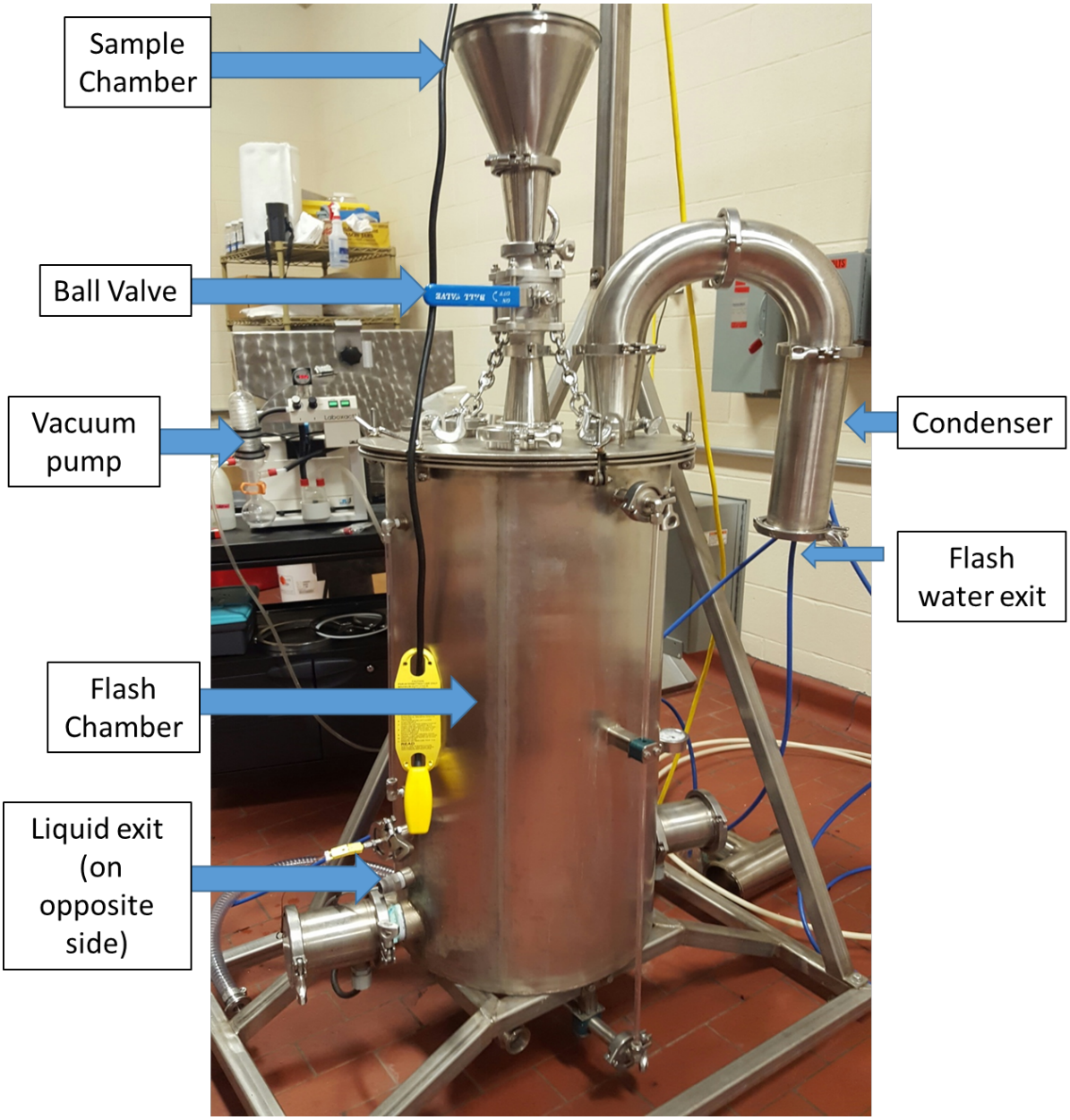


*Figure 6. Flash détente system*

The Eden Labs flash evaporation vessel (Seattle, WA) was evacuated to high vacuum, below 5.0 kPa, using a KNF Laboxact SEM 840 Vacuum System (Trenton, NJ). While industry

standard for flash processing is running the system continuously, the flash system used in the study ran in batch production mode. Opening of the ball valve released the contents of the sample chamber into the reaction vessel under high vacuum. The boiling point of water in the grape cells was below the temperature of the fruit cells at this reduced pressure. Since the fruit entered the chamber around 85-90$^0$C, the flash evaporation of the water caused a violent expansion of the grape cells, hypothetically releasing cellular components into the juice.

Before grape solids were removed from the system, subsequent batches were ran. Liquid from the process was removed by ball valve on the opposite side of the figure above and solids were kept in the system until all batches were ran. After being exposed to vacuum, or "flashed", the sample input valve was closed and the vacuum in the vacuum chamber was allowed to return to 1.9-5.0 kPa before the next portion of grapes was introduced. Following the last run of grape skin material, the experimental must was mixed back in with the free run juice and "flashed" juice by scraping the solids with a common kitchen rubber spatula into homogenous flash lots.

If pectinase treatment was selected for random treatment, it was applied to the mixture using 5 g per 4 liter batch. Fermentation proceeded as described in the section below.

**Fermentation**

The experimental must was pitched with Lalvin Bourgorouge RC 212 yeast (Burgundy, France). This yeast strain was selected for its ability to accentuate fruity aromas and to extract anthocyanins. Yeast, stored under refrigeration, was hydrated before adding to musts. Hydration consisted of stirring of five grams of yeast in 50 ml of water at 40-43$^0$C and allowed to hydrate for 15 minutes. The rehydrated yeast was split evenly between 8 liters of must. Musts were fermented for 8 days at 20$^0$C to ensure adequate fermentation to completeness in Midwest supply 8-liter plastic pails with an airlock, as shown below.


*Figure 7. Fermentation containers*

During fermentation, the cap, which describes the solid mass of grape skins and seeds that float to the top of the vessel during the fermentation process, were punched down once daily.

**Pressing / Racking**

Liquid was pressed at day 8 from skins and seeds using a Zambelli Enotech bladder press (Camisano Vicentino, Italy) shown in figure 8 below. The inside bladder was filled with water and this increase in volume in the closed system pressed the liquid apart from the grape skins.

*Figure 8. Zambelli Enotech bladder wine press*

After pressing, liquid was transferred from plastic pails to 4-liter glass containers, shown in figure 9 below (Midwest Supplies) and containers were labeled with the appropriate batch code and date of fermentation start.


*Figure 9. Wine transfer protocol*

Following transfer to glass containers, samples were racked after one week, a process that removes sediment from the bottom of the glass vessel from the liquid wine. Racking was performed twice more to remove the majority of sediment from the liquid wine. After racking, wines were bottled using a Learn to Brew® auto-siphon (3/8”) and plastic tubing into 375 ml dark green wine bottles.

## Gas Chromatography Volatile Analysis

### Chemicals and standards

Dichloromethane (HPLC grade, >99%) was purchased from Arcos organics (Fair Lawn, NJ). Hexane was purchased from Fisher Scientific (Pittsburgh, PA). Ethanol was purchased from Pharmco-AAPER (Brookfield, CT). The following standards (purity of >95%) were obtained from Sigma-Aldrich (St. Louis, MO): 1-Hexanol, Ethyl Acetate, Ethyl Hexanoate, Hexanoic

Acid, and Isobutanol. The following internal standards were obtained from Sigma-Aldrich (St. Louis, MO): 4-hydroxy-4-methyl-2-pentanone, 4-methyl-2-pentanol, 2-butanol, and 2-octanol.

*Table 1. Analytical standards purity for gas chromatography*

| Compound | Purity |
|---|---|
| **Standards** | **%** |
| 1-Hexanol | 99.9 |
| Ethyl Acetate | 99.0 |
| Ethyl Hexanoate | 99.5 |
| Hexanoic Acid | 99.0 |
| Isobutanol | 99.8 |

Volatile standards preparation

A solution of each of the standards was prepared by dividing the molecular weight of each stock compound by its declared purity, then calculating the weight of the standard needed to create a working stock solution. For each standard, the corresponding amount of the compound was then weighed into an appropriate volumetric flask and each flask was filled to volume with a wine model solution. The wine model solution contained 12% (v/v) ethanol, 5 g/l tartaric acid, and pH adjusted to 3.2 with sodium hydroxide. Synthetics wines samples containing volatile standards were extracted and analyzed using the procedure in the section below.

Sample preparation

A method modified from Ortega and others (2001) was used for free volatile analysis via gas chromatography. A dichloromethane micro-extraction process was used to extract free volatiles from the wine matrix. The extract procedure was done in a 15 ml centrifuge tube (Corning Centristar®) consisted of the addition of 5 ml of wine, 5 ml of milliQ water, 4.5 grams of ammonium sulfate salt, 300 μL of dichloromethane and 20 μL of internal standard. The

internal standard contained a mixture of four standards: 2-butanol, 2-octanol, 4-methyl-2-pentanone, and 4-methyl-4-hydroxy-2-pentanone at 140 μg/ml in ethanol.

The resultant mixture was shaken with a VMR analog shaker (Radnor, PA) for 60 minutes using a moderate to high shaking. The tubes were then centrifuged using a Fisher Scientific Centrific Centrifuge® (Waltham, MA) at 2500 rpm for 10 minutes at room temperature. The clear bottom of the dichloromethane layer was then collected placed into a 2 mL amber injection vial with a 250 μL pulled point glass insert and capped (Agilent). Samples were inspected for clarity before GC analysis visually.

GC system

A gas chromatography (GC) system with a flame ionization detector was used to analyze free volatile compounds. The GC system was manufactured by Agilent technologies (Santa Clara, CA) and consisted of a 6890N networked GC system, a 7683B series injector, a 7683 series auto sampler and air purifier from Alltech (Portland, ME). The GC system was operated by HP Agilent Chemstation software. The three gases used by the system were purchased from a Stillwater Steel and Welding Supply (Stillwater, OK). Hydrogen (UHP300) was used as carrier gas, helium (UHP300) was used as makeup gas, and air was used as the flame igniter.

DB-Wax fused silica capillary GC column (50m length x 0.32mm I.D. x 0.50 μm film thickness with a 5m pre-column) on which the separations were carried out was purchased from Agilent Inc. The oven temperature was maintained at 40°C for 5 minutes, raised at 3.0°C/min up to 200°C, then held for 5 minutes at 200°C.

The front inlet was set as split mode with hydrogen as a carrier gas at 30 ml/min. Other parameter settings were the detector set at 250°C, and operating the system at a pressure of 11.38 psi, and a column flow of $H_2$ gas at 3.0 mL/min. The sample injection volume was 2μL. The

extraction recovery was estimated by the internal standard solution containing 2-butanol, 4-methyl-2-pentanol, 4-hydroxy-4-methyl-2-pentanone, and 2-octanol. All volatile compounds were identified and quantified by comparisons with the retention times and peak areas obtained using the pure standard solutions. All samples were measured in duplicate.

Statistical Analysis

Statistical Analyses were performed using analysis of variance (ANOVA) for all data sets with Tukey's HSD to compare treatment groups and their significance. The alpha level was set at 0.05 for significance to be established, with a 95% confidence interval. The experimental design was set up as completely randomized with batches randomly assigned to batch numbers.

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# Chapter IV

## RESULTS AND DISCUSSION

Average pH measurements taken at day 38 following the start of fermentation are displayed in Table 2. No significant differences in pH were observed between treatments and with pectinase addition by batch type. The range of pH in the study was between 3.60 and 3.71, similar to the range of a study that examined pre-fermentation heat treatment on grape samples across many vintage years and grape varieties (Geffroy 2015).

### pH

*Table 2. Mean pH of samples comparison*

| Group | Control | | Thermovinification | | Flash Détente | |
|---|---|---|---|---|---|---|
| Treatment | Control (n=4) | Control with pectinase (n=2) | Thermovinification (n=2) | Thermovinification with Pectinase (n=2) | Flash Détente (n=6) | Flash Détente with Pectinase (n=4) |
| pH (±standard deviation) | 3.65±0.04 | 3.64±0.04 | 3.68±0.01 | 3.68±0.01 | 3.66±0.04 | 3.66±0.04 |

*Denotes a significant difference between treatment groups

Flash détente and thermovinification trials were not significantly different from the control batches, resulting in p-values of 0.48 and 0.16, respectively. In addition, there were no significant differences observed when comparing experimental treatments to one another (p-value=0.28).

The mesocarp of the grape contains the bulk of the organic acids, compartmentalized specifically in the vacuole of the mesocarp cells (Lund and Bohlmann 2006). It was hypothesized initially that there would be no significant change in pH because extraction of these organic acids occurs primarily during the crushing step and in the initial stages in the fermentation process. The data supports the hypothesis that pre-fermentation treatments have have no significant effect on the pH measurement values. Thus, experimental treatments do not significantly change the pH of the wine, but studies on other varieties of grapes may further this assertion.

## Alcohol Content by Volume

The mean percent alcohol by volume of the treatment groups taken at day 38 after the beginning of fermentation or in other terms, 30 days after pressing, is presented in Table 3. In general, alcohol by volume, taken in duplicate, following virtually all conversion of sugar to alcohol was nearly the same for all groups including treatment factors.

*Table 3. Mean alcohol by percent volume of samples comparison*

| Group | Control | | Thermovinification | | Flash Détente | |
|---|---|---|---|---|---|---|
| Treatment | Control (n=4) | Control with pectinase (n=2) | Thermovinification (n=2) | Thermovinification with Pectinase (n=2) | Flash Détente (n=6) | Flash Détente with Pectinase (n=4) |
| Alcohol % by Volume (±standard deviation) | 13.87± 1.05% | 13.23±0.04% | 14.29%±0.01% | 13.50±0.75% | 14.18±0.73% | 13.91±0.64% |

*Denotes a significant difference between treatment groups

The data exhibits that there were no significant differences among all test groups. Comparison of flash détente to control and thermovinification groups resulted in p-values of 0.25

and 0.24, respectively. In addition, when comparing thermovinification to the control groups, no significant difference was established as well (p=0.47).

As alcohol content in wines is a function of sugar concentration in the starting material, alcohol tolerance of the yeast during fermentation, and fermentation vigor, it was not anticipated to observe significant differences in alcohol content among the trial groups. Other factors may affect fermentation efficiency, however, this was not observed when comparing the data sets. Variation in alcohol content seen between batches of the same treatment was observed, but none was significantly different. One possible explanation is insufficient mixing of the batches when separating them into 8-liter batches before processing. However, because alcohol percentage content was not a major focus of this study, it is recognized as an unresolved issue. Further studies could validate if pectinase use significantly impacted alcohol percentage on a larger scale.

## Titratable Acidity

Table 4 displays the average titratable acidity measurements taken at day 38 after the start of fermentation, exactly 30 days after pressing of the wine from the grape skins.

*Table 4. Mean titratable acidity of samples comparison*

| Group | Control | | Thermovinification | | Flash Détente | |
|---|---|---|---|---|---|---|
| Treatment | Control (n=4) | Control with pectinase (n=2) | Thermovinification (n=2) | Thermovinification with Pectinase (n=2) | Flash Détente (n=6) | Flash Détente with Pectinase (n=4) |
| Titratable Acidity % (±standard deviation) | 0.65±0.05% | 0.66±0.08% | 0.66±0.04% | 0.65±0.01% | 0.65±0.02% | 0.69±0.06% |

*Denotes a significant difference between treatment

As expected, no significant differences were observed with regard to titratable acidity. Flash détente and thermovinification trials resulted in calculated p-values of 0.44 and 0.89, respectively, when compared to the control batches. A non-significant p-value of 0.55 was also calculated in experimental group comparison to one another. Most red wines fall within 0.60-0.70% acidity, comprised mainly of tartaric and to a lesser extent, malic acid (Boulton 1980). Thus, the titratable acidity values observed in the test wines were within a typical range for red wine. Given this information, it is certain that there is no significant differences using the experimental treatments with regard to titratable acidity.

## Yeast Assimilable Nitrogen

Yeast assimilable nitrogen measurement, the quantity of nitrogen nutrients available to the yeast during fermentation, is displayed in Figure 10.

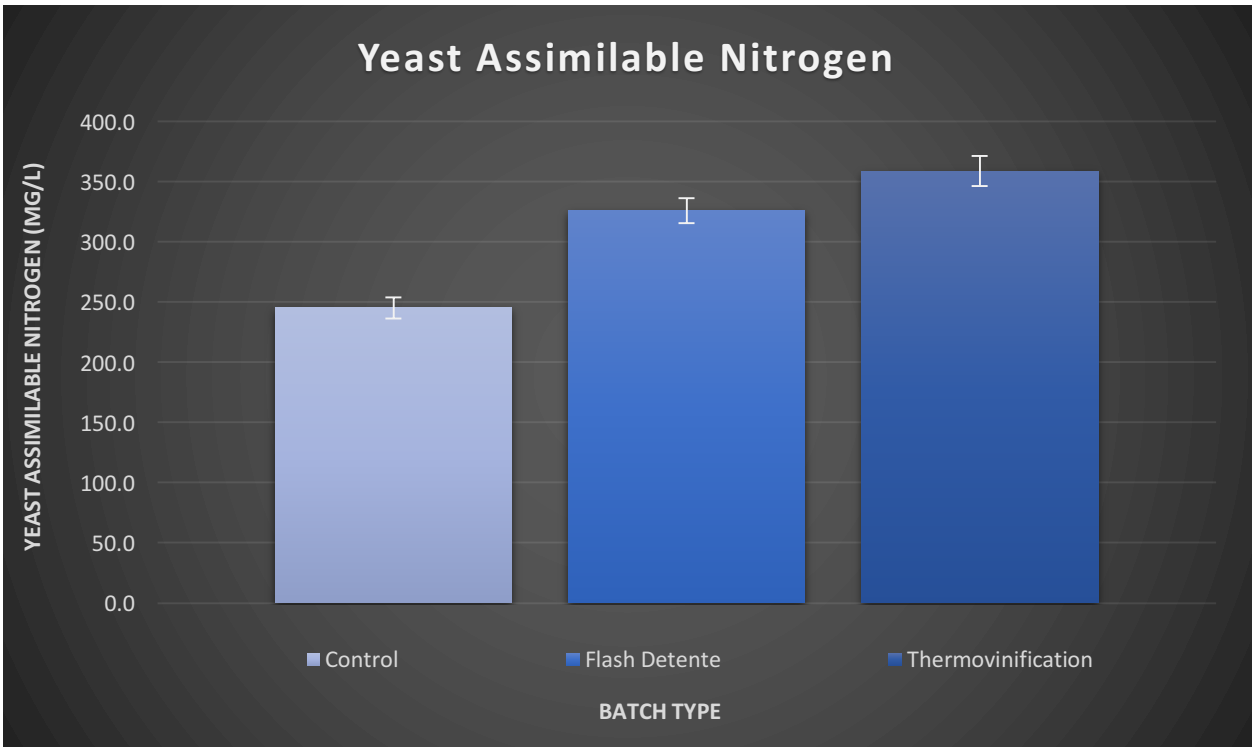


*Figure 10. Mean yeast assimilable nitrogen concentrations in juice samples*

1. n = 3 for control, n = 4 for flash détente and n=2 for thermovinification
2. Error bars indicate one standard deviation from the mean.

Table 5 below shows the average values determined for the yeast assimilable nitrogen test, with standard deviations of each.

*Table 5. Mean yeast assimilable nitrogen comparison*

| Batch Type | Control mean (n=3) | Flash Détente mean (n=4) | Thermovinification mean (n=2) |
|---|---|---|---|
| YAN (mg/L) | 245.0±8.8[c] | 325.9±10.4[b] | 358.8±12.4[a] |

- Letter differences indicate a significant difference between batch types ($p<0.05$)

Both flash détente and thermovinification samples were higher in yeast assimilable nitrogen values when compared to the control batches, which may be due to breakdown of the grapes during heating and/or vacuum treatment. Yeast assimilable nitrogen values were higher on average in the thermovinification batches, thus it may be possible that the extended heat treatment had a greater effect on the extraction process of these nitrogen nutrients. More studies are needed on this parameter to confirm this assertion. Significant differences were observed for all treatment batches when compared to one another. The average YAN value was 245.0 mg/L for the control batches, 325.9 mg/L for the flash détente batches, and 358.8 mg/L for the thermovinification batches. Though the results were significant for this parameter for each batch type, further studies with different types of red wine grapes could validate these findings on a larger scale.

A common practice in winemaking is to add 100-300 milligrams per liter of diammonium phosphate (DAP) to the juice or must at inoculation without measuring nitrogen concentration (Grape and Wine Institute, 2013). Since yeast assimilable nitrogen has a considerable impact on the flavor profile and thus style of the wine, it is important for winemakers to consider the

addition of DAP to create high quality wines. The process of both thermovinification and flash détente considerably increased the amount of yeast assimilable nitrogen, a measure of primary or alpha amino acids, ammonium ion and small peptides. The consequence is an indirect increase in the concentration of volatiles that may be produced during fermentation and through aging.

All trials with flash détente and thermovinification demonstrate effective balance between low YAN levels and excessive YAN levels. As stated in the literature review, high must YANs can stimulate overproduction of acetate esters, resulting in the perception of volatile acidity and suppression of varietal character (Grape and Wine Institute, 2013). Furthermore, high YAN musts are also associated with high concentrations of haze-causing proteins, urea, and ethyl carbamate, which are all considered faults by consumers (Grape and Wine Institute, 2013).

Low YAN concentrations can cause poor fermentation vigor and potentially lead to stuck or sluggish fermentation. In addition, with a low concentration of YAN, undesirable aromas may be formed, such as long chain volatile fatty acids, and the production of desirable fruity aromas, such as esters may be suppressed (Grape and Wine Institute, 2013). The control trials demonstrate the need for addition of DAP, but addition may cause undesirable effects in the sensory perception of the finished wines if the addition rate is not carefully controlled. Thus, the intermediate concentration of YAN achieved in these trials using both flash Détente and thermovinification may allow winemakers to produce a high-quality wine without the need for the additional nitrogen supplements.

**Color Data Analysis**

Color density is a measure of the combined absorbance values at 420 nm and 520 nm. The intensity at 420 nm is a measure of the yellow and brown pigments, while intensity at 520 nm is a measure of the red pigments. Color density of wine is also known as color intensity and

is the most common method to judge the overall color of a wine sample (Jackson 2008). Figure 11 below displays the color density absorbance values over three time points, day 1, 8, and 38.

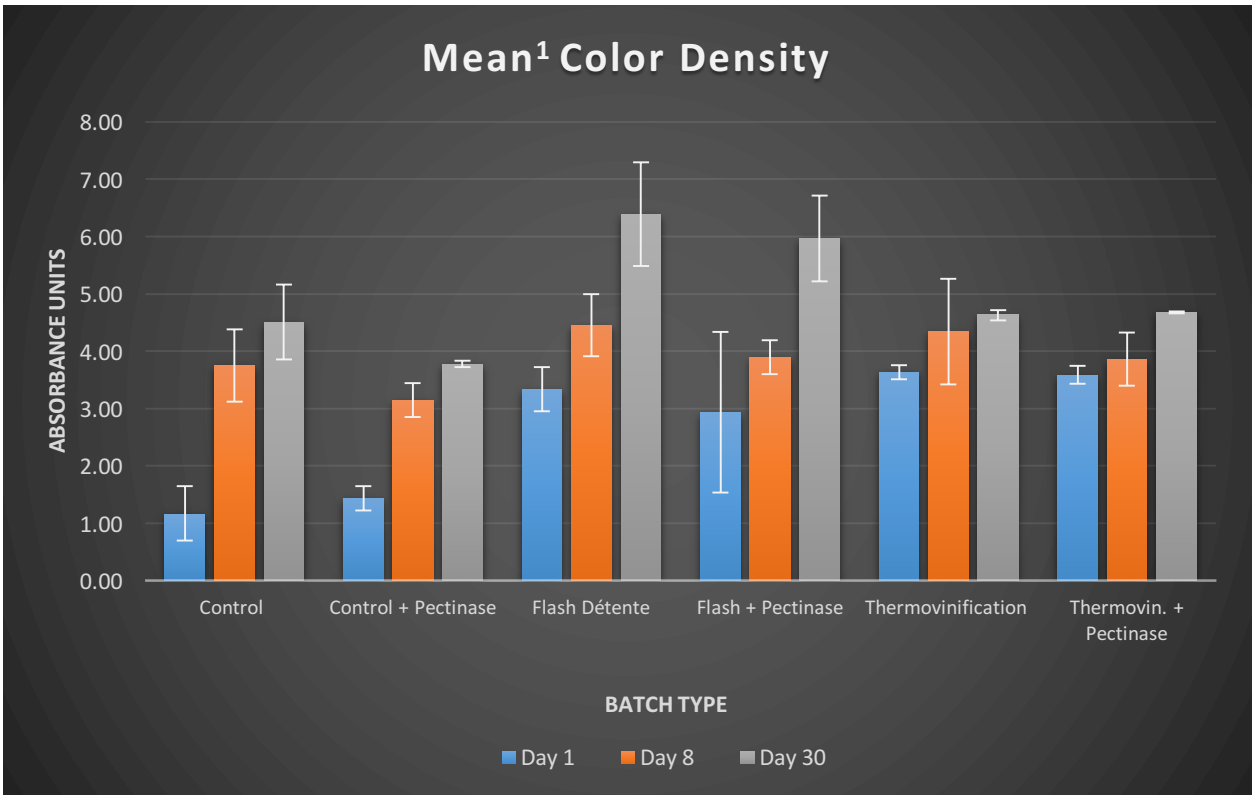


*Figure 11. Mean color density at days 1, 8, and 38*

1. n = 3 for control, n = 4 for flash détente, and n = 2 for thermovinification, thermovinification with pectinase, flash détente with pectinase, and control with pectinase (all measured in triplicate)
2. Error bars indicate one standard deviation from the mean.

As expected in all experimental and control trials, lower color density was apparent at day one versus day eight after the start of fermentation or at day 38. Alcohol extraction of color occurs as fermentation proceeds and sugars are converted by yeast to alcohol.

Initially, color was relatively low at day one after the beginning of fermentation for both of the control groups (control and control with pectinase), as might be expected given that color is extracted from the grape skins during the initial stages of fermentation when no treatment

other than crushing is applied. With the fermentation of sugars into alcohol, higher extraction of color from the skins was demonstrated, as the color intensified between day one and day eight for these two samples.

For the thermovinification trials, not much change between day one and day eight is apparent, probably due to the breakdown of the skin cells during heating after crushing and destemming. For the flash détente trials, a similar relationship is illustrated by the graph above. There was major variation observed between treatments initially, but this changed over time as the alcohol produced during fermentation allowed for more color extraction in the control batch and to a small degree in the flash détente and thermovinification batches.

At day 8, none of the treatments were significantly different from one another, suggested that there was an effect of processing when comparing the results at day 8 to day 38. At day 38, a negligible amount of residual sugar remained in the wines and the yeast had largely precipitated to the bottom of the fermentation vessel or had been removed by racking. At this point, flash détente batches were still significantly different from the control batches ($p<0.05$), and flash détente batches were significantly different from thermovinification batches ($p<0.05$). This evidence suggests that flash détente has a beneficial effect on the wine color density over the first part of a red wine's aging process when compared to heat treatment and to no treatment. Thus, it is reasonable to suspect that the vacuum step in flash detente has an effect on the ability of the grape skin to release its anthocyanin and other pigment constituents.

Next, pectinase use was compared to non-pectinase use for each batch type at day 38. Pectinase versus the absence of pectinase resulted in no statistical significance between batch types. Comparison of flash détente pectinase addition versus absence resulted in a calculated p-value of 0.89, while thermovinification pectinase addition versus absence resulted in a calculated

p-value of 0.49. In control batches, a calculated p-value of 0.21 resulted, comparing pectinase addition. Thus, based on this analysis at 38 days after the start of fermentation, the use of pectinase does not seem to influence the extraction of color from the grape skins.

Based on the results of color density, I would not recommend that winemakers add pectinase if the primary goal is to extract higher amounts of color from the grape skin cells. The significant increase of color absorbance from the flash détente procedure may have an impact on consumer preference of the wines, regardless of other quality factors. Further research in the form of a sensory panel is needed to further confirm the assertion that the wine color increase has an influence on the perception of higher quality wine product to the consumer. Flash détente may affect the overall quality character of red wines and is my assertion that it is a useful method to increase the amount of color density on a wine. Table 6 below has information related to the mean ± standard deviation at each time point of each batch type.

*Table 6. Mean comparison of color density*

| Batch Type | Day 1 | Day 8 | Day 38 |
|---|---|---|---|
| Control (n=3) | $1.17\pm0.47^{bB}$ | $3.75\pm0.63^{aA}$ | $4.51\pm0.65^{bB}$ |
| Control with Pectinase (n=2) | $1.43\pm0.22^{b}$ | $3.15\pm0.30^{a}$ | $3.78\pm0.06^{b}$ |
| Flash Détente (n=3) | $3.33\pm0.39^{aA}$ | $4.45\pm0.55^{aA}$ | $6.39\pm0.91^{aA}$ |
| Flash with Pectinase (n=2) | $2.94\pm1.40^{a}$ | $3.89\pm0.30^{a}$ | $5.97\pm0.75^{a}$ |
| Thermovinification (n=2) | $3.64\pm0.12^{aA}$ | $4.35\pm0.92^{aA}$ | $4.63\pm0.09^{bB}$ |
| Thermovinification with Pectinase (n=2) | $3.59\pm0.16^{a}$ | $3.86\pm0.46^{a}$ | $4.68\pm0.02^{b}$ |

- Results are displayed as mean ± standard deviation
- Lowercase letter differences indicate a significant difference between all batch types by column at day 1, day 8, and day 38 ($p<0.05$)
- Uppercase letter differences indicate significant difference between batch types (without pectinase comparison) by column at day 1, day 8, and day 38 ($p<0.05$)

Figure 12 below displays average free-colored anthocyanin absorbance values measured 30 days after pressing at day 38 after the beginning of fermentation. Anthocyanins and their

polymers and reaction products are the main color pigments found in red wines, providing significant information to guide winemaking processes related to color development. The data demonstrates that no statistically significant differences were seen in free-colored anthocyanin content among any of the process treatments at day 38.

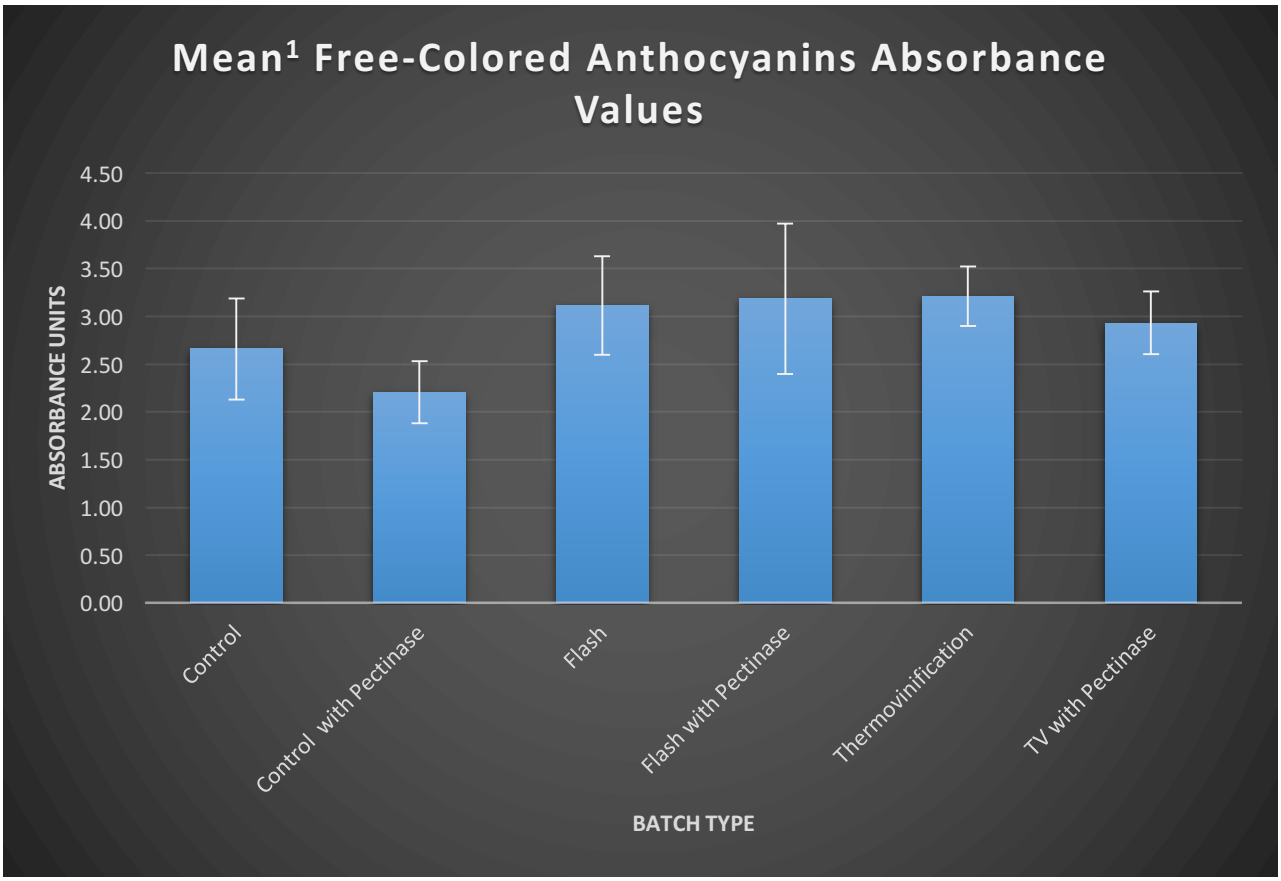


*Figure 12. Free-colored anthocyanins relative concentration means comparison at day 38*

1. n = 3 for control, n = 4 for flash détente, and n = 2 for thermovinification, thermovinification with pectinase, flash détente with pectinase and control with pectinase (all measured in triplicate)
2. Error bars indicate one standard deviation from the mean.

Free anthocyanin absorbance measurements exhibited a wide range of variance, and the exact reason is not fully understood, but the data demonstrates that there is no significant difference between any of the treatments tested. One possible explanation for the observed

values is that the more free anthocyanins were extracted by the flash détente and/or thermovinification treatments but that the polymerization and complexing of free anthocyanins that occurs over time during the ageing of red wines may have masked this to some degree.

Flash détente batches comparison to the control batches resulted in a calculated p-value of 0.22, while the thermovinification trials resulted in a calculated p-value of 0.26 when compared to the control. Based on the total color density measurements reported above, I expected to see a larger difference in free colored anthocyanins between flash détente and thermovinification, but the data demonstrates otherwise, with a calculated p-value between the experimental batch types of 0.82.

Further testing would be required to demonstrate this hypothesis, but the color data given below tends to support this idea as they tend to show higher values for polymerized and co-pigment compounds in the thermovinification and particularly in the flash détente samples.

Table 7 below displays the mean at day 38 of testing plus or minus the standard deviation.

*Table 7. Mean comparison of free-colored anthocyanin*

| Batch Type | Day 38 |
|---|---|
| Control (n=4) | $2.66\pm0.53^{aA}$ |
| Control with Pectinase (n=2) | $2.21\pm0.33^{a}$ |
| Flash Détente (n=6) | $3.11\pm0.52^{aA}$ |
| Flash with Pectinase (n=4) | $3.19\pm0.79^{a}$ |
| Thermovinification (n=2) | $3.21\pm0.31^{aA}$ |
| Thermovinification with Pectinase (n=2) | $2.93\pm0.33^{a}$ |

- Results are displayed as mean ± standard deviation
- Lowercase letter differences indicate a significant difference between all batch types by column at day 1, day 8, and day 38 ($p<0.05$)
- Uppercase letter differences indicate significant difference between batch types (without pectinase comparison) by column at day 1, day 8, and day 38 ($p<0.05$)

Figure 13 below displays the absorbance unit mean values of free colored anthocyanins plus polymerized, colored anthocyanins taken at 38 days or in other words, 30 days following pressing of the wine from the grape skins.

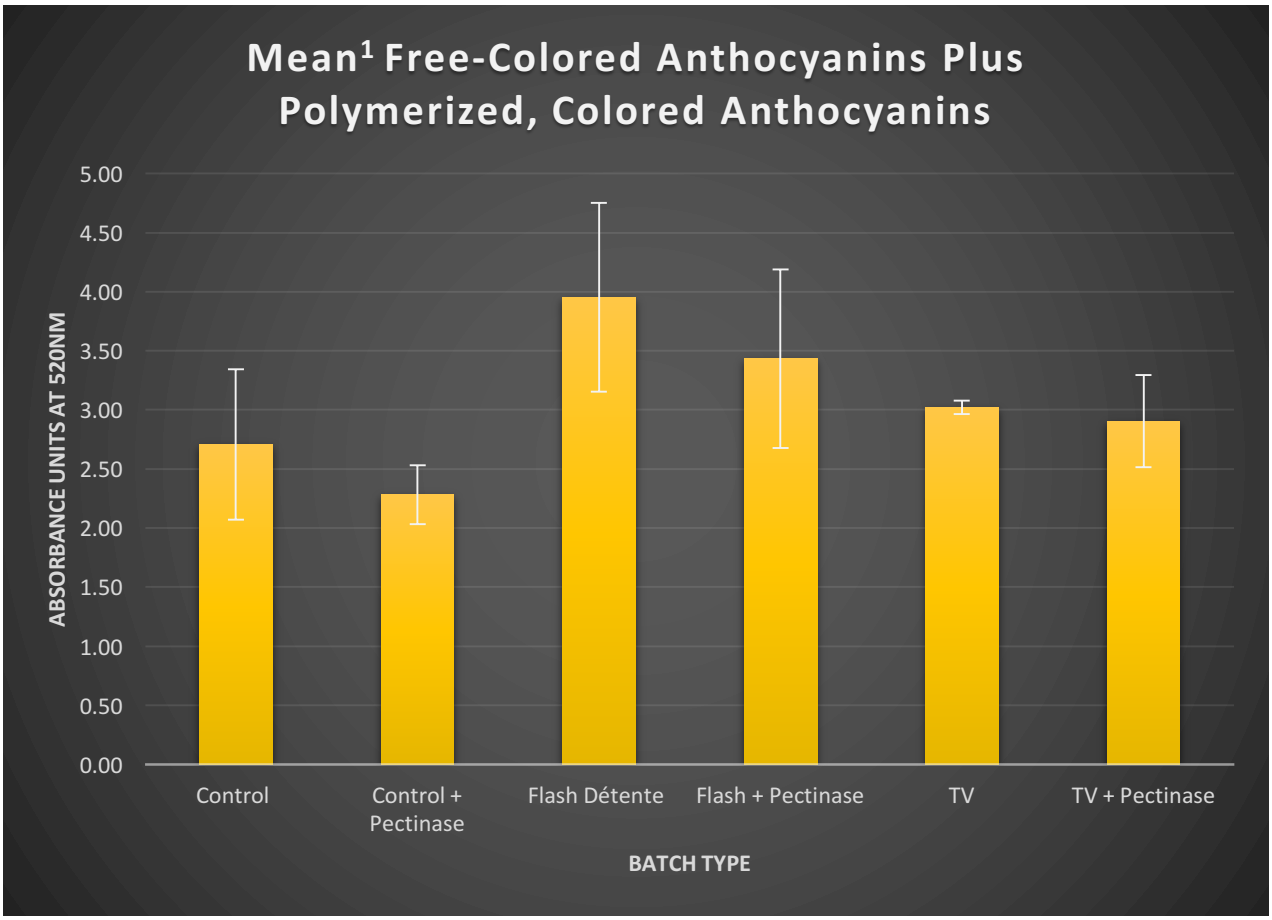


*Figure 13. Mean absorbance comparison of free-colored plus polymerized, colored anthocyanins*

1. n = 4 for control and flash détente with pectinase, n = 6 for flash détente, and n = 2 for thermovinification, thermovinification with pectinase and control with pectinase (all measured in triplicate)
2. Error bars indicate one standard deviation from the mean.

Comparing the batch types to one another (without pectinase comparison), a significant difference was calculated ($p = 0.04$), however mean separation techniques resulted in non-significance between means. Thus, no significant differences were calculated among treatments.

Nevertheless, the flash détente treatment, in particular, had a numerically higher average value. Again, this may indicate that flash détente, and to a lesser degree thermovinification, facilitates the extraction, polymerization, and/or complexing of anthocyanin pigments, but further research would be needed to confirm this on a larger scale. Table 8 below contains means absorbance values plus standard deviations at day 38 after the beginning of fermentation for reference.

*Table 8. Mean comparison of free-colored plus polymerized, colored anthocyanins*

| Batch Type | Day 38 |
|---|---|
| Control (n=4) | $2.71\pm0.64^{aA}$ |
| Control with Pectinase (n=2) | $2.28\pm0.25^{a}$ |
| Flash Détente (n=6) | $3.95\pm0.80^{aA}$ |
| Flash with Pectinase (n=4) | $3.43\pm0.76^{a}$ |
| Thermovinification (n=2) | $3.02\pm0.06^{aA}$ |
| Thermovinification with Pectinase (n=2) | $2.90\pm0.39^{a}$ |

- Results are displayed as mean ± standard deviation
- Lowercase letter differences indicate a significant difference between all batch types by column at day 1, day 8, and day 38 ($p<0.05$)
- Uppercase letter differences indicate significant difference between batch types (without pectinase comparison) by column at day 1, day 8, and day 38 ($p<0.05$)

During aging, it is expected the concentration of free anthocyanins will decrease and the concentration of polymerized, colored anthocyanins should increase in a proportional fashion as free anthocyanins polymerize into chains. Further studies on aging will determine the extent of this parameter and provide information about the stability of these anthocyanin compounds with the experimental treatments examined.

Figure 14 below displays absorbance unit measurements of flavone-phenols present at day 38 after the start of fermentation. The measurement of flavone-phenols is a measure of co-pigmentation between colored pigments and other, generally non-colored, organic molecules in solution. Overall, it has been reported that 30-50 percent of color in young red wines is accounted by co-pigmented anthocyanins; this association between the pigments and their co-

pigmentation factors involves the anthocyanin glucosides, and certain phenolic acids, flavonoids, and derivatives of the flavone subgroups (Boulton 2001).

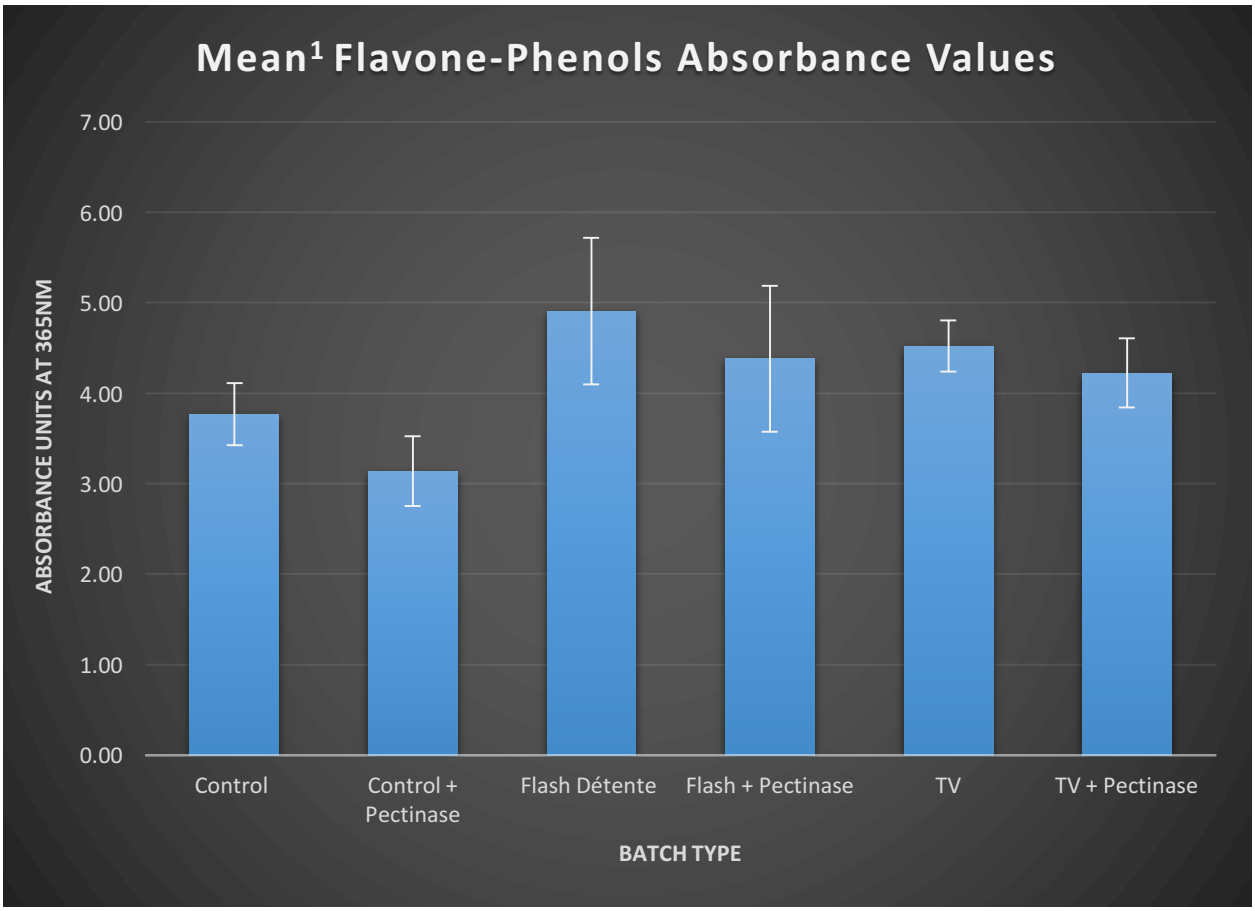


*Figure 14. Mean absorbance comparison of flavone-phenols*

1. n = 4 for control and flash détente with pectinase, n = 6 for flash détente, and n = 2 for thermovinification, thermovinification with pectinase and control with pectinase (all measured in triplicate)
2. Error bars indicate one standard deviation from the mean.

While the means were generally higher in concentration in both the flash détente and thermovinification groups, non-significance was established when comparing the experimental treatments and the control, minus the effect of pectinase addition (p=.08). Thus, the

concentration of the flavone-phenol measurements were not significantly different from one another.

There were no significant differences observed in co-pigment absorbance values between those samples treated with pectinase and those samples not treated with pectinase. Specifically, flash détente pectinase comparison resulted in a p-value of 0.37, thermovinification pectinase comparison resulted in a p-value of 0.47, and control pectinase comparison resulted in a p-value of 0.27 when compared to no pectinase use. Thus, pectinase use does not correlate to a statistically significant difference in flavone-phenol absorbance measurements. Table 9 below displays mean and standard deviation for the flavone-phenol absorbance measurements.

*Table 9. Mean comparison of flavone-phenols*

| Batch Type | Day 38 |
|---|---|
| Control (n=4) | $3.77\pm 0.31^{aA}$ |
| Control with Pectinase (n=2) | $3.14\pm0.39^{a}$ |
| Flash Détente (n=6) | $4.91\pm0.81^{aA}$ |
| Flash with Pectinase (n=4) | $4.38\pm0.80^{a}$ |
| Thermovinification (n=2) | $4.52\pm0.28^{aA}$ |
| Thermovinification with Pectinase (n=2) | $4.22\pm0.38^{a}$ |

- Results are displayed as mean ± standard deviation
- Lowercase letter differences indicate a significant difference between all batch types by column at day 1, day 8, and day 38 ($p<0.05$)
- Uppercase letter differences indicate significant difference between batch types (without pectinase comparison) by column at day 1, day 8, and day 38 ($p<0.05$)

Figure 15 below displays comparisons of absorbance values for the $SO_2$ test as measured at 30 days following pressing of the wine from the grape skins (day 38). The $SO_2$ test is a measure of the concentration of polymerized color compounds and is determined by determining the absorbance of the samples after adjusting the pH to 3.6. Overall, the values as observed at day 38 of fermentation/aging were much lower than those observed for free anthocyanins as well as the co-pigmented anthocyanins; this is a common occurrence in young wines. During aging,

the absorbance value of the $SO_2$ would normally be expected to increase as free anthocyanins begin to polymerize, but this factor was not investigated in this study.

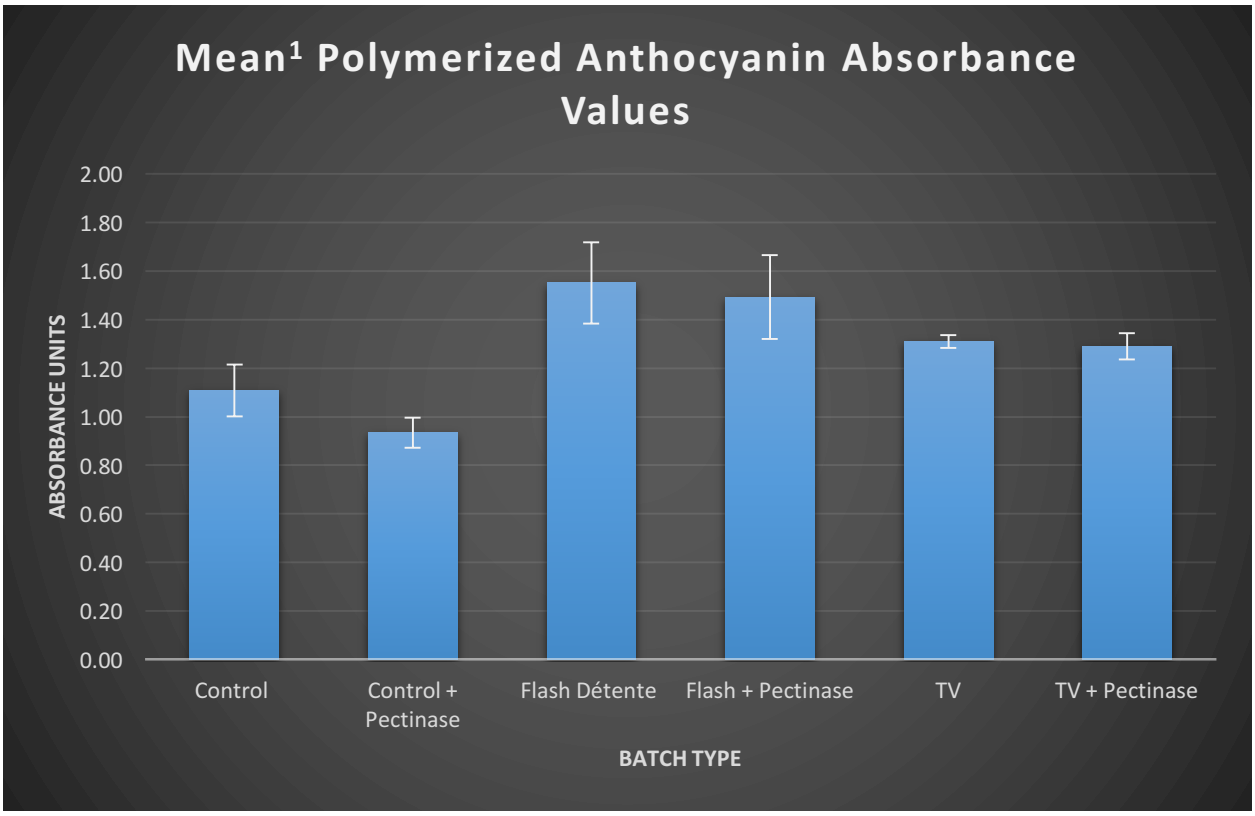


*Figure 15. Mean absorbance of polymerized anthocyanins*

1. n = 4 for control and flash détente with pectinase, n = 6 for flash détente, and n = 2 for thermovinification, thermovinification with pectinase and control with pectinase (all measured in triplicate)
2. Error bars indicate one standard deviation from the mean.

In comparing the batch types, the flash détente batches were significantly different from the control batches (p-value <0.01), while the thermovinification batches were not significantly different from the control batches or the flash détente batches. Thus, the use of flash détente did lead to higher observed concentrations of polymerized anthocyanin pigments in wines compared to control samples 30 days after pressing (day 38).

The use of pectinase enzymes have the effect of causing polymerized pigments to settle out of solution, thus causing them to be lost during multiple rackings. However, in this study, none of the comparisons within treatments showed statistically significant differences in polymerized pigment concentrations between pectinase treated and untreated wines. Flash détente with pectinase addition comparison resulted in a p-value of 0.64, thermovinification pectinase comparison resulted in a p-value of 0.70, and similarly, the control pectinase comparison resulted in a p-value of 0.22. Table 10 below displays means and standard deviations of the batch types examined.

*Table 10. Mean of polymerized anthocyanin absorbance values*

| Batch Type | Day 38 |
|---|---|
| Control (n=4) | $1.11\pm0.11^{bB}$ |
| Control with Pectinase (n=2) | $0.93\pm0.06^{b}$ |
| Flash Détente (n=6) | $1.55\pm0.17^{aA}$ |
| Flash with Pectinase (n=4) | $1.49\pm0.17^{a}$ |
| Thermovinification (n=2) | $1.31\pm0.03^{aAB}$ |
| Thermovinification with Pectinase (n=2) | $1.29\pm0.05^{a}$ |

- Results are displayed as mean ± standard deviation
- Lowercase letter differences indicate a significant difference between all batch types by column at day 1, day 8, and day 38 ($p<0.05$)
- Uppercase letter differences indicate significant difference between batch types (without pectinase comparison) by column at day 1, day 8, and day 38 ($p<0.05$)

Figure 16 shows absorbance measurements at 420 nm at three time points, day 1, 8, and 38. These data allow us to examine possible relationships among color, time, and processing treatment. Absorbance at 420 nm is a function of yellow and brown pigments present in the wine samples.

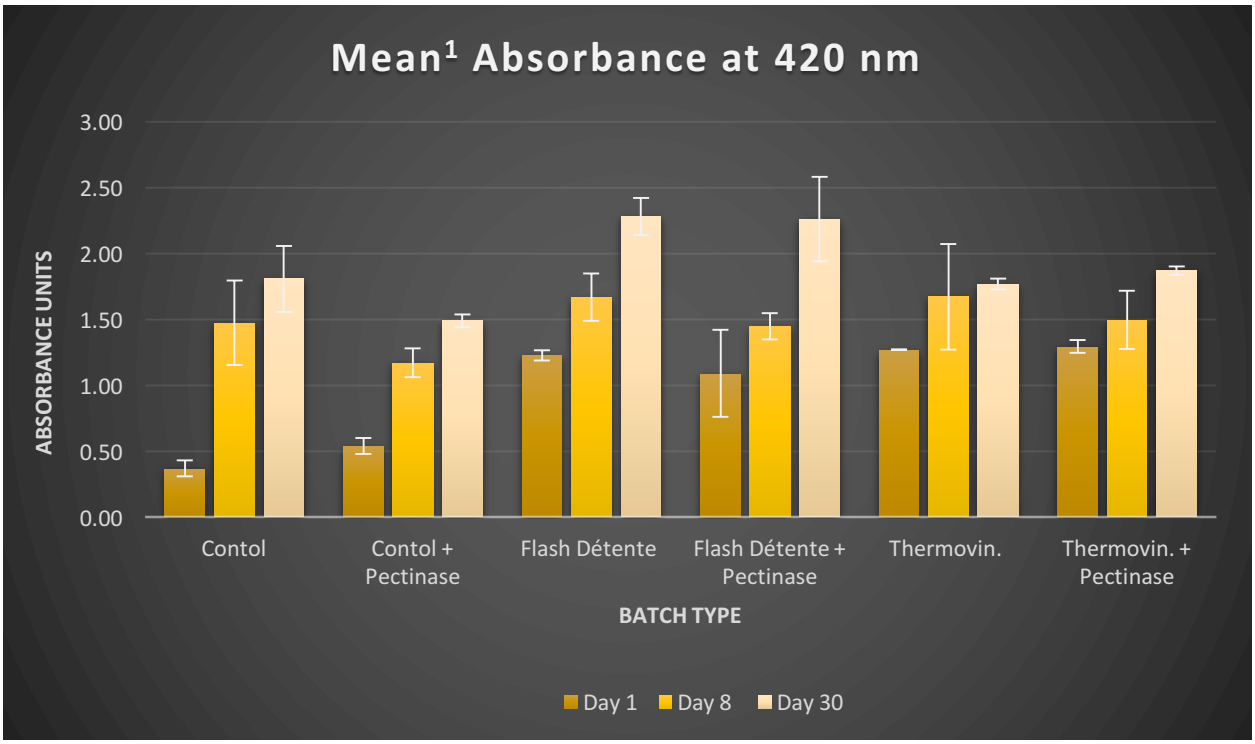


*Figure 16. Mean absorbance values at 420 nm*

1. n = 3 for control and flash détente and n = 2 for flash detente thermovinification, thermovinification with pectinase and control with pectinase (all measured in triplicate)
2. Error bars indicate one standard deviation from the mean.

All batches increased in yellow and brown pigment concentration over time. Initially, at day 1, both flash détente and thermovinification samples exhibited significantly higher concentrations of yellow-brown pigment colors as compared to the control. No differences were seen among treatments at day 8. By day 38, the flash détente wines had statistically higher yellow-brown pigment concentrations than both the thermovinification and the control wines. Interestingly, the overall magnitude of the differences between both treatments and the controls decreased over time. It is possible that the alcohol produced during fermentation may have facilitated the extraction of these yellow-brown pigments – the largest increase for the control wines was seen between day 1 and day 8. It is also possible that pressing served to help extract

these pigments and this effect was more pronounced in the control samples that has experienced less initial tissue disruption as compared to the flash détente and thermovinification treatments. Table 11 below displays mean absorbance values and standard deviations at 420 nm for days 1, 8, and 38 tested in triplicate for each batch.

*Table 11. Mean absorbance values at 420 nm*

| Batch Type | Day 1 | Day 8 | Day 38 |
|---|---|---|---|
| Control (n=3) | $0.37\pm0.06^{cB}$ | $1.47\pm0.32^{abA}$ | $1.81\pm0.25^{bcB}$ |
| Control with Pectinase (n=2) | $0.54\pm0.06^{b}$ | $1.17\pm0.11^{b}$ | $1.49\pm0.05^{c}$ |
| Flash Détente (n=3) | $1.23\pm0.04^{aA}$ | $1.67\pm0.18^{aA}$ | $2.42\pm0.14^{aA}$ |
| Flash with Pectinase (n=2) | $1.09\pm0.33^{a}$ | $1.45\pm0.10^{a}$ | $2.26\pm0.32^{a}$ |
| Thermovinification (n=2) | $1.27\pm0.00^{aA}$ | $1.67\pm0.40^{aA}$ | $1.87\pm0.04^{bB}$ |
| Thermovinification with Pectinase (n=2) | $1.30\pm0.05^{a}$ | $1.50\pm0.22^{a}$ | $1.89\pm0.03^{b}$ |

- Results are displayed as mean ± standard deviation
- Lowercase letter differences indicate a significant difference between all batch types by column at day 1, day 8, and day 38 ($p<0.05$)
- Uppercase letter differences indicate significant difference between batch types (without pectinase comparison) by column at day 1, day 8, and day 38 ($p<0.05$)

Further comparing the two experimental treatments, the total concentration of yellow-brown pigments and the increase seen over time are generally similar for both flash detente and thermovinification at day 1 and day 8, but the flash détente wines showed a greater increase in yellow-brown pigment concentration from day 8 to day 38 after fermentation started. These results may suggest either that there is some function of pressing that influences the color between thermovinification and flash détente – pressing may have extracted more yellow-brown pigments in the flash détente-treated samples, or possibly that the duration of heat in thermovinification may have had an effect over time on the development of color as measured at 420nm. This effect may also have been caused by differing rates of anthocyanin polymerization, or there may be an undiscovered factor responsible for this phenomenon. This relationship merits

further study to understand the mechanism at action among the three time points and between the experimental treatments.

Figure 17 displays the absorbance measurements at 520 nm over three time periods. Absorbance at 520 nm is commonly used to quantify red pigmentation present in the wine samples and is taken as a major indicator of quality in red wines. Thus, these data allow us to examine the potential relationships between red color, time, and processing treatment tested.

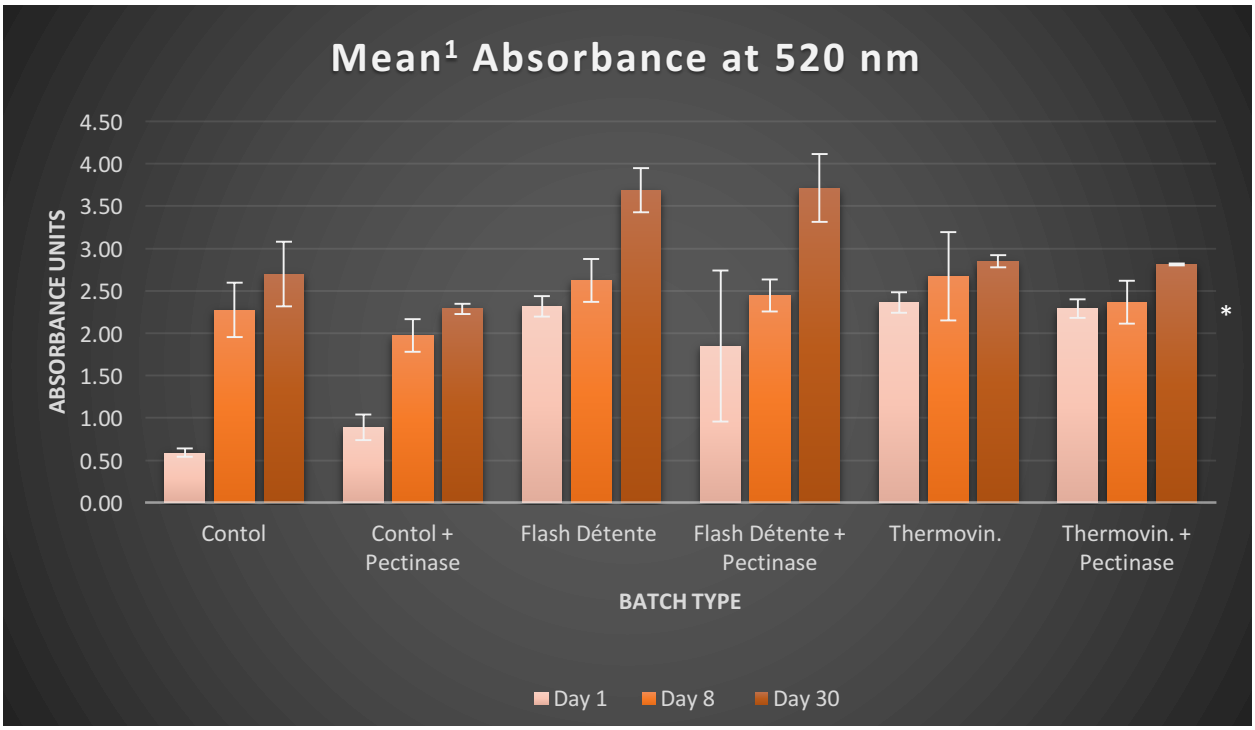


*Figure 17. Mean absorbance values at 520 nm*

3. n = 3 for control and flash détente and n = 2 for flash detente thermovinification, thermovinification with pectinase and control with pectinase (all measured in triplicate)
4. Error bars indicate one standard deviation from the mean.

Overall, the red pigment concentration increased in all samples over time. Initially, flash détente and thermovinification batches exhibited more red pigment compared to the control batches, but as time progressed, alcohol formation in the control batches may possibly have allowed for the extraction of the pigments into the liquid phase. Comparing treatments over time,

flash détente resulted in a higher concentration of pigmentation at 520 nm at day 38 following pressing, while thermovinification and the control mean values were nearly identical at day 8 and 38. For all batch types, pectinase use compared to no pectinase use resulted in no significant differences at any of the time points tested. Results with means and standard deviations tested in triplicate are displayed below in Table 12 for reference

*Table 12. Mean absorbance values at 520 nm*

| Batch Type | Day 1 | Day 8 | Day 38 |
|---|---|---|---|
| Control (n=3) | $0.59\pm0.05^{cB}$ | $2.28\pm0.32^{aA}$ | $2.70\pm0.38^{bcB}$ |
| Control with Pectinase (n=2) | $0.89\pm0.15^{b}$ | $1.97\pm0.19^{a}$ | $2.29\pm0.06^{c}$ |
| Flash Détente (n=3) | $2.32\pm0.12^{aA}$ | $2.62\pm0.25^{aA}$ | $3.69\pm0.26^{aA}$ |
| Flash with Pectinase (n=2) | $1.85\pm0.89^{a}$ | $2.44\pm0.19^{a}$ | $3.71\pm0.40^{a}$ |
| Thermovinification (n=2) | $2.37\pm0.12^{aA}$ | $2.67\pm0.52^{aA}$ | $2.85\pm0.07^{b}$ |
| Thermovinification with Pectinase (n=2) | $2.29\pm0.11^{a}$ | $2.37\pm0.25^{a}$ | $2.81\pm0.01^{bB}$ |

- Results are displayed as mean ± standard deviation
- Lowercase letter differences indicate a significant difference between all batch types by column at day 1, day 8, and day 38 ($p<0.05$)
- Uppercase letter differences indicate significant difference between batch types (without pectinase comparison) by column at day 1, day 8, and day 38 ($p<0.05$)

Overall, the results seen for red pigments mirror those previously described for yellow-brown pigments almost exactly. Once again, wines produced using flash détente showed significantly higher concentrations of pigments at day 38, specifically red pigments in this case, compared to both thermovinification and control treatments. As noted above, this effect merits further study to understand the mechanism at action among the three time periods and between the experimental treatments.

Figure 18 below displays the mean absorbance values of color hue after 38 days after the beginning of fermentation. The color hue of a wine is a measure of the ratio of the absorbance values at 420 nm divided by the absorbance values at 520 nm. Color hue is generally used an

indicator of how the ratio of the two absorbance values change over time and can indicate browning or oxidation of wine samples.

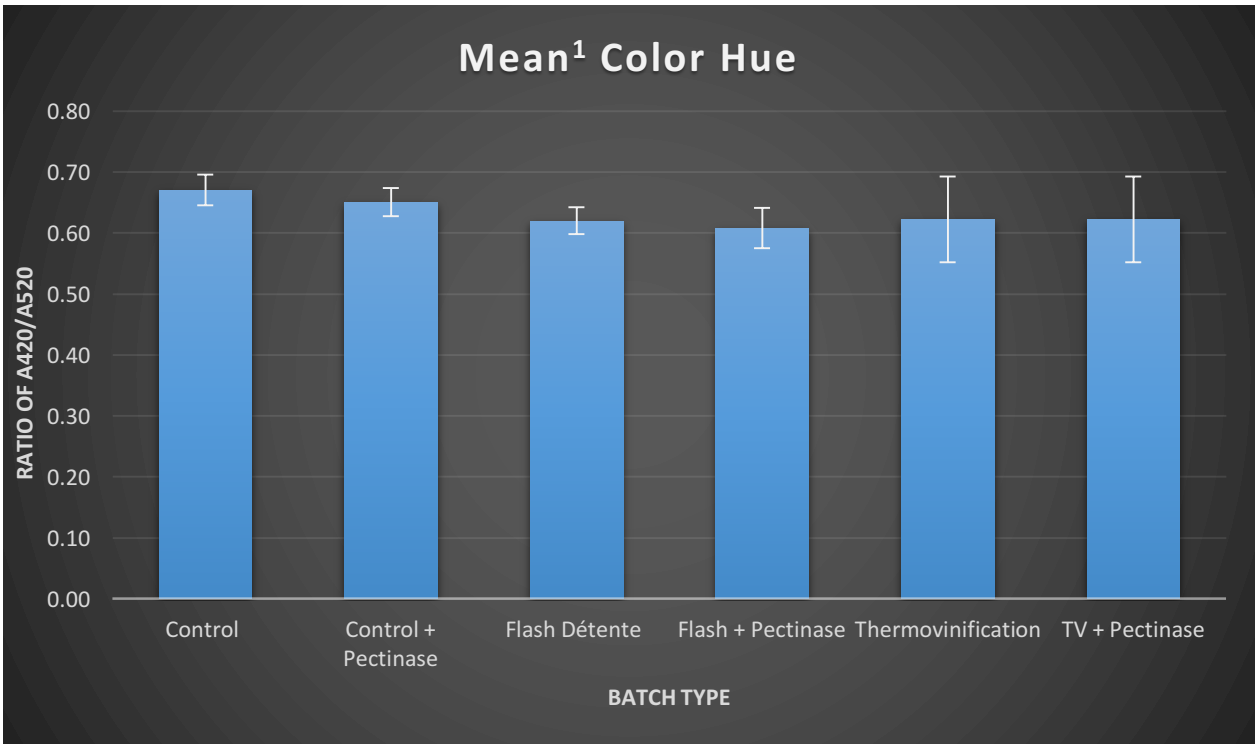


*Figure 18. Mean color hue values*

1. n = 4 for control and flash détente with pectinase, n = 6 for flash détente, and n = 2 for thermovinification, thermovinification with pectinase and control with pectinase (all measured in triplicate)
2. Error bars indicate one standard deviation from the mean.

Pectinase use was found to have no significant impact on the color hue in this study. Comparison of flash détente pectinase addition resulted in a calculated p-value of 0.50, thermovinification pectinase comparison resulted in a calculated p-value of 0.47, and the control pectinase comparison resulted in a calculated p-value of 0.48. If the batch types are compared to one another, statistical analysis demonstrates no significant difference between batches with a p-value of 0.14. Thus, for all practical purposes, color hue was not affected by batch type or

pectinase treatment at 30 days after pressing (day 38 total). Table 13 below displays color hue means and standard deviation for all batch types.

*Table 13. Color hue means*

| Batch Type | Day 38 |
|---|---|
| Control (n=4) | $0.67\pm0.03^{aA}$ |
| Control with Pectinase (n=2) | $0.65\pm0.02^{a}$ |
| Flash Détente (n=6) | $0.62\pm0.02^{aA}$ |
| Flash with Pectinase (n=4) | $0.61\pm0.03^{a}$ |
| Thermovinification (n=2) | $0.62\pm0.07^{aA}$ |
| Thermovinification with Pectinase (n=2) | $0.62\pm0.07^{a}$ |

- Results are displayed as mean ± standard deviation
- Lowercase letter differences indicate a significant difference between all batch types by column at day 1, day 8, and day 38 ($p<0.05$)
- Uppercase letter differences indicate significant difference between batch types (without pectinase comparison) by column at day 1, day 8, and day 38 ($p<0.05$)

This result is not surprising considering the age of the wines tested. Ordinarily, an increase is expected in the hue value for a red wine as aging occurs, although the usefulness of these values is limited when relating the hue values to wine quality attributes (Birse 2007). This particular test may prove useful for evaluating oxidation and subsequent browning after the experimental wines produced in this experiment have undergone aging.

**Gas Chromatography Data Analysis**

Results for the gas chromatography experiment of wine volatiles are expressed in mg/L. The retention times of the volatile compounds investigated are shown in Table 8. Attempts were made to analyze other volatiles for which standards had been obtained, but these were detected at levels below the minimum quantification level. Thus, they were not included in the results or discussion. Measurements were made 3 months after aging began. The five major and minor volatile compounds that were analyzed by GC analysis (Table 14) are known to significantly influence red wine quality and character.

*Table 14. Average retention time of pure GC standards*

| Compound Standard | Standard Retention Time (min) |
|---|---|
| 1-Hexanol | 26.7 |
| Ethyl Acetate | 6.97 |
| Ethyl Hexanoate | 21.2 |
| Hexanoic Acid | 46.1 |
| Isobutanol | 14.6 |

**Ethyl Acetate**

The most significant and most concentrated ester in wines is ethyl acetate, which imparts an aroma described as solvent, nail polish or vinegar (Rapp 1998). At high concentrations, it is an undesirable compound, but it can enhance a wine's perceived complexity at lower concentrations (Rapp 1986). The concentration of ethyl acetate in the red wine samples tested ranged between 37.2 and 53.3 mg/L. The mean ethyl acetate concentration grouped by batch type is displayed in Figure 19 below.

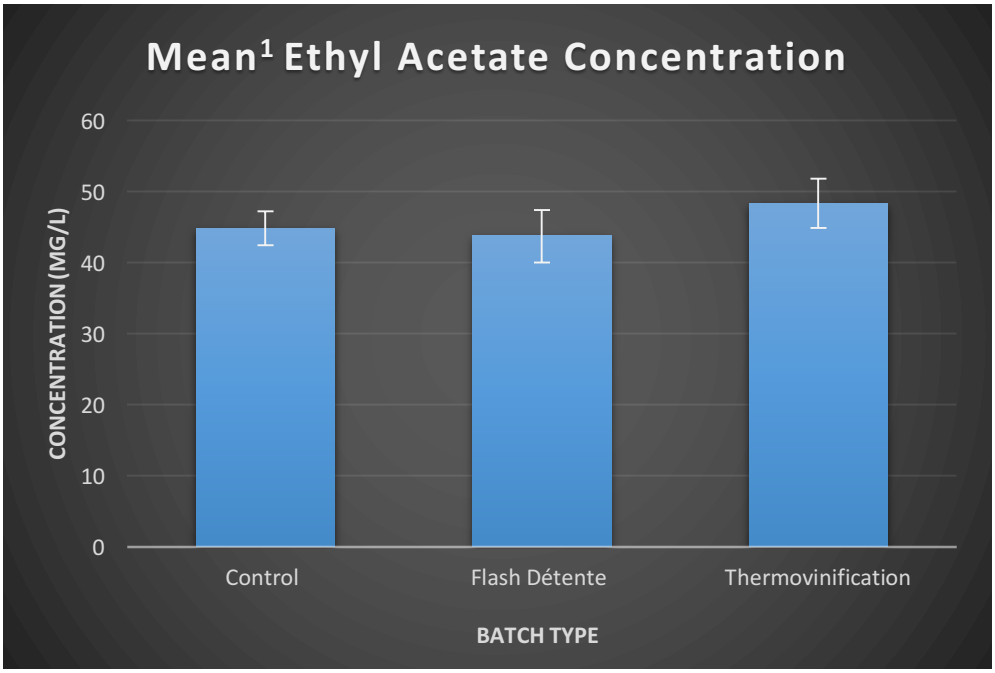

*Figure 19. Mean ethyl acetate concentration*

1. n = 8 for control, n = 19 for flash détente, and n = 5 for thermovinification (all measured in duplicate)
2. Error bars indicate one standard deviation from the mean.

Statistical analysis of the treatment groups shows a significant difference between treatment groups, with ANOVA giving a p-value of 0.02. Using Tukey HSD, the only significant difference seen was between thermovinification and flash détente groups. In other words, there is no difference between the control and treatment groups, but there is a significant difference among the two treatment groups. Table 15 below displays mean concentration (mg/L) of ethyl acetate measured in triplicate.

*Table 15. Ethyl acetate mean concentration*

| Batch Type – Measured in triplicate | Mean concentration (mg/L) |
|---|---|
| Control (n=8) | 44.85 ±2.40$^{ab}$ |
| Flash Détente (n=12) | 43.74±3.72$^{b}$ |
| Thermovinification (n=4) | 48.38±3.49$^{a}$ |

- Letter differences indicate significant difference between batch types ($p<0.05$)

Relating this back to aroma and the impact on the quality of the wine, thermovinification increased the concentration of ethyl acetate, known for its vinegar-like presence, above that found in the flash détente trial groups. Flash détente batches were no different from the control, indicating that it is neither less nor more desirable than existing methods of production in terms of ethyl acetate concentrations.

**Ethyl Hexanoate**

Another compound in the ester family is ethyl hexanoate, which conveys a green apple or fruity aroma (Rapp 1998). Significant difference between the treatments could indicate that there is a quality improvement among treatments, given there is an increase in this fruity volatile. The

concentration of ethyl hexanoate is between 0.2 mg/L and 1.1 mg/L in the samples examined. The mean ethyl hexanoate concentration (mg/L) grouped by batch type is displayed in Figure 20 below.

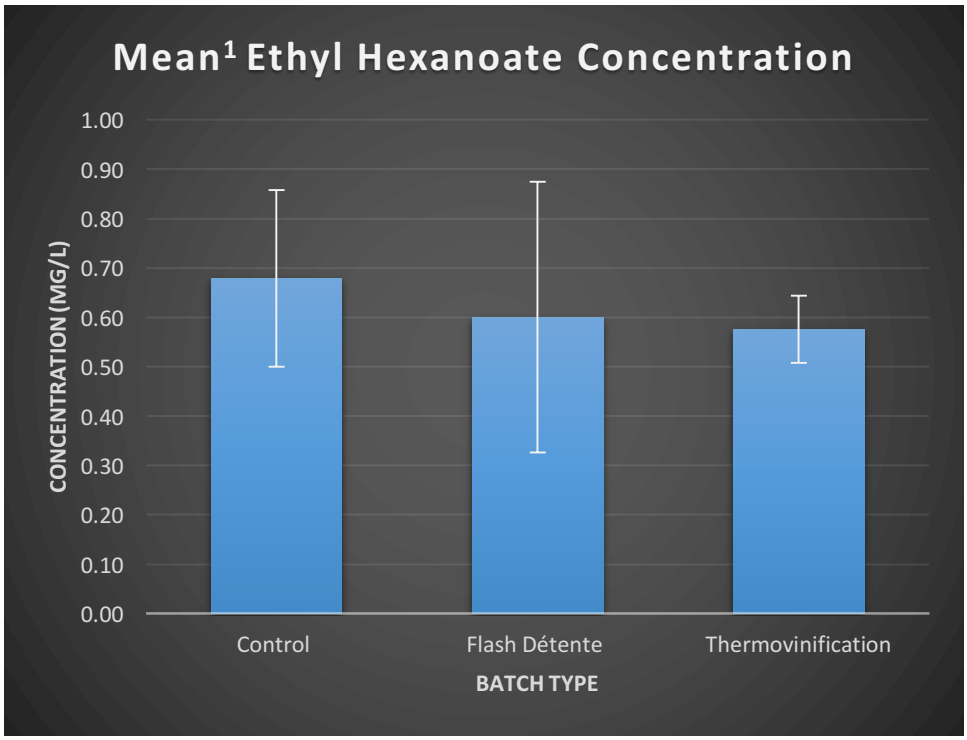


*Figure 20. Mean ethyl hexanoate concentration*

1. n = 8 for control, n = 19 for flash détente, and n = 5 for thermovinification (all measured in duplicate)
2. Error bars indicate one standard deviation from the mean.

Statistical analysis showed no significant differences between any of the treatment groups, giving a p-value of 0.67. In other words, the tested processing treatments had no impact on this green apple fruity-aroma volatile. Mean concentrations of ethyl hexanoate are displayed in the table below for reference.

*Table 16. Mean ethyl hexanoate concentration*

| Batch Type – Measured in triplicate | Mean concentration (mg/L) |
|---|---|
| Control (n=8) | 0.68 ±0.18[a] |
| Flash Détente (n=12) | 0.60±0.27[a] |
| Thermovinification (n=4) | 0.58±0.07[a] |

- Letter differences indicate significant difference between batch types ($p<0.05$)

A wide range of variance is present within measurements for ethyl hexanoate, possibly due to the low concentration of this volatile compared to others examined.

**1-Hexanol**

The volatile compound 1-hexanol imparts an aroma described as green or grassy (Villamor 2012). A lower concentration of 1-hexanol would generally indicate a higher quality wine as an excessively green or grassy aroma is considered a quality defect in most wines. The average concentration ranged from 0.5 mg/L to 2.1 mg/L in the experiment. The average concentrations (mg/L) of 1-hexanol are displayed in Figure 21 below.

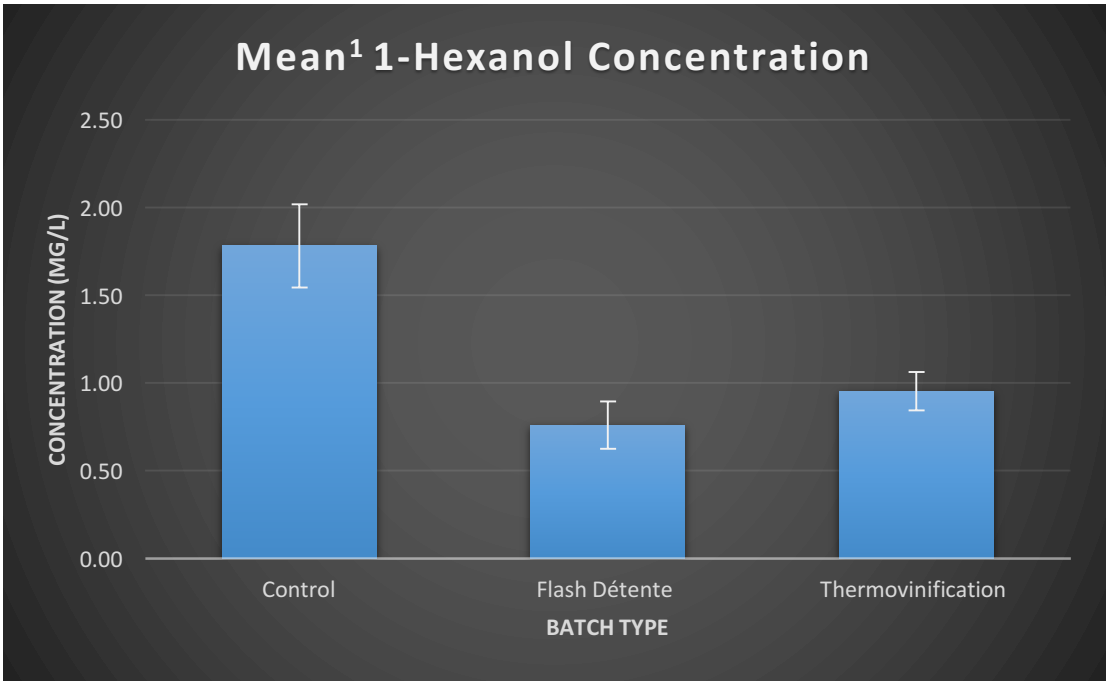


*Figure 21. Mean 1-hexanol concentration*

1. n = 8 for control, n = 19 for flash détente, and n = 5 for thermovinification (all measured in duplicate)
2. Error bars indicate one standard deviation from the mean.

Statistical analysis demonstrated a highly significant p-value of <.0001, indicating a difference between the batch treatments. From the figure it is apparent that the difference is between both experimental treatments and the control batches. Tukey's HSD demonstrates there is a p-value of <0.01 for the difference between the flash détente and the control batches and a p-value of <0.01 for the difference between the thermovinification and control batches. The mean concentration (mg/L) of 1-hexanol is displayed in table 17 below.

*Table 17. 1-Hexanol mean concentration*

| Batch Type – Measured in triplicate | Mean concentration (mg/L) |
|---|---|
| Control (n=8) | 1.78 ±0.24[b] |
| Flash Détente (n=12) | 0.76±0.13[a] |
| Thermovinification (n=4) | 0.95±0.11[a] |

- Letter differences indicate significant difference between batch types (p<0.05)

Although sensory evaluation would be necessary to confirm this, the lower 1-hexanol concentrations seen in both the flash détente and thermovinification treatments may indicate that both processes have the potential to improve the perceived sensory quality of red wines made from Oklahoma grapes by reducing grassy or vegetal aromas and flavors.

**Hexanoic Acid**

Hexanoic acid is an acid found in wines that imparts an aroma described as fatty type odor that is described as unpleasant in high concentrations (Rapp 1998). The average concentration measured ranged from 2.9 mg/L 4.7 mg/L in this experiment. The average concentration of each batch type is displayed in Figure 22 below.

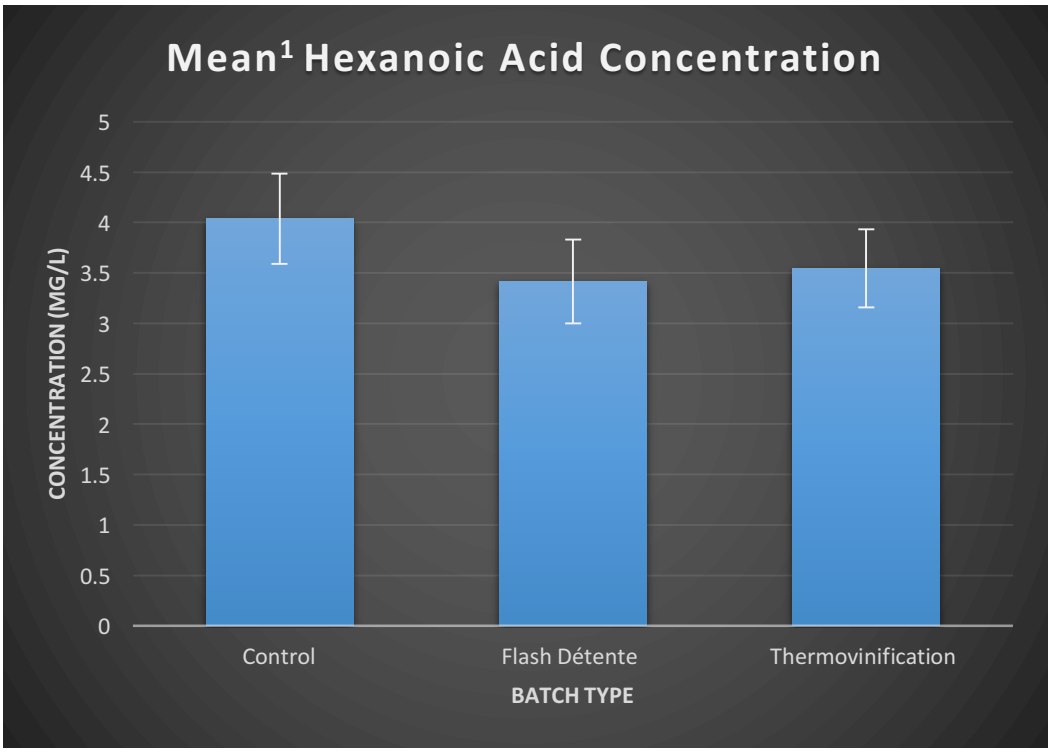


*Figure 22. Mean hexanoic acid concentration*

1. n = 8 for control, n = 19 for flash détente, and n = 5 for thermovinification (all measured in duplicate)
2. Error bars indicate one standard deviation from the mean.

Statistical analysis indicated that were no significant differences observed in hexanoic acid content among the tested treatments (p-value=0.13). Other studies conducted on this volatile over many seasons and across wine grape varieties, have clearly indicated that concentrations of this compound may vary by a factor of 2 to 3 (Geffroy 2015). Table 18 below displays mean concentration of hexanoic acid in the wine samples.

*Table 18. Mean hexanoic acid concentration*

| Batch Type – Measured in triplicate | Mean concentration (mg/L) |
|---|---|
| Control (n=8) | 4.04 ±0.45[a] |
| Flash Détente (n=12) | 3.45±0.42[a] |
| Thermovinification (n=4) | 3.55±0.39[a] |

- Letter differences indicate significant difference between batch types (p<0.05)

The results of this experiment did not demonstrate much variability in hexanoic concentration by treatment; more studies would be needed to understand the possible impact of processing treatments on this volatile compound over years and among different varieties of grapes.

**Isobutanol**

Isobutanol is a volatile compound that imparts an aroma variously described as wine-like, solvent-like, or bitter (Rapp 1998). This compound contributes desirable complexity to wine at concentrations below 300 mg/L. The concentration of isobutanol in the experiment ranged between 85.0 mg/L and 99.6 mg/L in the wines. The average isobutanol concentration is displayed in Figure 23 below.

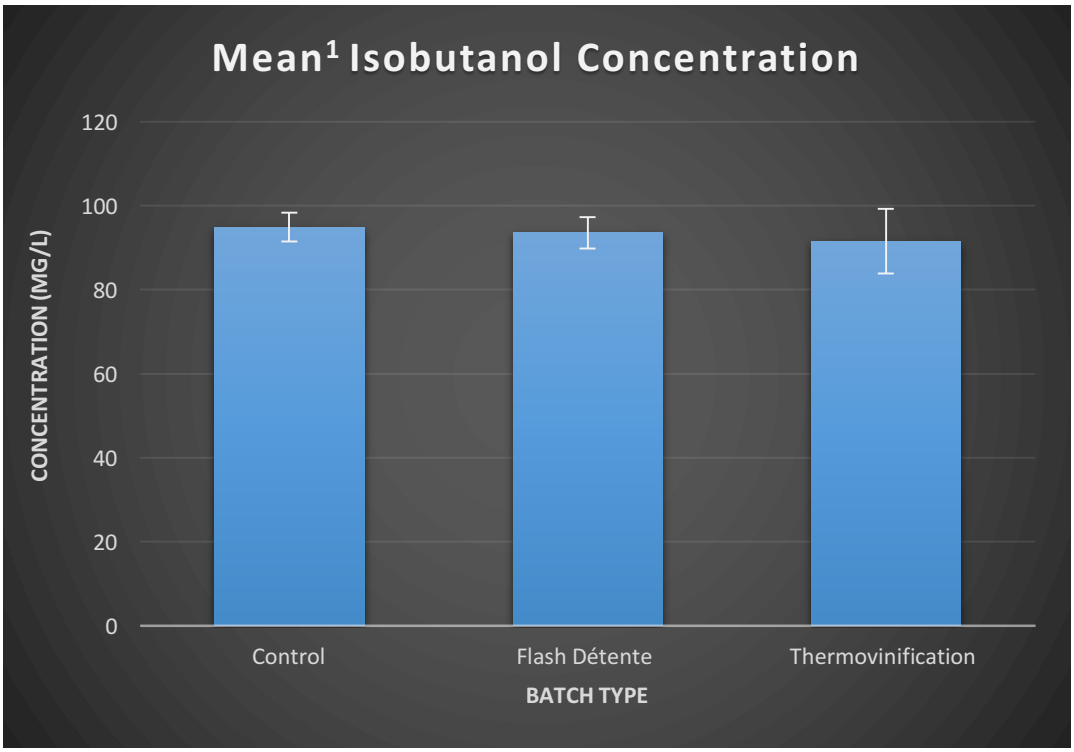


*Figure 23. Mean isobutanol concentration*

1. n = 8 for control, n = 19 for flash détente, and n = 5 for thermovinification (all measured in duplicate)

2. Error bars indicate one standard deviation from the mean.

Because of isobutanol's influence on the complexity of wine aroma, and because the aroma of isobutanol is itself difficult to describe with precision, it is challenging to isolate and adequately describe all of the effects that this volatile compound may have on a wine's aroma and flavor. Table 19 below displays mean concentration for isobutanol for each batch type.

*Table 19. Mean isobutanol concentration*

| Batch Type – Measured in triplicate | Mean concentration (mg/L) |
|---|---|
| Control (n=8) | 94.87 ±3.42[a] |
| Flash Détente (n=12) | 93.54±3.75[a] |
| Thermovinification (n=4) | 91.55±7.65[a] |

- Letter differences indicate significant difference between batch types ($p<0.05$)

No statistically significant differences were seen among the treatments tested (p-value = 0.42). Thus, neither thermovinification nor flash détente had a significant effect on the measured concentration of isobutanol in the wine samples.

**Discussion of methodology**

While flash détente processing is conducted in a continuous production process, this study used batch processing to process grape skin and associated pulp. Recognizing this parameter is important and differences may arise when comparing the methodology utilizing batch versus continuous processing. Furthermore, due to limitations of heating systems, an open container was utilized as compared to a closed system in continuous processing. Overall the system used worked well, but there were some problems/inefficiencies. These problems and possible solutions are listed below.

1. Heating was in batch mode in a steam jacketed vessel. Heating through this method led to a large variation in temperature samples, leading to overheating in

some parts of the grapes. Furthermore, agitation was necessary to decrease variation, which may have further destroyed the grapes further.

2. Straining was manual throughout the whole process. Since grapes were strained after heating and then transferred to the system sample container, heat may have decreased during the process. A possible solution to this problem could be grapes moving parallel to the straining equipment to avoid blockage of the strainer.
3. Heating was done in an open oxygen environment when heating of grape skins and associated pulp. Volatiles may have been released at this point, and not primarily driven off at the point of condensation in the flash vessel. A potential solution could be to heat samples in a closed container with a stirring element and then a mechanical pump to displace samples from the heating element.

Solving these issues may lead to increased efficiency and better results replicable to industrial operations. The batch system helped understand the process related to color formation, though further studies are needed in continuous production mode to validate volatile composition findings contained in this paper.

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# Chapter V

## CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

This study focused on the impact of two processing methods designed to help disrupt grape tissue at the start of the fermentation process and thereby enhance color and volatile compounds in the finished wine. Overall, the data demonstrate the utility of flash détente to develop deeper red-colored wines with reduced grassy and vegetal aromas and flavors. Thermovinification also demonstrated the potential to accentuate finished wines with an enhanced aromatic profile although its impact on color was not as great as the flash détente treatment.

With the possible exception of Yeast assimilable nitrogen (YAN), none of the standard enological parameters examined, i.e. pH, titratable acidity, and alcohol by volume, were expected to be significantly different among the processing treatments examined and the results confirmed these expectations. YAN was higher in both experimental processing treatments, possibly due to the breakdown of the grape skin cell's physical structure, which may have allowed for a higher concentration of nitrogen sources to be available to the yeast for fermentation. In addition to quantifying essential yeast nutrient content, thus helping to avoid incomplete or "stuck" fermentations, YAN also provides information about the aromas that may result during and after fermentation both because a healthy and vigorous fermentation can enhance desirable aroma compound creation and avoid off-aroma compound creation and

because some of the nitrogenous compounds measured may participate in off-aroma compound formation if concentrations are too high. The results of this study indicate that use of either flash détente or thermovinification may reduce or eliminate the need for added yeast nutrients and thereby reduce both the incidence of stuck fermentations and the formation of sulphidic off-flavors that may result in lower quality wines.

The specific objectives and results of this study related to color, aroma compound concentrations, and pectinase use are presented below.

1. Flash détente and/or thermovinification were originally hypothesized to enhance the color of wines. Data indicate that the significant color differences were observed and these came largely from differing concentrations of free and polymerized anthocyanins as measured by absorbance values at specific wavelengths of 420 and 520 nm. Increases in these absorbance values indicate that Cabernet Sauvignon wines made using both flash détente and thermovinification were generally more deeply pigmented than control wines and that flash détente wines were highest in free anthocyanins at week 38. This research may enable the wine industry to tailor applications of the flash détente and thermovinification processes for specific grape varieties and blends. This may allow winemakers to resolve common issues related to color formation with regard to grapes grown in the area.
2. Flash détente and/or thermovinification were originally hypothesized to liberate fruity aromas and decrease the concentration of undesirable aromas in wines. The volatiles analyzed demonstrated a reduction in the green and grassy-type aroma for both flash détente and thermovinification treatments, though no increase in the concentration of fruity esters was observed. More studies are needed with a wider array of volatiles in

order to determine whether or not aroma composition is significantly changed with the flash détente and/or thermovinification processes. In addition, sensory panels will need to be conducted to understand consumer preference for these wine products and to determine if the observed reductions in undesirable aroma compounds translate to higher subjective sensory scores.

3. The use of pectinase was originally hypothesized to assist in further extracting color compounds from grape skins during fermentation. However, in this study all measurements comparing color of wines made with pectinase demonstrated that pectinase had no statistically significant effect on color. Thus, based on these results, the use of pectinase to improve wine color cannot be recommended, particularly as the use of pectinase involves added cost and requires specialized knowledge. This study showed that apart from flash détente and thermovinification treatments, extraction of color appeared to be largely impacted by changes in alcohol content during fermentation.

Overall, this research may help winemakers in the state of Oklahoma to produce higher quality, richer colored wines made with grapes grown in the state. Due to issues related to color formation, and laws that prohibit the addition of many coloring agents in wine, flash détente in particular may provide a process with which to create naturally deeper red hues in red wine. Furthermore, with the market at-large demanding more natural food and beverage products, it may provide an effective means to satisfy the demand for high-quality wines without color additives, thus enhancing marketing potential.

Given the limited sample size of this study, it may be beneficial to have wineries test this process on a larger scale using additional grape varieties over several seasons in order to verify

the findings contained in this study. Flash détente demonstrated that it has the ability to produce more deeply colored red wines at day 38, but larger samples are needed for testing over time.

Suggestions for future research include quantifying methoxypyrazine compounds in wines produced using the flash détente and thermovinification process and to compare them to wines produced using standard winemaking procedures. These earthy, grassy compounds impart a sensory attribute to wines that is highly disagreeable to most consumers. They could not be tested using the methods employed in this study due to their extremely low concentrations; typically, these compounds are present at levels of ng/L.

In addition, future studies might profitably focus on the use of flash détente and thermovinification to produce wines suitable as blending stock for winemakers. Furthermore, an important area that warrants further research is the sensory evaluation of the experimental wines made during this study using sensory panels with both trained and untrained sensory panelists. Trained sensory panels could verify analytical findings of this experiment, while untrained panels could demonstrate the utility of the processes in marketing and consumer quality perception factors.

In summary, this study constitutes research that highlights the potential advantages of flash détente and thermovinification as processing pre-fermentation techniques. It also serves to familiarize winemakers with these relatively uncommon winemaking processes and thus may stimulate interest among smaller wineries. With that in mind, if sufficient interest exists among these smaller winemakers, another potentially fruitful avenue of research would be the design and testing of a relatively small-scale, affordable system for flash détente processing that would be more practical than the laboratory-scale device used in this study. Such a system could be extremely useful in helping to stimulate interest and drive adoption of the flash détente process.

VITA

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