EVALUATION OF THE COLOR COMPOUNDS PRESENT IN RUBAIYAT WINE AS FUNCTION OF SKIN CONTACT TIME DURING FERMENTATION

By

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Bachelor of Science in Biochemistry

Oklahoma State University

Stillwater, Oklahoma

2018

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 2020

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ACKNOWLEDGEMENTS

I would first like to thank my graduate advisor, Dr. William McGlynn, without his encouragement and knowledge I never would have been able to make it this far in my graduate career.

In addition, I want to say thank to the other members on my committee, Dr. Tim Bowser and Dr. Ranjith Ramanathan. Dr. Bowser's door was always open and his feedback was invaluable. Dr. Ramanathan's lab was the first lab I conducted research in as an undergraduate. In that lab, I learned how to proper conduct research and I cannot thank him enough for that opportunity.

I would like to extend a thank you to the faculty and professors of the Food Science program at Oklahoma State University for the knowledge they bestowed upon their students.

Lastly, I would like to thank my fellow graduate students. The good times we shared in the office are memories I will remember fondly for the rest of my life.

Name: PARKER WILSON

Date of Degree: JULY, 2020

Title of Study: EVALUATION OF THE COLOR COMPOUNDS PRESENT IN RUBAIYAT WINE AS FUNCTION OF SKIN CONTACT TIME DURING FERMENTATION

Major Field: FOOD SCIENCE

ABSTRACT: This research evaluated a hybrid grape originating from Oklahoma State University to determine the physical and chemical characteristics of the wine it produced and its viability as a coloring agent in commercial winemaking. The grape variety is known as Rubaiyat and it was first introduced in 1975 at Oklahoma State University by Dr. Herman Hinrichs. Rubaiyat has been proposed as a teinturier (coloring wine) for commercial winemaking due to the grape's deep purple/blue skin and the presense of pigments in the grape flesh. However, prior to this research, there have not been any data recorded to determine the suitability of the Rubaiyat grape for this particular purpose. The criteria evaluated in this project included standard assays of wine characteristics, the total phenolic content, and colorimetric analysis with a focus on the effect of skin contact time on extraction of anthocyanins and other pigments. To do this, standard assays of quality such as pH, titratable acidity, and total alcohol content were used to determine the general characteristics and quality of the Rubaiyat wine produced. The winemaking process used in this experiment was kept simple to focus on the biochemical interactions between the grape and the wine. The process included destemming/crushing, adding yeast, and then taking samples daily until the wine was pressed. Samples were then taken weekly and the wine was also racked (decanted) weekly. Color analyses showed that pigment extraction, was essentially complete after two days of maceration. Also, spectrophotometric testing demonstrated that red pigments have the highest rate of extraction. The wine produced from the Rubaiyat grapes did not have the concentration of pigments that would make it a useful teinturier grape. Beyond this, the Rubaiyat wines produced in this study was typical in terms of basic physical and chemical characteristics when compared to the more popular European grape varieties.

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CHAPTER I

INTRODUCTION

Wine is a beverage that has been consumed for millennia and is still enjoyed today for many reasons. Many fruits are used to make wine, however, grapes are by far the most popular and commercially significant fruit used for winemaking. In addition to wine, grapes are utilized for many other purposes. Table grapes are grown and eaten fresh, unfermented grape juice is popular and consumed frequently, dried grapes are used to produce raisins, and grape skins can also be utilized to produce spirits such as grappa (a popular Italian spirit made from pomace). Due to these facts, grapes are arguably one of the most important horticultural products grown today. In 2016, the U.S. alone produced approximately 4 million metric tons of grapes, on roughly 250,000 hectares, that were crushed for wine, with a farm value of approximately $4.1 billion. The amount of wine that the U.S. produced has only accounted for roughly 10% of the world's total volume of wine in recent years (Lapsley, Alston, & Sambucci, 2019). In total, the value of all U.S farm production in 2016 was $357 billion from a total of 370.1 million hectares. This means the wine industry contributed 1.1% of the total value of farm production and did it using only 0.07% of the total land (Lapsley et al., 2019). Grapes are the preferred fruits for winemaking because of their balanced

bioactive components, which includes varying species of phenolic compounds such as anthocyanins and tannins, high amounts of natural sugars and acids, and a natural microflora conducive to successful fermentation. The phenolic compounds constitute one of the most important quality parameters for wine because these components contribute to organoleptic characteristics such as color, astringency, bitterness, and aroma (Monagas, Bartolome, & Gomez-Cordoves, 2005). Phenolic compounds are synthesized in grapes as a plant defense against ultraviolet radiation, and infections by pathogens, parasites, and predators (Sacchi, Bisson, & Adams, 2005). The relative assortment of these above mentioned components in the berries of each grape variety defines what is known as "varietal character" (Lund & Bohlmann, 2006). Each different grape variety has a different varietal character and winemakers must consider this in order to produce complex and desirable wines for consumers.

By far the most common species of food grape, *Vitis vinifera* is native to the Mediterranean region, Central Europe, and southwestern Asia, from Morocco and Portugal north to southern Germany and east to northern Iran (Trust & Robinson, 2012). Cultivars of *Vitis vinifera* form the basis of the majority of wines produced around the world. Most of the wine varieties familiar to wine consumers in the United States are *Vitis vinifera*, which is cultivated on every continent except for Antarctica, and in all the major wine regions of the world (Trust & Robinson, 2012). Hybrid grapes are a cultivar of grape that is the product of crossing two more different grape species together. For winemaking, hybrid grapes are less popular in comparison to the more popular and commonplace *Vitis vinifera* species of grapes. Hybrids are not as desirable or as well-known as the *Vitis vinifera* species, however, many existing hybrid cultivars exhibit many

benefits for the growers. Cold hardiness and disease resistance are two of the most sought after traits of hybrids. In a study done by the University of Minnesota, crosses between V. *riparia* and French hybrids or V. *vinifera* wine cultivars have resulted in very cold hardy and disease resistant seedlings. The new V. *riparia* derivatives demonstrated much greater survival than the existing varieties, with the best selections, e.g. MN 1131 (V. *riparia* x 'Seyval') having up to 88% live nodes (Hemstad & Luby, 1998). Although hybrids grapes provide potential benefits to the wine industry in many locations, information regarding the quality of wines made from hybrid grapes is relatively rare.

The amount of wine produced from the more traditional regions of California, Washington, and Oregon accounts for more than 90% of all the wine made in the U.S. (Cooke, 2018). Due to the potential profitability, as well as an increased interest in wine culture, new non-traditional winemaking regions have been emerging within the U.S. These new regions include, but are not limited to, the midwest, east coast, and more southern states including Oklahoma and Texas (Li, Gómez, Brent Ross, & Chaddad, 2019). Some of these non-traditional regions face challenges in producing wines that consistently meet the consumers' quality expectations. These challenges may include sourcing high-quality grapes, consumer perceptions regarding the status of hybrid grapes, and securing the services of skilled winemakers (Pennell, 2019).

Appearance is one of the first characteristics of a products consumers notice, which is why color is one of the major attributes that affect the consumer perception of quality (Francis, 1995). Two classes of phenolic compounds, called anthocyanins and polymeric pigments, are responsible for the red-purple coloration of red wines (Sacchi et al., 2005). The original grape anthocyanins, which are responsible for the initial purple-

red color of young red wines, are displaced progressively during aging by more stable pigments through what are presumed to be irreversible chemical reactions. These more stable pigments are responsible for the brick-red color of the more aged wines (Vivar-Quintana, Santos-Buelga, & Rivas-Gonzalo, 2002). Anthocyanins and polymeric pigments can be found mainly in the hypodermal cells of the grape berry, which are a tightly-packed layer of flattened cells found just under the outer layer of grape berry skin cells (Guan et al., 2016). Maceration, which is the initial grape tissue degradation that occurs during early fermentation, begins the process of extracting these and other compounds from the hypodermal cells. Maceration, begins after the grapes have been destemmed and partially crushed to produce a material termed "must", which consists of partially crushed grape berries and free juice. Yeast is added to the must and consumes the sugars present to begin the initial fermentation. Digestive enzymes produced by the yeast break down grape berry tissues during maceration and the alcohol and other by-products produced from fermentation facilitate the extraction of phenolic compounds from the hypodermal cells (Pinelo, Arnous, & Meyer, 2006).

The purpose of this research project was to assess the winemaking potential of Rubaiyat, a hybrid grape novel to Oklahoma, as well as to examine some of the grape's physicochemical properties in order to build a foundation for future research and to explore the grape's possible uses in commercial winemaking. To accomplish this, the chemical composition of this novel grape variety was examined to determine must pH, titratable acidity, sugar content, and color density. These physical attributes are important because they directly contribute to the quality and consistency of finished wine products. Wine was also made and alcohol content, overall total phenolic content, and indirect

quantification of total anthocyanins and related pigments were conducted over time during the winemaking process. Total phenolics content measures not only the phenolic components but all of the reducing components *in vitro*. This is a standard assay performed to quantify total phenolics and, indirectly, the presumed total antioxidant capacity of a sample. Antioxidants are believed to provide potential health benefits and can react with damaging reactive oxygen species within the human body. By examining the specific anthocyanin and other phenolic components of this wine, we expect to obtain information that can be directly used for commercial winemaking to enhance color, flavor, and/or mouthfeel.

The following specific study objective were tested:

1. Examine the relationship between the amount of time the pomace is allowed to remain in the must – commonly termed skin contact time in winemaking – and the increase of color density, presumably due to anthocyanin extraction.
2. Quantify the total phenol/polyphenol content of the pressed wine and observe how the anthocyanins and other phenolic compounds interact with each other during fermentation.
3. Evaluate the color characteristics of the wine made from Rubaiyat grapes, to evaluate the cultivar's potential as a teinturier (coloring grape) in commercial winemaking.

CHAPTER II

REVIEW OF LITERATURE

Background

Wine is a fermented beverage that has been and is still consumed for religious and recreational reasons. Historically, the vast majority of wine was made from grapes and this is still true today with a few other variants. Grapevines and stems are referenced in the Bible and ancient Hebrew texts. Fossils found in Europe show that grapevines date back to the beginning of the Quaternary Age roughly 2.5 million to 11 thousand years ago (Goor, 1966). Wine residues found in Hajji Firuz Tepe, in the northern Zagros Mountains of Iran are suspected to date back to the mid-fifth millennium B.C.E. and through archeological records, wine is confirmed to date back more than at least 7.5 thousand years (R. S. Jackson, 2008).

Wine starts in the vineyard. Grapes must first be grown before they can be harvested and used to make wine. However, viticulture is not as simple as putting seeds in the ground and waiting for grapes to grow. Grapes require specific conditions in order to grow and to be of good winemaking quality. The term terroir is used to define an interactive ecosystem, which includes soil, climate, and vine (Seguin, 1988). Terroir relates the sensory attributes of wine to the conditions in which the grapes are grown, which is why it is an important concept in viticulture. There are a few conditions that fall under the scope of terroir. First, the precocity of the grapevine variety should be ideal for the surroundings to allow the grape to achieve complete ripeness by the end of the growing season. Secondly, for quality, the vine needs to experience conditions such as water deficit stress or low nitrogen availability in order to limit

yield and vigor (van Leeuwen, 2010). These conditions can be met through a multitude of avenues creating a great many possibilities for new wine development.

The terroir of Oklahoma is well suited for grape cultivation. However, there are a few hindrances that affect grapes in the state. There are native grape varieties that have been found in Oklahoma such as, *Vitis aestivalis* Michx. (summer grape), *Vitis mustangensis* Buckley (mustang grape), *Vitis riparia* Michx. (riverbank grape) and *Vitis rupestris* Scheele (rockgrape) (Munson, 1909). Due to the cold winters and hot summers, varietals in Oklahoma must have considerable cold and heat tolerance; they should ideally also have resistance or tolerance to diseases such as black rot, powdery mildew, and botrytis bunch rot (Stafne, 2006). In addition, Oklahoma has several varieties of soil from sandy to clay. The pH of the soil also varies within different regions of the state from strongly acidic to basic.

There are different techniques that growers use to circumvent these hindrances and allow for optimal maturation and vine vigor. Rootstocks are commonly used for these purposes. Grapes can be grown from their own rootstock as rooted cuttings, or scions may be grafted onto cultivated rootstocks of a different variety. Rootstocks can help with pest resistance, and aid in overcoming soil deficiencies. One drawback to the use of rootstocks is that that if grafted plants suffer freeze damage to below the graft union, the vineyard operator would have to replace or re-graft those vines (McCraw, McGlynn, & Striegler, 2005).

In addition to the challenges presented by climate and disease inherent to terroir, natural pests must also be taken into account when choosing varieties to plant. Animals, birds, and insects are common pests of grapes and vary depending on the location. In Oklahoma a multitude of birds, and insects feed on grapes and can damage grapevines. Some pests, such as phylloxera, can be deterred by using resistant rootstocks as mentioned earlier or with the use of chemical deterrents. The use of insecticides has raised concerns regarding the safety and overall

quality of the wine produced. However, studies have shown that the proper use of insecticides had no long term effect on the quality or content of wine after it had been produced. Winemaking, with or without maceration, has been shown to eliminate or significantly reduce the insecticide residue on processed grapes (Cabras et al., 1995). Predation by birds and animals may be deterred through the use of noise machines or other physical methods. Today, many consumers prefer to support the use of more natural deterrents such as falconry or nest boxes. Conversely, the use of live ammunition and chemical deterrents such as methyl anthranilate spray were more negatively received (Herrnstadt, Howard, Oh, & Lindell, 2016).

Grape Anatomy

Before conducting an accurate assessment of any particular grape varietal, one must have a basic understanding of grape anatomy and physiology. Grapes are berries; the definition of a berry is a fleshy fruit produced from a single flower containing one ovary (Creasy & Creasy, 2016). Examined from a macro perspective, a grape is made of three different parts, the skin, flesh, and seeds. The grape's skin creates a barrier against the diffusion of phenols and aromas (Pinelo et al., 2006). The exocarp (epicarp), or outer portion of the grape, is composed of a waxy layer called the cuticle, and the skin which incorporates the epidermis and hypodermis (Figure 1).

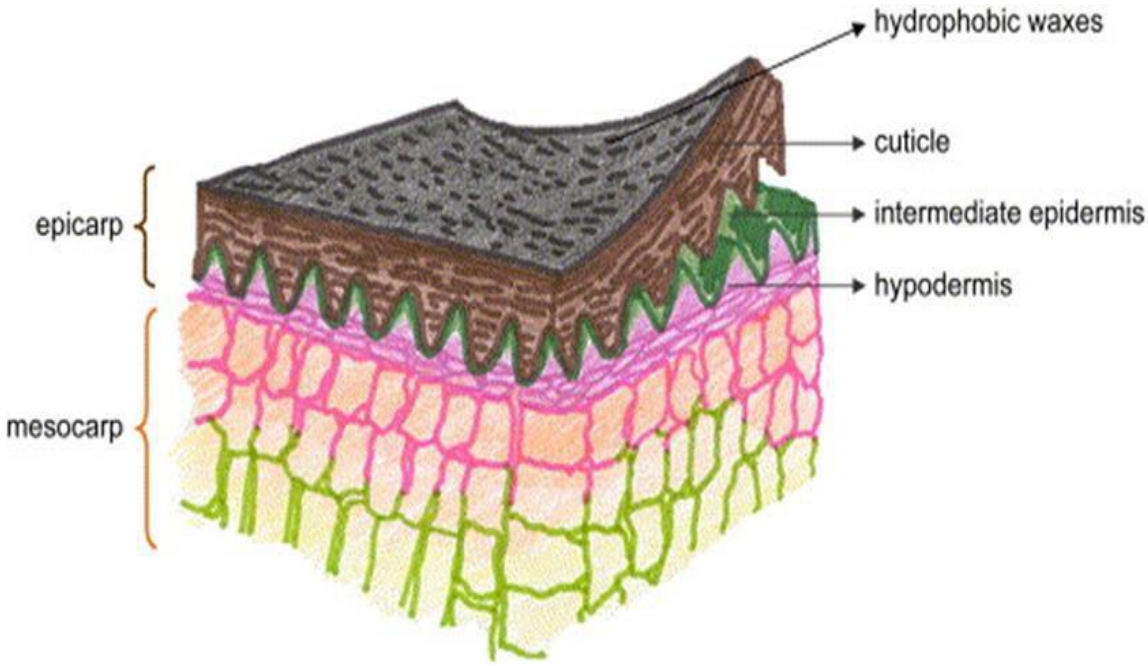


Figure 1: Cutaway diagram of the outer layers of a grape berry. (Pinelo et al., 2006)

The cuticle is the waxy layer on the skin of the grape and this layer consists of a structural component, a thin layer of hydroxyl-fatty acid esters, intracellular waxes, and a thin amorphous layer of complex long-chain lipids. This cuticle acts as a protective barrier against pathogens and it reduces water loss (Commenil, Brunet, & Audran, 1997). The epidermis contains stomata that facilitate gas exchange. However, it does not contain the same concentration of stomata as the leaves, so heat dissipation is limited, which can cause damage in hot weather conditions. The diffusion of water is also made difficult, thus grapes have a higher risk of splitting compared other fruits (Creasy & Creasy, 2016). Directly beneath the epidermis is a layer of varying thick cells called the hypodermis. This portion of the exocarp is where most of the phenolic compounds are found. The phenolic composition of wine is what determines the wine's color, sensory, and potential health-promoting properties. During the winemaking

process these phenols are extracted and the extent of extraction depends on how the phenols are bound to the skin (Pinelo et al., 2006).

While the grapes' skin contains most of the phenolic components, the flesh of the grape houses large vacuoles and these vacuoles are where sugar develops and accumulation occurs. Sucrose is degraded into glucose and fructose as the grape berry matures and these two monosaccharides are in the highest concentrations (Davies & Robinson, 1996). Organic acids and tannins are also found in the flesh, however they are considered to be insignificant due to their low concentrations (Conde et al., 2007). There are specific verities of grapes that also have some phenolic compound accumulation in the flesh of the grape as well. This causes the flesh to turn red or have a reddish hue (Guan et al., 2016).

Within the flesh of the grapes are the seeds. Typically, there are 0-4 seeds per grape and this can vary based on the success of fertilization, which heavily relies on the environmental and nutritional conditions at bloom time (Kennedy, 2002). The seeds are comprised of a coat, endospore, and embryo. The coat is a protective layer, and the endosperm comprises the bulk of the seed and provides nutrients to the embryo during maturation (Kennedy, 2002). As for winemaking, the seeds are known to contain tannins. The most prominent tannin is catechin which comprises roughly 50% of the total grape seed's tannin content (Guendez, Kallithraka, Makris, & Kefalas, 2005).

Molecular Composition

Each grape varietal contains unique concentrations of various chemical compounds and it is up to the winemaker to choose a grape that will produce the desired wine. Red grapes differ from white grapes by the color of their skins; this color stems from their different chemical compositions. Red wine grapes are the focus of this study.

As discussed previously, grape skins contain the majority of the phenolic compounds found in the grape berry. Phenolic compounds are classified as compounds that incorporate a phenol group, which is an aromatic organic compound that has the molecular formula C_6H_5OH (Rice-Evans, Miller, & Paganga, 1996). Phenolic compounds of wine fall under two classifications, flavonoids and nonflavonoids. Nonflavonoids include compounds such as hydroxybenzoates and hydroxycinnamates. Flavonoids include flavonols (e.g. quercetin, myricetin), flavan-3-ols (catechin, epicatechin) as well as flavan-3-ol derivitaves such as procyanidins, and anthocyanins (Minussi et al., 2003). Flavonoids are formed from the aromatic amino acids tyrosine and phenylalanine, with the addition of malonate. The basic structure consists of 15 carbon atoms arranged in three rings, these rings are known as A, B, and C rings. The various classes of flavonoids differ in the level of oxidation as well as substitutions within the three rings (Pietta, 2000).

Flavonoids are thought to be responsible for the phenomenon known as the "French Paradox". The paradox was the observation of low coronary heart disease among the French despite a diet of foods with high cholesterol and saturated fats (Ferrières, 2004). The hypothesis that flavonoids are responsible for this observation has not been definitively proven. However, flavonoids have the ability to act as antioxidants by free-radical-scavenging (Minussi et al., 2003). There have been many studies that have shown antioxidants have the capacity to prevent health issues such as coronary heart disease (Estruch, 2000). Free radicals known as reactive oxygen species (ROS) are very reactive and rapidly attack nearby cells (Pietta, 2000). This is one of the reasons why wine has been researched so extensively and is understood to benefits ones' health, when consumed in moderation.

Another subset under the classification flavonoid are the anthocyanins. The concentration of anthocyanins present represents the main difference between the chemical compositions of red and white grapes. Anthocyanins are the pigments that produce the red color

of grapes and are the foundation of the red-brown pigments in aged wines. Figure 2 shows basic anthocyanin structure. There are many species of anthocyanins that vary by nature of the substituents on the B-ring as well as the number and presence of residual sugars (Ribéreau-Gayon, 1982).

Anthocyanins are one of the two chemical components that have been identified as the largest contributors to overall wine quality, with tannins being the other major component. Tannins are amphipathic molecules having both hydrophobic aromatic rings and hydrophilic hydroxyl groups (Adams & Scholz, 2008). It is well understood that tannins are responsible for the astringent or dry feeling from drinking wine. This is caused by the hydrogen bonding and hydrophobic interactions that inhibit salivation, which causes the sensation of oral astringency (Hanlin & Downey, 2009).

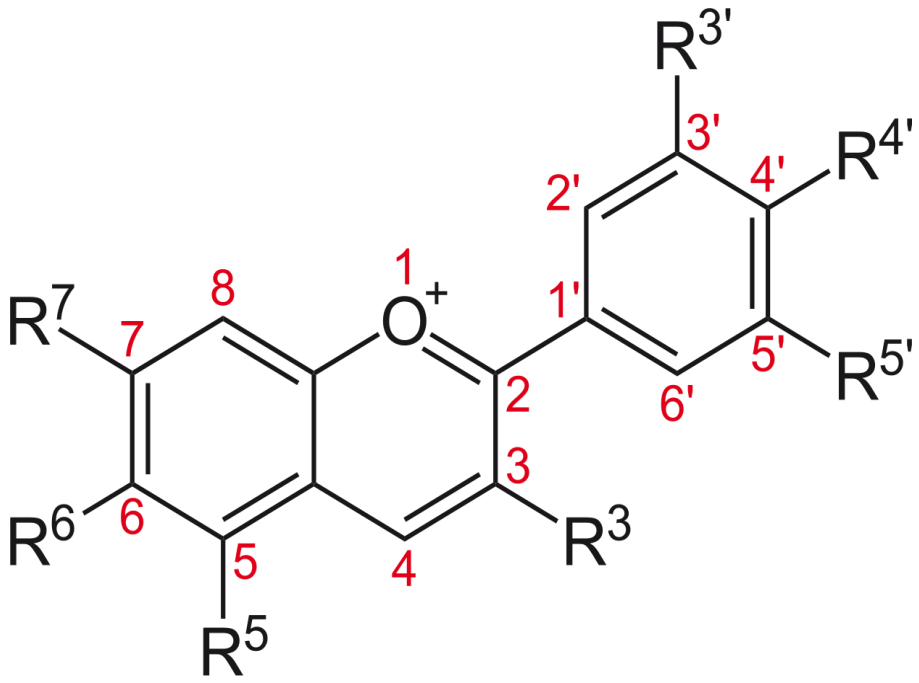


Figure 2: Basic structure of an Anthocyanin (NEUROtiker / Public domain)

Relevance of Anthocyanins

It was noted above that that anthocyanins are the majoring contributors to the familiar hues of red wine. Anthocyanins are water-soluble and are found within the hypodermis of the grape skin and in the flesh of specific varietals. Anthocyanins are extracted from the epidermal cells in the early stages of fermentation. To be more specific, maceration is the point when phenolic compounds are extracted from the grape skins. After the grapes have been destemmed and crushed, the must and skins are left to sit for a specified amount of time (Romero-Cascales, Fernández-Fernández, López-Roca, & Gómez-Plaza, 2005). The amount of time depends on the desired flavor and color profile of the wine. As the winemaking process continues and the wine ages, anthocyanins and other phenolic compounds undergo structural transformations via oxidation and condensation reactions (González-Manzano, Rivas-Gonzalo, & Santos-Buelga, 2004). These transformations are generally unavoidable and to some degree desirable. Interestingly, anthocyanins are inherently more stable at lower pH due to the natural activity of organic acids (Somers, 1971). Many studies have been done to try and slow the degradation of anthocyanins and the other phenolic compounds. Winemakers are always interested in maintaining and extending wine stability in order to preserve a wine's desirable sensory attributes and extend its window of market acceptability.

Color additives are widely used in the winemaking industry today to achieve the desired color attributes. The additive that is the most commonplace for these purposes is known as "Mega Purple", which is a grape concentrate used to enhance color and mask the flavor of pyrazines. Made from rubired grapes and saturated with sugar. Mega Purple is also used to round out the flavor of a wine that does not have a desirable body. Rubired grapes are known as teinturier grapes, or grapes that have greater concentrations of deep color pigments (Bell, 2016, August 26). Mega Purple is typically used for cheaper commercial wines to make the final product look as if it is of higher quality. However, it is not possible to know which winemakers

are using Mega Purple because it is not required for them to label its use. There is a negative stigma about Mega Purple because it is seen as a cheap way to mask a poor product or simplify a complex wine so that it is more appealing to a wider range of consumers. Instead of using Mega Purple, a more traditional method of enhancing color employed by some winemakers is to simply blend the juice from teinturier (coloring) grapes into their wines. This has the effect of giving wine improved color without relying on a wine additive as such.

Pigment Analysis

Anthocyanin pigments are well understood to contribute to the specific perception of color we see when we observe a wine sample. It is possible to quantify the intensity of color, degradation of color, and other biochemical traits or changes having to do with pigments using a spectrophotometer. Spectrophotometry is a quantitative measurement of the interaction of ultraviolet, visible, and infrared radiation. There are several modes of spectrophotometric measurement, these include absorption, reflectance, transmittance, emittance, scattering, and fluorescence (Germer, Zwinkels, & Tsai, 2014). The principle of spectrophotometry is the basis of colorimetry. Colorimetry is used to determine the concentration of color compounds in solution by application of the Beers-Lambert Law. This law states that the absorption of the solution is equal to the molecular extinction coefficient multiplied by the concentration or molarity and the path length of the beam of light passing through the solution. There are three phases of colorimetry: color matching; color difference evaluation; and prediction of color appearance (Choudhury, 2014). For the first two phases, a pair of specimens are compared for relative difference in color while being viewed in the same conditions, color values are quantified using color difference formulas that are typically automatically calculated by electronic colorimeter instruments. This is possible because various colorimetric attributes can be derived from various spectra measured under controlled lighting conditions. Because the lighting conditions must be identical in order to derive useful spectra data, one cannot compare

the appearance attributes of an object under two light sources (Choudhury, 2014). In order to compare similarities or differences the experimentation must be performed under one light source and in specific conditions. Even so, color matching and differentiation are difficult because the complexity and subjectivity of color perception. To remove as much subjectivity as possible, researchers have derived numeric interval scales for hue, and ratio scales for brightness, lightness, colorfulness and chroma (Heckaman & Fairchild, 2006). Applying these scales to the evaluation of wine samples allows enologists to quantify color without subjectivity. This allows for consistency, reproducibility of product, and a more empirical avenue of developing new colors or enhancing an existing wine. From a marketing standpoint, this is important because color is a large aspect of the quality and consumer perception of wine.

History of Grapes and Wine in Oklahoma

Oklahoma has a climate that can be quite favorable for growing certain varieties of grapes. The number of grapes grown in the region in 1907 and 1908 was estimated to be 1501 ha and 2195 ha (OGGWMA, 2005). Specific varieties of grapes were successfully grown in Oklahoma. However, due to the climate, it was difficult for grape growers to maintain a multitude of other varieties and species. The grape and wine industry started to significantly decline around the early 1920s. This decline was due to the introduction of prohibition and other laws such as the "Bone Dry Law" (Brown, 1974). After the beginning of prohibition came the Dust Bowl and following that came the Great Depression, which all had a hand in the decline of grapes and grape products in Oklahoma. Around 1933, Oklahoma A&M University started to conduct research on American and French grape varieties (Stafne, 2006). Herman Henrichs, a horticulturist at Oklahoma State University, introduced several cultivars in the 1970s. These were all the products of the grape breeding research being done at OSU. The grape industry was stagnating in the 1980s. However, research was still being conducted (Stafne, 2006). Nothing had significantly changed until the steady rise of grape production that occurred during the mid-

1990s. This increase of grapes led to more wine and in turn led to the increase of vineyards in Oklahoma (Stafne, 2006). In around 1997 there were 170 acres of grapes being grown in the entire state of Oklahoma. The number increased to 375 acres in 2002 and 597 acres in 2007 (USDA, 2009). As the years progressed there was a second decline in the number of grape-producing acres in the state. The total number of acres dropped in 2012 to 456 but has seen another rise to 556 in 2017 (USDA, 2017).

Many species of grapes are grown and used in Oklahoma but the majority consists of the traditional European species, *Vitis vinifera*. Despite this fact, there are other hybrids that have been seeing an increase in plantings and have the potential to be developed further. Approximately 80% of grapes grown in Oklahoma are the European, *V. vinifera,* and hybrids make up approximately 15%. Red grapes are preferred by growers with 60% of total acreage consisting of red grapes (GRAPES, 2019). The most popular grape grown in Oklahoma is the European Cabernet Sauvignon cultivar. Following Cabernet Sauvignon in terms of acres planted are Merlot, Shiraz, Riesling, Chardonnay, and lastly Zinfandel. Other plantings include both hybrids and other American grape species, but the previously stated varieties are the most abundant (GRAPES, 2019). All of these cultivars are being grown in the state currently. However, it is arguable that the majority of the European grapes are being grown based on their familiarity to consumers and the industry and not because they are especially well suited to Oklahoma's climate.

Rubaiyat

The grape of interest for our research has been named Rubaiyat. This cultivar was developed by Herman Henrichs during his breeding experiments. Rubaiyat is a grape that was developed by crossing between two different grapes, Seibel 5437 and "Bailey." Seibel grapes were classified as a group of varying wine grapes developed by the French horticulturist Albert

Seibel. Although not commonly used as the primary grape to make wines, they are still used commonly as blended grapes (Robinson, 1986). Seibel 5437 specifically was a black grape with medium-sized clusters and berries. In addition, Seibel 5437 was cold hardy and disease resistant with good vigor (Stafne, 2006). "Bailey" is also a black grape with medium to large clusters and was developed by T.V. Munson, who was a grape breeder active in Texas during the late 19th and early 20th centuries (Stafne, 2006).

Rubaiyat also has some notable progenitors, such as the "Concord", "Muscat of Alexandria", and "Alicante Bouschet". The overall genetic make-up of Rubaiyat is 37.5% *V. lincecumii,* 31.25% *V. Vinifera,* 18.75% *V. lambrusca,* 6.25% *V. rupestris,* and 6.25% *V riparia (Stafne, 2006).* The initial cross between Seibel 5437 and "Bailey" was in 1952. Shortly after the seed was germinated in 1953 and grown in a nursery for two years. It was then planted at the Pecan and Fruit Experimentation Station, Perkins, Oklahoma in 1955. The first fruit appeared in 1957 (Stafne, 2006).

Rubaiyat is a dark-colored grape that can be described as having deep blue, almost black pigments. The clusters are medium in size with a long shoulder and short to medium shank. Rubaiyat ripens around mid-August, however it may be left on the vine for sugar accumulation if needed. The vine and berries are cold hardy, have good to very good disease resistance, and possess medium vigor. Given that the skin and flesh of this grape have strong dark pigments, the juice also is typically dark and has a deep red color.

As noted above, the majority of the grapes grown in Oklahoma are *V. vinifera* cultivars, Cabernet Sauvignon, Merlot, Shiraz, etc. These grapes are grown for their notoriety and popularity. However, they are not necessarily best suited for the growing condition in Oklahoma. Hybrids, such as Rubaiyat, can offer better cold resistance and disease resistance than the *V. vinifera* cultivars. In addition, Rubaiyat was developed to be grown in Oklahoma so

the vineyards in the state may find the vines easier to manage. Hybrids may also be useful and beneficial as blending grapes. Blending grapes can add complexities to the finished wine product and because Rubaiyat has such deep pigments, it could possibly make up for a product that lacks pigments or be combined with other wines in a blend to create a product with a more intense and complex color.

Winemaking Process

Wine is a fermented fruit beverage that has been a part of a variety of different cultures throughout history. Typically, the majority of wine has been made from grapes however nearly any fruit can be made into wine. Wine grapes are known as such because they are specifically grown to be used for making wine. The bulk of red wine grapes are grown between the 30 and 50 degrees north and south latitudes. This is because red wine grapes require specific climates and conditions to grow effectively and those regions are in general found between the 30 and 50 degrees north and south latitudes. Through the growing process, grapes need to be carefully monitored to reduce the risk of losing a crop to predators and weather changes. Best growing practices and the ability to survive adverse weather events vary among grape varieties.

Wine grapes are harvested when they have met the grower's specific desired qualities. For example, red wine grapes can be harvested when they reach a specific degrees Brix, pH, color, taste, size and total acidity. There is a rather consistent range of time for each varietal to reach the typical levels that will produce the highest quality of wine. However, some grapes will occasionally have to be left on the vine in order to accumulate more sugar and doing this will reduce the acidity. Once the desired conditions have been met the grapes are harvested and weighed. Wine grapes are not typically washed after they are harvested because the natural microbiota, yeast most importantly, will be removed and this can affect the fermentation process even though additional yeast is added (Cavazza, Franciosi, Pojer, & Mattivi, 2007).

Some winemakers also believe that washing grapes will reduce the sophistication of the finished product. If pesticides are used, as long as the harvest is done after the allotted pre-harvest interval the chemicals will not affect or linger in the wine during or after production (Herrnstadt et al., 2016). Pathogens are not considered an issue due to wines low pH, alcohol content, and high organic acid content (Waite & Daeschel, 2007). After harvest, the red grapes are then destemmed and crushed. The must and skins are transferred into fermentation vessels and here is where the fermentation process begins.

Yeast is added to the fermentation vessels along with the skins and must. The levels added and type of yeast depend on the winemaker's preferences or desired results. At this point, the red grapes begin the first fermentation step in the winemaking process. As the must and skins rest in the vessel, polyphenolic compounds, tannins, and color compounds are extracted. This step is known as maceration. When grapes are first crushed the color of the must is clear or greyish, with the exception of teinturiers, and the color will depend on the concentrations of anthocyanins extracted from the grape skins. It is a common misconception that white wine is only produced from white grapes. Red grapes are also used to make white wine. When using red grapes to make white wine the winemaker either avoids maceration entirely or heavily limits the time the skins are in contact with the must. Rosé is a style of wine that is a hybrid of white wine and red wine. In order to achieve Rosé, maceration may be extended but not to the extent of red wine production. The total time of maceration depends on the winemaker. The longer the maceration period the more robust the wine. However, longer maceration times typically result in long ageing times so that the wine has time to age and round out the robustness from maceration. A wine that is made to drink as a young wine will have a shorter maceration time. The color of the wine is pigment dependent so the amount of time to extract amount of pigment from the skins varies. A skin contact time of 4-7 days is usually enough time for the wine to reach its deepest color.

During maceration, the first round of fermentation begins. Fermentation or ethanol fermentation is a process that occurs in anaerobic conditions; it involves the conversion of simple sugars to CO_2 and ethanol. The sugar content of the grapes is important for this step because it is the rate-limiting factor for fermentation. Once all of the sugar has been used, fermentation will stop. If the grapes do not have a high enough sugar content, winemakers will add sugar. Fermentation is a biochemical pathway that is utilized when the organism, such as yeast, does not have access to oxygen. Glycolysis is not technically a part of fermentation. However, they work together in this situation. Glycolysis is a pathway that breaks down glucose and converts it into pyruvate. In glycolysis, there is also a net gain of 2 ATP and 2 NAD^+ (Fothergill-Gilmore & Michels, 1993). ATP is used as energy for other pathways and NAD^+ is an electron carrier that donates or accepts electrons. Glycolysis is not as efficient for ATP production as aerobic respiration. However, it is enough for yeast to survive until oxygen becomes available. After glycolysis, in anaerobic conditions, pyruvate will be used in the fermentation pathway to produce ethanol and CO_2. Fermentation also uses the NAD^+ from glucose and reduces it to NADH. NADH is then recycled and used for glycolysis to again, produce NAD^+. Fermentation is an exothermic reaction and this is important to note as it affects the final quality of the wine (Katahira, Mizuike, Fukuda, & Kondo, 2006). During maceration, the winemaker may choose to "punch down the caps". This refers to pushing the grape skins down below the surface of the must. The grape skins rise due to the CO_2 that is produced during fermentation. This is done to mix the must and get the skins more must contact but more importantly to release the trapped heat produced during fermentation. If this is not done the wine may develop undesirable cooked flavors. Off-flavors may also be generated by spoilage organisms that can grow in the undisturbed cap.

After maceration has concluded, nearly all of the free sugar will have been metabolized. The young wine is then pressed out of the skins and remaining must and moved to

settling/fermentation vessels, or sometimes bottled. It is common for winemakers to use oak or other wood barrels for aging, this is to add complexities to the finished product flavor and is not a necessary part of the winemaking process. While the wine is aging, precipitates will start to form and fall to the bottom of the container. These will be composed mostly of dead yeast cells but will possibly include organic acid salts and other complexes of tannins, proteins, and the like. This sediment falls to the bottom of the vessel and the wine atop is decanted off periodically and moved to another container leaving the sediments behind. The sediment is referred to as "lees" and the process of transferring the wine to another container is termed "racking the wine"

Red wines may also undergo a second round of fermentation during aging, called malolactic fermentation. This fermentation is facilitated by lactic acid bacteria and it converts malic acid into lactic acid. This conversation does reduce the tartness or astringency of the wine and is common in making red wine. The conversion of malic acid to lactic acid is facilitated by malolactic enzymes and is simply the decarboxylation of L-malate to L-lactate (Bauer & Dicks, 2004).

It is also standard practice in winemaking to add sulfur dioxide, and this is often done at the beginning of fermentation, periodically during aging, or prior to final bottling. The added sulfur dioxide serves a number of functions depending on the timing and amount added. It may be used in sufficient concentrations to kill yeast and halt fermentation. Sulfur dioxide also aids in preventing the wine from spoiling during aging by inhibiting wild yeast and spoilage bacteria. Finally, sulfur dioxide can also increase the stability of the wine pigments. Wines color may fade as it ages and this is due to anthocyanins reacting and binding with other phenolic compounds. Sulfur dioxide can slow this process.

Wine Quality

Wine is a difficult product to grade or classify as a specific quality due to the many subtle factors that can influence the wine's attributes. Typically, market price is what is used for the average consumer to determine the quality of a wine. However, price is not necessarily a reliable indicator of quality because it can be influenced by fashion, tradition, availability and personal preferences (D. Jackson & Lombard, 1993). At its core, wine is composed of mostly water. To be more specific, water accounts for 75 to 95% of a wine's total volume. The varying 15 percent is dependent on the concentrations of alcohol, organic acids, phenolics, pectins, and mineral salts, and these can differ from wine to wine (Conde et al., 2007). All of the factors that can influence wine quality, and the degree to which these factors contribute to a wine's perceived quality, remain the subjects of ongoing research.

Grapes are well suited for wine production. Some of the factors that make grapes natural wine precursors are their high accumulation of sugars that provide nutrients for natural yeast to grow and their high concentration of acids to reduce or limit the growth of harmful pathogens. The high natural sugar content leads to high alcohol contents after fermentation and this also serves to limit the growth of pathogens and spoilage organisms and thereby helps to produce a product with a long shelf life. Lastly, the chemical properties of grapes also impart a unique complex of aromas, colors, and flavors to the finished wines (Kunkee, 1991). Thanks in part to grapes' natural physico-chemical characteristics, they will naturally ferment into wine by simply crushing them in a container, as long as the environment around the container is acceptable. Thus, it is entirely possible that our ancestors discovered wine by accident. However, it occurred, since wine first became a part of society people have been investigating techniques to improve wine quality. Today we know that quality largely depends on soil composition, growing climate, growing season, light, microorganisms on the grapes surface, and human interaction (Kunkee, 1991). Year by year grapes can be grown in the same region, but it takes intense supervision and consistent human interaction to ensure that a consistent

product is produced. Through this research, we hope to further extend our knowledge of how to grow better grapes and make better wines. Due to the nature of our research as highlighted in the research objectives presented above, throughout this paper we will focus primarily on red grape and red wine quality characteristics, especially those related to color.

CHAPTER III

METHODOLOGY

Rubaiyat Harvest and Storage

Rubaiyat grapes are not grown commercially and were developed by Herman Hinrichs in 1952 at Oklahoma State University by crossing two grape hybrids. The grapes used in this study were collected on August 22nd, 2019 from the Cimarron Valley Research Center located in Perkins, Oklahoma (see Figure 3). The harvest was limited because a portion of the vines did not accumulate as much sugar due to a red blotch virus and there was a slight issue with damage from birds and Green June Beetles. Approximately 90 kg or roughly 200 pounds of Rubaiyat grapes were harvested. Degrees Brix was measured before harvest to determine sugar accumulation. All of the grapes were handpicked and placed into polyethylene bags. After all of the grapes were harvested, they were immediately taken to the Rober M. Kerr Food & Agricultural Products Center in Stillwater, Oklahoma and stored in a -10 freezer.

Figure 3: Rubaiyat grapes collected from the Cimarron Valley Research Center in Perkins, Oklahoma

Must Adjustments

The grapes were removed from the freezer and given time to thaw a day before they were destemmed and crushed. The crusher/destemmer (Enoitalia model ENO 10, VIA Provinciale Pisana 162 - 50050 Cerreto Guidi (FI)) destemmed and slightly crushed the grapes to produce the initial run off of juice. The juice and grape material, or must, was then combined in a stainless steel collection vessel. The must was adjusted to meet specific parameters for quality and consistency. First, a small amount of SO_2 was added to aid in the control of microbial agents and color stabilization. The SO_2 added was at industry concentrations of 50ppm and not in a high enough concentration to affect the wild yeast. The sugar concentration of the must was determined using an Anton Paar, Abbemat 200 refractometer, (Anton Paar, Ashlan, VA). The general equation that was used to adjust the Brix was S=.125(v)(B-A) where S is grams of sugar needed, v is the volume in liters of juice, B is desired Brix, and A is actual Brix. Lastly, the pH was

adjusted to approximately 3.36. Tartaric acid was used to adjust the must to the desired pH.

Preparation of juice samples

Once the adjustments were made the must was mixed until it was roughly homogenous, the must was distributed evenly between 4, five-gallon buckets. These fermentation vessels were fixed with airtight lids and airlocks. Each vessel was stored in the same area of the wine lab at Oklahoma State University and at the same temperature of 23℃ to limit the possibility of sample variation.

Pitching Yeast

Pitching yeast is the process of adding yeast to the must. The yeast used in this experiment was Lalvin ICV-D47 wine yeast (*Saccharomyces cerevisiae*) and stored in a 41-44℃ refrigerator prior to use. Hydration of the yeast was started after the must adjustments had been made and the yeast was pitched immediately after the hydration process. The hydration process consisted of adding a single packet of yeast to 50mL of water at 35-37℃, gently stirring the mixture to avoid clumping, then allowing the mixture to rest for 20 minutes. A single 5g packet of yeast was hydrated and added to each of the five-gallon fermentation vessels.

Fermentation

The fermentation process used for this experiment was separated into two stages. The first stage lasted for 8 days in order to ensure adequate fermentation and extraction. During fermentation, a significant amount of gas was being produced and the evolution

of CO2 was visibly aggressive. The first stage of fermentation was deemed complete through visual inspection when the gas production had for the most part halted completely. The fermentation was done in five-gallon plastic buckets secured with airlocks and sealed with Parafilm®. Every day during the first stage of fermentation, the grape skins, seeds, and other material rose to the top of the juice due to the gas that was being produced. This “cap” was pushed down daily to reintroduce the solids into the juice (Figure 4).

Figure 4: Before and after the caps were punched down

The second stage of fermentation began after the grape skins were pressed and the juice was moved into 5-gallon glass carboys (Figure 5). Potassium metabisulfite was added at a rate of 1.47 grams per five gallons of wine following the pressing of the wine off of the skins. Potassium metabisulfite was added according to common winemaking standards so as to not interfere with the yeast but also aid in the inhibition of microbial growth. This stage of fermentation lasted 4 weeks and it was important during this step to limit oxygen and allow for the yeast to precipitate out of solution. Due to the lack of

sugar and high levels of alcohol, the yeast began to die and form what is referred to as "lees" or dead yeast and other precipitates. The wine was racked off of the lees to prevent off-flavors and bacterial growth. This was accomplished by using a hand pump siphon to decant the wine from the lees resting at the bottom of the carboy. For each 5-gallon carboy, this racking process was done once per week throughout this stage of fermentation (Figure 6).

Figure 5: Resting containers with airlocks

Figure 6: Racking the wine with a siphon

Sample Collection

Sample collection started on the day of crushing after the must adjustments and yeast had been pitched. This was done by using a pipette to fill seven 4mL amber vials and one 15mL plastic conical tube from each of the 5-gallon buckets used throughout maceration. All samples were dated, labeled, and sealed with Parafilm®, then placed in a -10 freezer until they were used for further analysis. This process was repeated every day throughout the 8 days of maceration/first stage of fermentation. After the skins had been pressed and the wine was moved into the second stage fermentation vessels, the sample collection interval increased to once a week rather than once a day, following the same sample collection procedures.

pH

Must/wine pH was determined using an Accumet, AB 15 pH meter, (Buffalo, New York) using the protocol provided by the manufacturer. The standardization was also done following the protocol from the manufacture. The pH was recorded for each fermentation vessel in duplicates on day 8 and 36 after the grapes had been crushed.

Titratable Acidity

The titratable acidity was measured on day 8 and 36 after the grapes had been crushed. The titratable acidity was measured manually. In a 250mL beaker, 125mL of deionized water was added and then to that 5mL of the wine sample was introduced. A magnetic stir bar was added to create a steady vortex, while the pH probe was placed in the diluted sample. The pH was recorded and the dilution was titrated to a pH of 8.2 with 0.1 N NaOH. The volume of NaOH added was recorded in mL and using the formula:

% tartaric acid (1g/100mL) = [((mL of NaOH added) (.01 N NaOH) (milliequivalent factor of tartaric acid)/ (mL of wine)) (100)

Two duplicates were taken per fermentation vessel and the results averaged.

Percent Alcohol

The alcohol content was determined on day 36 after the grapes were crushed using an Anton Paar, Alcoholizer Wine M, (Ashlan, VA). The sample was prepared in 50mL beakers and covered until they were ready to run through the alcoholizer. Approximately 40 mL of wine was used during alcohol determination. Between each

sample, the alcholizer was rinsed with deionized water. The samples were taken in duplicates and the reading were averaged.

Color Density

The color density of the wine was determined for each of the fermentation vessels. Samples were taken every day throughout maceration which included day 0 - day 8. Day 0 represented the day that the grapes were crushed and the must adjustments had been made, Day 8 refers to the day wine was pressed off of the grape skins. Color density was also determined for day 36. All of the readings were taken using a Spectramax M3 Plate Reader. Clear bottom 96 well plates were loaded with 200µL of filtered and diluted samples, duplicates were loaded, measurements were taken at 420nm and 520nm, and then the results were averaged for each different wavelength. For all of the trials, DI water was used for a blank (Figure 7).

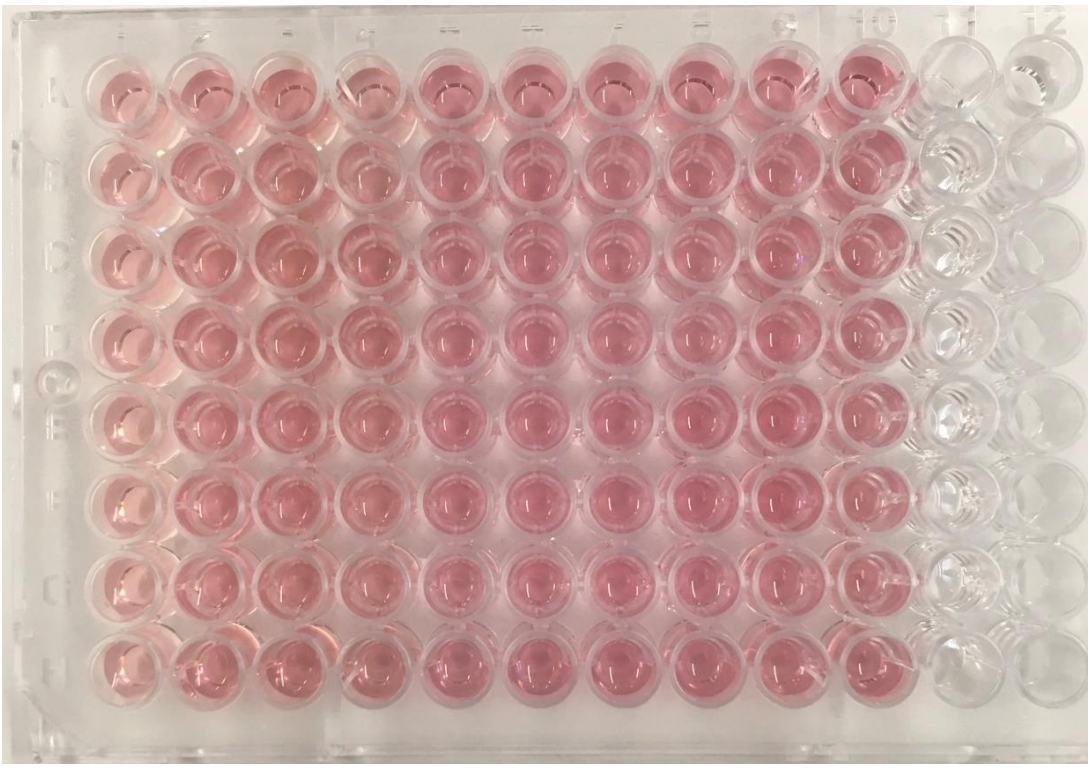


Figure 7: Well-plate for color density

In order to account for turbidity, three different methods for removing particulate were tested and compared to test effectiveness. The first method included using cheesecloth to filter the wine samples and then the samples were left at room temperature and the supernatant was decanted off of the settled particulate. The supernatant was then diluted and the samples were loaded into the wells of the plate (see figure 1 and table 1 in the appendices). The second method including filtration with cheesecloth and the centrifugation for 1 minute at 5000 rotations per minute to facilitate the separation of supernatant and participate. The supernatant was then decanted, diluted and then loaded into the well plate (see figure 2 and table 2 in the appendices). Lastly, the samples were filtered with a .45-micron syringe filter. The filtered wine samples were then diluted and

loaded into the wells of the plate. Due to the lack of accuracy with the first two methods, the .45-micron filter was used for all of the color density testing.

Folin-Ciocalteu Assay

The Folin-Ciocalteu assay was used to determine the total reducing capacity of Rubaiyat wine. This assay is a colorimetric reaction that uses gallic acid as a reference to determine the total reducing capacity of a sample.

Reagents

Folin-Ciocalteu (FC) reagent, sodium carbonate, and gallic were purchased from Sigma-Aldrich (St. Louis, MO). The sodium carbonate was used to create a 20% stock solution.

Sample Preparation

Samples of wine were taken from each of the 4 different fermentation vessels. The wine used in this assay was taken from days 15, 22, 29, and 36 after the grapes had been pressed. Four milliliters of wine from each of the sampling days were filtered through a .45 micron syringe and diluted by a factor of 10 in preparation for further analyses.

Procedure

The standard curve was prepared by creating stock solutions of gallic acid at specific concentrations in order to use as a reference to determine the GAE or gallic acid equivalent for the wine. The stock solutions were prepared in ascending concentrations of 0g/L, 1g/L, 2g/L, and 3g/L of gallic acid respectively. Two milliliters of FC reagent and

500µL of stock gallic acid solution were combined and vortexed in a 15mL conical vial. This mixture was allowed to sit for 3-5 minutes and then 2mL of the 20% sodium carbonate solution was introduced. The subsequent reaction was light sensitive; thus the final solution was incubated at room temperature for two hours without light. After incubation, 1mL from each of the different concentrations were transferred into microfuge tubes and spun at 10,000rpm for 3 minutes to remove any possible salt that had formed. Then, using a microplate reader the absorbance was determined at 765nm. All of the standards were done in duplicate and the results were averaged. The standard curve was prepared in Microsoft Excel (Excel version 2016).

The protocol for the wine is identical to the gallic acid standard curve. However, the assay was done again on a separate day, also in duplicate, for repeatability. After the absorbance was determined, by using the slope of the gallic acid standard curve the concentration of GAE was determined for the wine samples.

Statistical Analyses

Data were analyzed using either paired t-tests in Microsoft Excel (Excel version 2016) or by using the GLM analysis of variance procedure of the SAS/STAT software (Version 9.4 of the SAS System for Windows. Copyright © 2016 SAS Institute Inc., Cary, NC, USA.); means were separated using the Duncan means separation test at a significance value of 0.05.

CHAPTER IV

RESULTS AND DISSCUSION

Wine pH

The mean pH values were taken on the day the grapes were destemmed and crushed (Day 0), as well as the last day of maceration when the must was pressed (Day 8), and one month after the end of maceration (Day 36) are shown below in Table 1**.** The mean pH values shown in Table 1 represent the average pH of all the fermentation vessels for that particular day. There was a significant difference observed from Day 0 to Day 36. The p-values corresponding to days 8 and 36 were .112 and .023, respectively.

Table 1. Average pH of all wine samples for that day (n=4)

Day of Sample Collection	Day 0	Day 8	Day 36
pH ± Std. Dev	3.38±0.015	3.41±0.025	3.42±0.017

This increase in pH from Day 0 to Day 36 could be due to the loss of potassium ions from the skins of the grapes during the maceration process. Grapes with higher potassium concentrations have shown to produce wines with higher pH values (Somers, 1975). Or the degradation of strong acids during the fermentation process may have also had an effect (Boulton, 1980). There are also other possibilities as to why there might be an increase to pH during the winemaking process, however, the observed increase in pH was not investigated due to the practical insignificance of the variation.

Wine pH affects the quality and microbial stability of wine and should be monitored throughout the winemaking process. One phenomenon that pH affects is termed copigmentaion. This is the phenomenon of free anthocyanins or pigments and other noncolored organic components forming molecular associations or complexes (Boulton, 2001). This phenomenon has a direct influence on the overall color of wine and mouthfeel due to tannins binding with pigments. One previous study showed that a higher pH resulted in a loss of color intensity and increased browning during storage for nine months whereas wine with lower pH had a greater loss of free, unpolymerized anthocyanins, which indicated greater polymerization in lower pH wine (Sims & Morris, 1984). Due to this, one goal during this research was to maintain a wine pH that was low enough while still being in the appropriate range of typical red wines. Typically, the wine will fall in the pH range of 3-4 with white often having a lower pH than red wine. Red wine will typically fall within the pH range of 3.3 to 3.6.

Wine Titratable Acidity

The mean percentages of titratable acidity in Rubaiyat wine were calculated for samples taken on the last day of maceration (Day 8) and one month after the end of maceration (Day 36). Table 2 shows the average of the titratable acidity measurements taken on those particular days and the corresponding standard deviation. Statistical analysis revealed a p-value of 0.000006, indicating that there is a significant difference between the titratable acidities of the samples.

Table 2. Average titratable acidity of wine for that day (n=4)

Day of Sample Collection	Day 8	Day 36
% Titratable Acidity ± Std. Dev	1.02±0.02%	0.89±0.02%

These results were largely expected. It is known that the titratable acidity of the wine will fall due to precipitation of acids via the binding of tartaric acid with the "lees" (Robinson & Harding, 2015). Tartaric acid is the most predominant organic acid in wine and that is why titratable acidity is typically represented as g/L tartaric acid. Organic acids have a critical role in the winemaking process, including quality and the physical, biochemical, and microbial stability of wine (Volschenk, Van Vuuren, & Viljoen-Bloom, 2006).

The typical titratable acidity of the red wines varies from 0.60-0.70% acidity (Boulton, 1980). The wine that was produced from the Rubaiyat grapes in this study had a significantly higher average titratable acidity, which is common among hybrid grapes. However, there has not been enough data collected from Rubaiyat wine over different growings seasons to confidently suggest this would always be the case. More research is required to determine if these levels of titratable acidity will be consistent over multiple growing seasons.

Wine Percent Alcohol

The percentage of alcohol by volume was taken twice and then averaged for each of the fermentation vessels used for this study, as shown in Table 3. The samples were taken at one month after the end of the maceration (Day 36). Percent alcohol was taken

once the large majority of sugar had been metabolized by the yeast and the initial fermentation was nearing completion. This was determined by examing the fermentation vessels for by-products of fermentation or once the yeast stopped producing CO_2. Because the alcohol content was only determined for a single period, there cannot be a comparison between varying periods. However, vessels 3 and 4 were significantly different from vessel 1. Compared to vessels 1, 3, and 4 had p-values of .04 and .02, respectively. Vessel 2 was not significantly different from any of the other vessels. The differences in alcohol content shown to be statistically different however there were no functional differences in wine quality due to the slight differences observed in alcohol content.

Table 3. Average alcohol percentage by volume (n=2)

Group	Vessel 1	Vessel 2	Vessel 3	Vessel 4
Percent Alcohol ± Std. Dev	13.92± 0.03%	13.83± 0.04%	13.79± 0.03%	13.76± 0.03%

In the winemaking process, fermentation begins when the yeast is "pitched" or introduced to the must. Before pitching, the yeast was first hydrated in a beaker containing 50mL of warm water and allowed to rest for 20 minutes before being pitched into the must. The order of pitching was in ascending order, i.e 1, 2, 3, then 4. Due to the preparation of the yeast, there was an approximately 5 minute time period between pitching. A few factors could affect the fermentation process and the efficiency of alcohol production, such as the sugar content of the must, the temperature of the must and environment, competition from the natural yeast present on the grapes themselves, insufficient mixing, and so on. It was concluded that the difference in alcohol content was most likely due to unequal sugar concentrations amongst the fermentation vessels. In this

instance, the sugar concentration of the must was adjusted as a whole and then distributed into the four fermentation vessels. It would be very difficult to ensure that each vessel had exactly the same concentrations of sugar but also the same proportions of solids to liquids. The vessels were all kept in the same environment and at the same temperature. Nonuniform mixing is another possible cause, however, this is a well-known issue and several precautions were taken to avoid such an occurrence. The effeciency of the yeast could have also influenced the rate of alcohol production, but since the differences were not shown to interfere with the other assays, there was no further investigation.

Folin Ciocalteu Assay of Total Phenolics

The Folin Ciocalteu assay is a colorimetric reaction that is used to determine the total phenolic content or antioxidant capacity of wine and other agricultural products. The Folin Ciocalteu (FC) reagent will interact with benzoic and cinnamic acid derivatives, flavonoids, anthocyanins, antioxidants, tannins, and the other phenolic components of the wine. As the FC reagent is reduced, the interaction will produce a blue chromophore consisting of a phosphotungstic-phosphomolybdenum complex (Blainski, Lopes, & De Mello, 2013). Colorimetric assays are commonly used due to fact they are easy, inexpensive, and provide rapid results. However, this colorimetric assay requires the use of a reference substance to determine the total phenolic content of the desired sample. Figure 8 shows the standard curve that was used as a reference to determine the total phenolic content of the Rubaiyat wine.

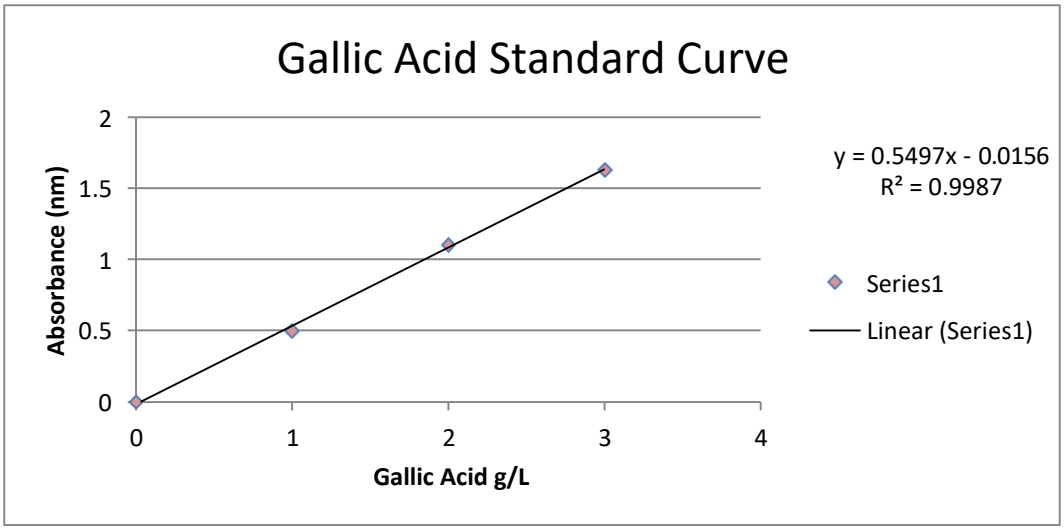


Figure 8. Gallic acid standard curve reference

Gallic acid is commonly used to represent the total phenolic content when using this particular assay. This is because gallic acid is typically a prominent phenolic compound present in wine and has been shown to best represent the total phenolic of wine samples (Singleton, Orthofer, & Lamuela-Raventós, 1999). The gallic acid standards used to create this curve had concentrations of 0, 1, 2, and 3 g/L, respectively. These concentrations were selected because they overlap with the average gallic acid equivalent (GAE) of red wine. The slope of this graph was created by plotting the absorbance collected from the standards versus their controlled concentrations. The absorbances obtained from the unknown wine samples can be used with the slope of this graph to determine the GAE of the sample.

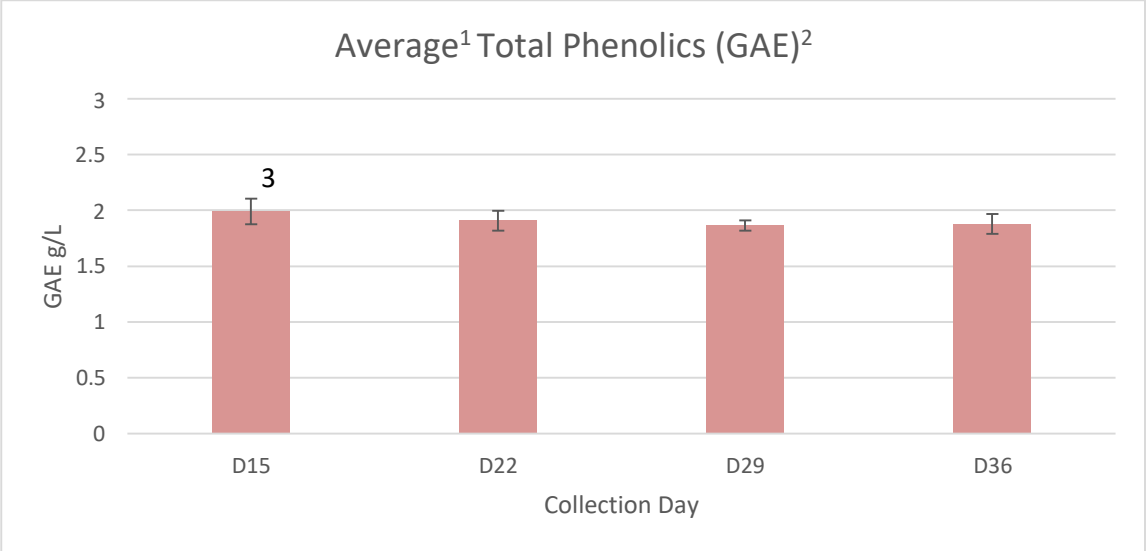
The absorbance alone cannot be used to determine the GAE of and unknown and that is why the reference is required. However, we can see that there is a slight decline over time in the absorbances. Because the absorbance and GAE concentrations are directly proportional, it can be assumed that the difference will also be seen once the absorbances

have been converted to GAE using the slope equation of the standard curve. Below is the corresponding Table 4 which contains the values and standard deviations for the absorbance of the wine samples.

Table 4. Absorbances of the wine samples after the FC Assay (± standard deviation, n=4)

Collection Day	D15	D22	D29	D36
Absorbance (nm)	1.015±0.063	0.972±0.049	0.95±0.026	0.958±0.049

Figure 9 and Table 5 below represents the average GAE of the wine samples and the days that those samples were collected. The total phenolic content of wines contribute directly to many aspects of quality that are important to winemakers and consumers. For example, phenolics contribute to the astringency, bitterness, and other physical sensations known as the "body" of the wine, as well as the color of the wine (Lee, Nomura, Patil, & Yoo, 2014). As time progressed, there was a slight decline in the GAE concentration of the samples. This decline from Day 15 and Day 36 was determined to be significant with a p-value of 0.0067.



1. n = 4 for all of the sample collection days and each sample were measured in duplicates
2. Gallic acid equivalents
3. Error bars indicate one standard deviation away from the mean

Figure 9. GAE of Rubaiyat wine

Table 5. GAE averages of Rubaiyat wine (± standard deviation, n=4)

Collection Day	D15	D22	D29	D36
GAE (g/L)	1.99±0.116	1.91±0.089	1.87±0.048	1.88±0.089

During this time of the winemaking process, the wine was aging and being racked weekly. The wine was racked at this point in the process because “lees” or yeast, excess grape material, organic acids, phenolic compounds, and other solids begin to precipitate out of solution. If the wine is left to sit on the lees, off-flavors, and aromas could develop. However, there are winemaking techniques that allow the wine to rest on the lees and the variance in techniques can be regional.

The slight decrease in the GAE concentration observed over this particular period is not unexpected. As the lees that are forming, falling to the bottom of the rest container, and then left in the container as the wine is racked, small amounts of organic acids and phenolic compounds are likely being left behind. If the winemaker chooses to do a malolactic fermentation, then more lees may be formed. However, the lees stop forming eventually. The amount of lees that precipitate out of solution may be different with every new batch of wine made even though the same grapes are used. This loss is difficult to predict and the differences from batch to batch may be significant. In this case, the decline in the GAE concentration was relatively minor.

While a statistically-significant difference was observed between days Day 15 and Day 29, a significant difference was not observed between days 22-36. Because of this, it can be assumed that the most significant change in GAE concentration was between Day 15 and Day 29. This corresponds to the time during which the majority of the lees fell out of solution. After the wine was racked on Day 29, a significant decrease in the amount of sediment was apparent the next time the wine was racked the following week.

Table 6 below shows data collected at Oklahoma State University as a part of a wine analysis program provided by Dr. William McGlynn (personal communication, May 28, 2020). The data was meant to show similarities and differences between the phenolic content of other wines that wer,e and were not, grown in the area. The location "OK" means that the grapes were grown in Oklahoma, the variety indicates whether or not the grapes used to make the wines were *Vitis vinifera* or hybrid varieties. All of the wines tested were red wines.

Table 6. Total phenolics data collected from commercial wine (g/L)

Location-Variety-Color	OK Grapes - Vinifera - Reds	OK Grapes - Non - Vinifera - Reds	American (non-OK) Grapes - Vinifera - Reds
Average:	3.20	2.19	2.38
Standard Deviation	0.32	0.78	0.40
Highest value	3.54	2.95	2.8
Lowest value	2.91	1.27	1.99
n	3	4	4

The average GAE concentration of rubaiyat on the final day of sample collection was 1.88 (g/L). Compared to the values of Oklahoma vinifera and non-Oklahoma vinifera, the wine made from Rubaiyat grapes does not fall within the highest/lowest values for GAE concentration. However, the Rubaiyat wine does fall within the range of Oklahoma-Non-Vinifera- Reds, which makes sense because the Rubaiyat were grown in Oklahoma and it is not a vinifera variety. Thus, the average GAE concentration observed for the Rubaiyat wine was relatively low, but does fall within the range seen for other Oklahoma-Non-Vinifera-Reds previously tested.

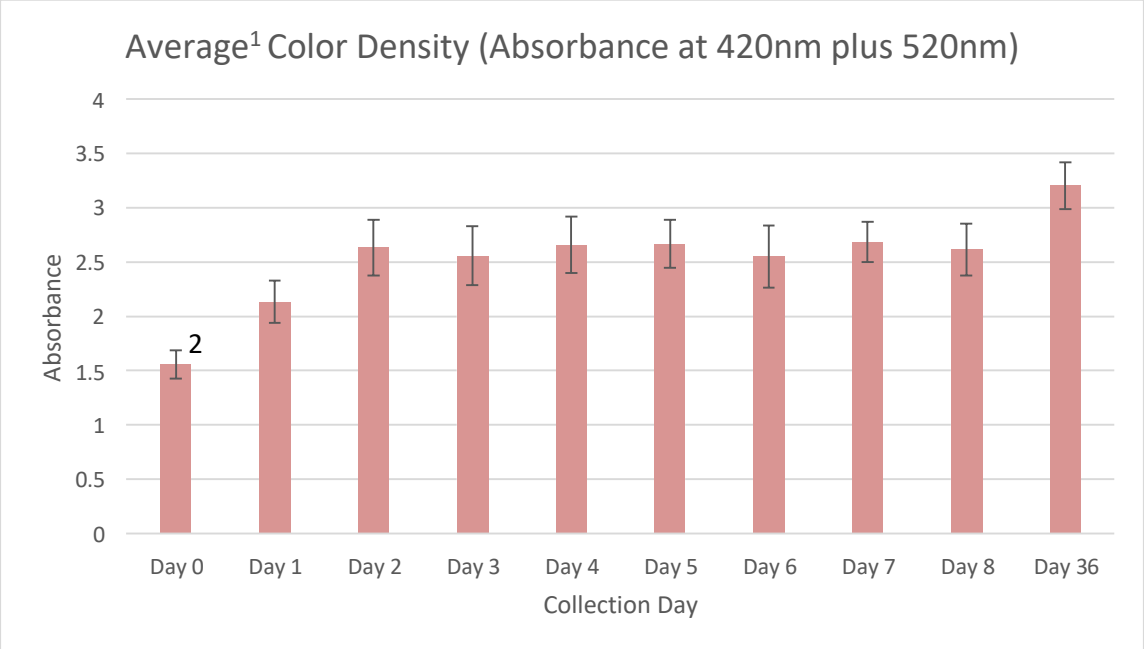
Wine Color Evaluations

Total Color Density

The color density of the wine was measured by combining the absorbances of the wine sample at 420nm and 520nm. These wavelengths are used because they correspond with the most prevalent colored pigments for red wine. A wavelength of 420nm corresponds with yellow/brown pigments, while 520nm corresponds with red pigments (Kelebek et al., 2006). The color of red wine comes primarily from the grape skin. Some

grapes will have a darker flesh that can also slightly contribute to the color, however, the compounds associated with color are found at the highest concentrations in the skin.

Maceration is the process of allowing the grape skins to remain in contact with the grape juice during initial fermentation. As fermentation proceeds, the skin tissue begins to break down and the polyphenolic compounds, tannins, and color compounds present in the skins are extracted. The rate of extraction and specific proportions of the various compounds extracted are a function of contact time, the ratio of water and alcohol in the wine, and other factors that will likely vary from batch to batch and over time such as temperature, degree of batch agitation, and so on. Thus, the intensity of wine color may vary a great deal depending on the maceration treatment applied. Figure 10 shows the change in color density throughout the 8 days of maceration and the color density for 4 weeks after the grapes were pressed.



1. n = 4 for all of the sample collection days and each sample were measured in duplicates

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

Figure 10. Average total color density (420nm plus 520nm)

As expected, the color density was more intense at the end of maceration than on the day the grapes were crushed. It is also to be expected that the absorbance values would stabilize at some point during an extended maceration treatment and remain fairly constant for the remainder of the maceration time; this was observed to occur.

Rubaiyat grapes may be classified as teinturier grapes, or grapes that have a pigmented flesh. The flesh of a non-teinturier grape typically has more clear or greyish juice when crushed. Teinturier grapes tend to have more anthocyanins within the grape's flesh which is why the flesh has more reddish color (Guan, 2016).

Day 0 represents the day that the grapes were crushed, the must was equally distributed into 4 different fermentation vessels, and yeast was introduced to the must. Within the first few days of maceration, the color density reached its peak and stabilized. Between day 0, day1, and day 2, each day the absorbances were significantly different from one another with p-values of 0.00001 and .001, respectively. From day 0 to day 2 the must had a significant increase in the color density and this is suspected to be due to several reasons. First, fermentation has begun and alcohol has begun to develop within the must. Alcohol aids in the extraction of anthocyanins because anthocyanins have hydrophobic characteristics making them more soluble in organic solvents, such as ethanol (Khoo, Azlan, Tang, & Lim, 2017). The natural organic acids of the grape's juice may also aid in the extraction of color compounds.

From day 2 to day 8 we did not observe any significant difference between the absorbance values. This indicates that the extraction of anthocyanins and other color compounds had reached a maximum by day 2. The rate of extraction from day 0 to day 2 in terms of absorbance increased 0.538nm per day. The average absorbance of the wine increased 69% over the first two days and then remained stable for the duration of the maceration period.

After the last sample had been taken at day 8, the wine was pressed and then moved into the aging containers. There was a significant difference between the color density of day 8 and day 36 with a p-value of 0.0002. We believe this change in the color density is due to the physical pressing of the grape skins. The pressing process involves physically pressing the grapes to liberate the juice from the remaining grape tissues. This process tends to physically rupture the skin of the grapes, in our case we pressed the skins up against a steel mesh surface using a rubber bladder. The physical stress most likely pressed the residual juice from the skins which were more concentrated with anthocyanins. It is unlikely that the difference is due to other factors because the wine has no source in which to draw more anthocyanins from after the skins have been pressed. Anthocyanins can polymerize and form complexes with other colorless flavonoids, this process is called copigmentation. It is unlikely that the increase in absorbance from day 8 to day 36 was caused by copigmentation, because copigmentation is a phenomenon that has a greater effect on the stability of anthocyanins than on their overall concentration (Reynolds, 2010).

The skin contact time and the effect this has on the color density in wine made from Rubaiyat grapes is shown in the data presented. Currently there is limited research

available in the literature that illustrates the skin contact time vs. color density for hybrid grape varieties. Data regarding the more commonly used Vinifera grapes is also limited, perhaps because preferred maceration treatments may vary a great deal among winemakers based on the style of wine the winemaker is attempting to make. The relatively few existing studies that examine color density as it relates to the skin contact time are difficult to compare to the data presented here for several reasons. For example, most other studies intoduce other elements to the winemaking process that either promote or inhibit extraction during the maceration process i.e. cold soaking, flash detene, exraction enzymes, etc. For the purpose of this study, we were examining the rate of extraction using Rubaiyat grapes during maceration and not the additional means of color extraction. If more data become available in the future, winemakers may be able to utilize the information to accurately predict the desired color density of their wine based on skin contact time. They may also have greater insight into the grape varieties that would be ideal to use to make blending wines or to add to their initial fermentations. Table 7 below presents the color density means and standard deviations for each collection day

Table 7. Average color density (± standard deviation absorbance at 420nm plus 520nm, n=4)

Collection day	Day 0	Day 1	Day 2	Day 3	Day 4
Absorbance	1.55±0.130	2.13±0.195	2.63±0.258	2.56±0.270	2.65±0.258
Collection day	Day 5	Day 6	Day 7	Day 8	Day 36
Absorbance	2.66±0.219	2.55±0.285	2.68±0.184	2.61±0.237	3.20±0.215

Table 8 below shows data points for color density that were acquired by the Oklahoma State University wine analysis program and provided by Dr. William McGlynn (Personal communication, May 28, 2020). This data is meant to show a general comparison of color density between the wine made from rubaiyat grapes and other wines that were, or are, on the market. The location "OK" means that the grapes were grown in Oklahoma, the variety meaning if they are, or are not, apart of the *Vitis vinifera* or the European varieties. All of the wines tested were red wines.

Table 8. Color density data collected from commercial wine

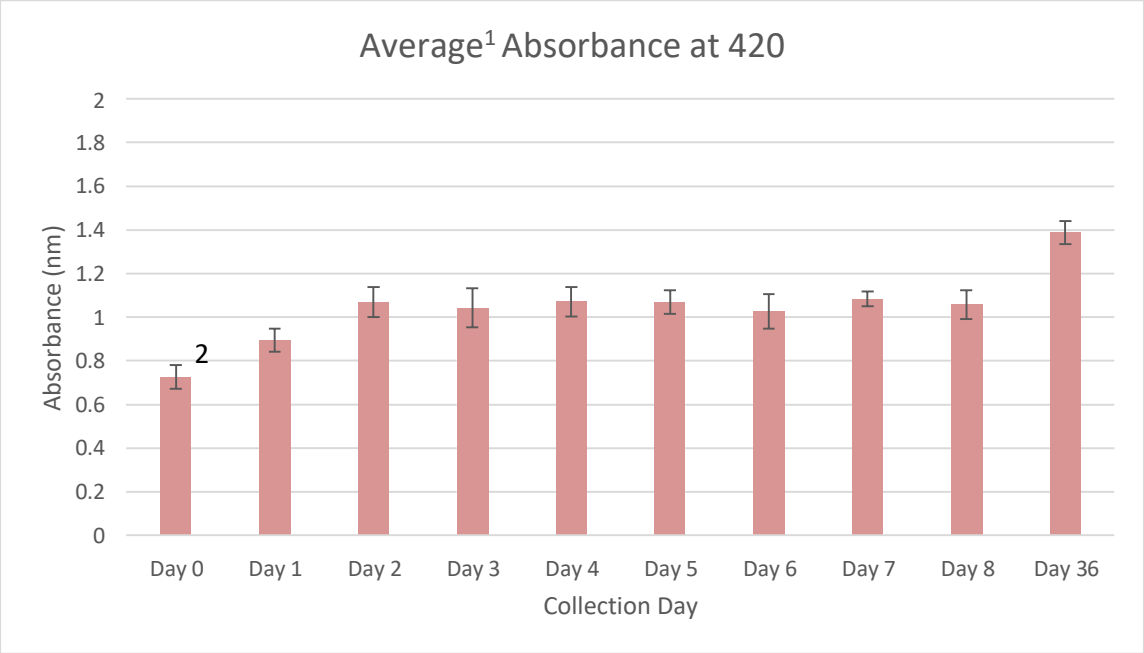
Location-Variety-Color	OK Grapes - Vinifera - Reds	OK Grapes - Non - Vinifera - Reds	American (non-OK) Grapes - Vinifera - Reds
Average:	8.45	5.45	4.76
Standard Deviation	2.12	2.42	0.82
Highest value	9.7	7.5	5.56
Lowest value	6	2.66	3.78
n	3	4	4

The color density of Rubaiyat wine compared to the other non-vinifera grapes grown in Oklahoma show that rubaiyat fall somewhere in the middle of the samples shown above. This was fairly unexpected because one of our original hypotheses was that Rubaiyat grapes would produce an intensely dark wine based on reports in the literature (Stafne, 2006). However, this was not that case and the data suggest that Rubaiyat would not work as well as a substitute for coloring agents in commercial winemaking. Further testing of grapes from additional growing locations and additional growing seasons would be necessary to provide greater insight into this issue.

Unfortunately, it is difficult to make additional comparisons between the Rubaiyat wine produced in this study and commercial wine color data, given that we do not know the commercial producers' winemaking practices. In particular, we do not know whether or not the winemakers used color enhancing additives. It is also difficult to compare the Rubaiyat wine directly to other wines because of the Rubaiyat wine had not yet undergone aging, as would typically be the case with red wines. The wines presented were commercial samples that did have an opportunity to age for an extended time before testing whereas the Rubaiyat wine did not.

Concentration of Yellow-Brown Pigments

Figure 11 shows the average absorbance at 420nm from days 0-8 and day 36. In red wine, the intensity at 420nm is used to measure the yellow/brown pigments in wine. As red wine ages, it is a general rule that the absorbance at 420nm will increase. However, to see significant changes due to aging, the wine must age for a few years, possibly decades.



1. n = 4 for all of the sample collection days and each sample were measured in duplicates
2. Error bars indicate one standard deviation away from the mean

Figure 11. Average absorbance taken at 420nm

Over the 8 days of maceration and 4 weeks after the skins had been pressed, the absorbance at 420nm increased, plateaued, and then increased again. It is apparent from these results that it took 2 days for the yellow/brown pigments to be fully extracted from the crushed skins. In those two days, the absorbance increased by an average rate of 23% per day. There was a significant difference from day 0 to day 1, day 1 to day 2, however, there was not a significant difference from day 2 to day 3. The p-values for each of those were calculated to be 0.00003, .00001, and .522, respectively. After the second day, no significant differences were observed between any of the collection days except for day 8 to day 36. Day 8 to day 36 had the highest increase in absorbance with a p-value of

9.97 E-08. The steady increase in absorbance from day 0 to day 2 can most likely be attributed to the alcohol being produced from the yeast in fermentation. The lack of change from days 2 to 8 indicates that the extraction had reached its peak and could not liberate more yellow/brown pigments from the skins. The day at which extraction will plateau may vary depending on several factors and further research will have to be done on these grapes to determine if the observed plateau is consistent. The largest increase between days 8 and 36 is again most likely due to the grapes being pressed. The physical extraction of anthocyanins aids in the overall effectiveness. Some believe that the highest quality wine is the unpressed runoff that can be collected before the grapes are pressed. However, that wine may be lacking in pigments compared to the pressed wine. This is speculation and cannot be authoritatively concluded from the results of this study.

Table 9 below shows the average absorbance and standard deviations at 420nm for the first 8 days of maceration and 4 weeks after the grapes have been pressed.

Table 9. Average absorbance taken at 420nm (± standard deviation, n=4)

Collection Day	Day 0	Day 1	Day 2	Day 3	Day 4
Absorbance	0.73±0.054	0.90±0.052	1.07±0.069	1.04±0.089	1.07±0.067
Collection Day	Day 5	Day 6	Day 7	Day 8	Day 36
Absorbance	1.07±0.053	1.03±0.08	1.08±0.035	1.06±0.07	1.39±0.053

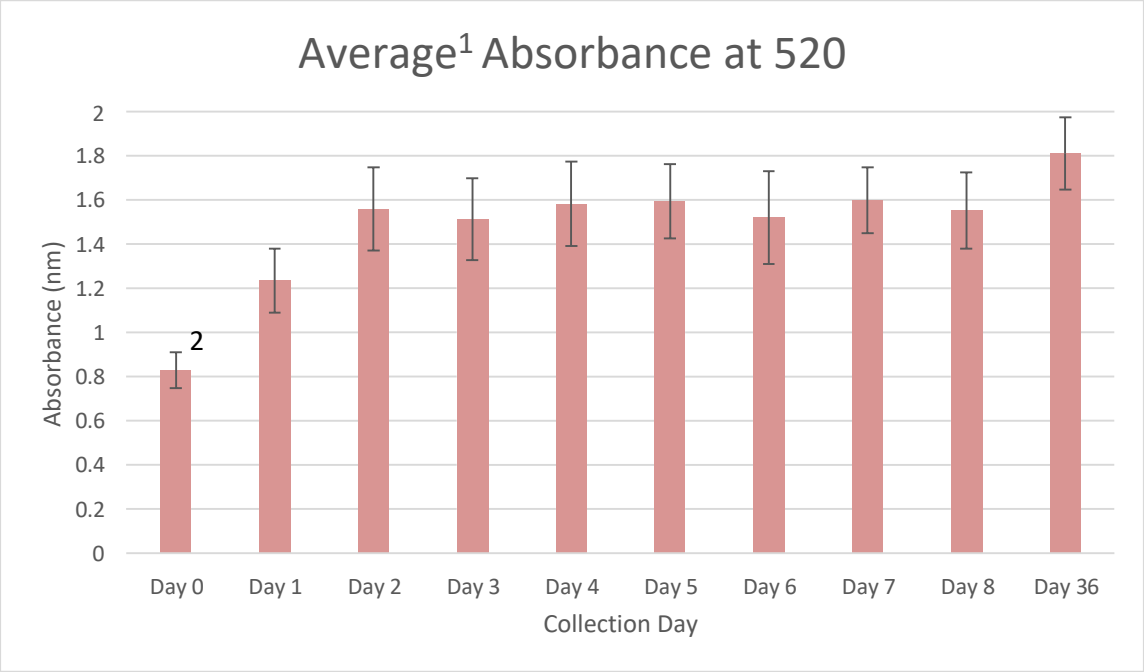
.

As wine ages, the monomeric anthocyanins began to bind with other monomeric anthocyanins to for polymeric anthocyanins (Reynolds, 2010). Polymeric anthocyanins are partly responsible for the color change from a young ruby red to a more dulled brick red. This gradual and typically rather slow change in color will cause the absorbance of

the wine at 420nm to increase and the absorbance at 520 to decrease. That is because the wine is losing the redder pigments for the more polymeric anthocyanins which are more of a yellow/brown color.

Concentration of Red Pigments

Figure 12 below shows the average absorbance at 520nm over the 8 days of maceration and 4 weeks after the skins had been pressed. The absorbance at 520nm represents the intensity of the red pigments in the wine. Young wines will have a higher concentration of red pigments due to the lack of anthocyanin-anthocyanin complexes. The formation of these complexes will gradually cause the absorbance at 520nm to decrease (Wrolstad, 1993).



1. n = 4 for all of the sample collection days and each samplewere measured in duplicates
2. Error bars indicate one standard deviation away from the mean

Figure 12. Average absorbance taken at 520nm

Overall we can see that the absorbance at 520nm is higher than the absorbances and 420nm. Again, we see that there was a steady increase in absorbance from day 0 to day 2, and then after that the absorbances plateau. The rate of extraction increased compared to the absorbance at 420. The average increase per day during the 2 days for the absorbance at 420 was 23% and the average per day increase for 520 is 44%. This difference in extraction is most likely due to the higher concentrations of red pigments in the skin that are characteristics of young red wines. The threshold of extraction was reached by the time the day two samples were collected and then once again there is an increase in absorbance between days 8 and 36. Table 10 below shows the average

absorbance and standard deviations at 520nm for the first 8 days of maceration and 4 weeks after the grapes have been pressed.

Table 10. Average absorbance taken at 520nm (± standard deviation, n=4)

Collection Day	Day 0	Day 1	Day 2	Day 3	Day 4
Absorbance	0.83±0.082	1.24±0.145	1.56±0.189	1.51±0.185	1.58±0.191
Collection Day	Day 5	Day 6	Day 7	Day 8	Day 36
Absorbance	1.59±0.168	1.52±0.209	1.60±0.15	1.55±0.172	1.81±0.163

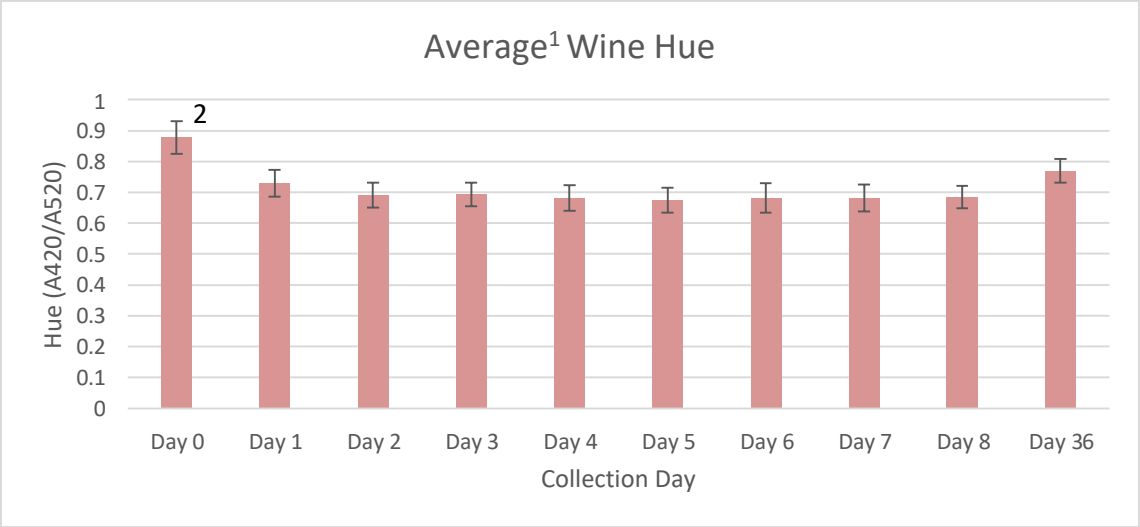
Although there are some differences between the data for the absorbance readings at 420nm and 520nm, they both show that the rate of extraction peaks early in the fermentation process. Because the absorbance at 520nm is mainly a function of the concentration of non-polymerized, free anthocyanins we would expect absorbance at 520nm to be high in a young wine and to decrease over the extended ageing period. In this case higher absorbance values were obsierved at 520nm compared to 420nm. This makes sense because the grapes and must are young and there are more red pigments in the wine.

Overall we observed a greater rate of extraction for red versus yellow-brown pigments during the early stages of maceration in wines made from Rubaiyat grapes. This is likely due to the high concentration of free anthocyanins present in the must. Additional research would need to be conducted to determine if the observed rates of extraction are repeatable for different growing seasons.

Wine Hue

Figure 13 below shows the average color hue for days 1-8 of maceration and 4 weeks after the grapes were pressed (day 36). Color hue value is calculated by dividing

the absorbance at 420nm by the absorbance at 520nm. This calculation provides a ratio between the two absorbances to illustrate the differences between them. This is typically done to show the changes in perceived "redness" over time. If the two values are equal then the hue will be 1. If the hue is above 1 then the absorbance at 420nm is greater than 520nm and vice versa. Hence, a lower hue values indicate a greater perceived wine "redness."



1. n = 4 for all of the sample collection days and each sample were measured in duplicates
2. Error bars indicate one standard deviation away from the mean

Figure 13. Average wine hue

The hue for day 0 indicates that on this day the two absorbances were the most equal and this makes sense because very few pigments have been extracted from the skins. Between Day 0 and day 1 we do see a significant difference with a p-value of 0.00005. For day 1, we see the hue drop significantly because the red pigments were present in higher concentration than the yellow-brown pigments. This caused the absorption at 520nm to rise higher and faster than at 420nm, which caused the hue to

decrease. A significant difference was not observed with days 1-8 all having p-values above 0.05, indicating again that extraction rates for all pigments were similar during maceration. However, there is a significant difference between days 8 and 36 with a p-value of 0.0007. This change indicates that the absorbance at 420nm saw a greater increase than at 520nm. Presumably, if the increase to the absorbance on day 36 is due to pressing, then it could be concluded that after the skins were pressed, they released more brown/yellow pigments than red pigments. However, this change could also be due to oxidation or polymerization of anthocyanins. Table 11 below shows the values for the average color hue on day 1-8 of maceration and for 4 weeks after the grapes were pressed.

Table 11. Average color hue (± *standard deviation,* n=4)

Collection Day	Day 0	Day 1	Day 2	Day 3	Day 4
Color Hue	0.87±0.052	0.73±0.044	0.69±0.04	0.69±0.038	0.68±0.041
Collection Day	Day 5	Day 6	Day 7	Day 8	Day 36
Color Hue	0.67±0.041	0.68±0.048	0.68±0.044	0.68±0.036	0.77±0.038

Typically, wine hue is an analysis used to evaluate wine that has aged or is aging. Hue is a particularly useful test to determine the effects of oxidation as wine ages. However, this data could be useful for winemakers who want to make a vibrant young red wine. With more research in a similar vein, winemakers could have a larger catalog of techniques for customizing and creating new and improved quality wines. With data that shows the average rates of extraction and specific pigments being extracted, a

winemaker could potentially create wines with highly specific color attributes to maximize quality and saleability.

Table 12 below shows color hue data for commercial wines that was collected at Oklahoma State University and provided by Dr. William McGlynn (Personal communication, May 28, 2020). The wines that were sampled had completed the ageing process and were ready to be sold to the public. Because of this, the color hue data cannot be directly compared to the data collected for the Rubaiyat wine due to the fact the commercial wines have been aged and the anthocyanins have presumably polymerized, formed stable complexes with tannins, and so on due to the oxidation reactions that occur during ageing. However this data can provide some insight into the ultimate color quality results we might expect to see with the Rubaiyat wine. We anticipate that future studies will evaluate the color parameters of the aged Rubaiyat wine.

Table 12. Color hue data collected from commercial wine

Location-Variety-Color	OK Grapes - Vinifera - Reds	OK Grapes - Non - Vinifera - Reds	American (non-OK) Grapes - Vinifera - Reds
Average:	1.20	1.33	1.02
Standard Deviation	0.16	0.79	0.11
Highest value	1.38	2.51	1.19
Lowest value	1.07	0.81	0.94
n	3	4	4

Since the rubaiyat wine was produced from Oklahoma grapes and it is not in the *vinifera* family, the OK Grapes - Non - Vinifera – Reds category affords the most direct comparisons. There is a obvius diffrence in the averages between all of the different commercial wines and the Rubaiyat wine with the Rubaiyat wine being notably more red

than the commercial wines. This is almost certainly because, as mentioned prevoiusly, the Rubaiyat wine is young and has not had the time to develop the more brown/yellow pigments that are associated with older red wines.

CHAPTER V

CONCLUSIONS AND FUTURE RESEARCH

The purpose of this study was to evaluate the basic quality attributes of wine made from the Oklahoma-native Rubaiyat grape variety and to research the effects of skin contact time on the color intensity of the Rubaiyat wine produced. By using this information, our goal was to determine if blending Rubaiyat wine with other, less highly-pigmented wines could offer winemakers an alternative to the use of artificial coloring additives. This could have potential benefit to Oklahoma and regional winemakers because Rubaiyat grapes are well suited to be grown in a midwestern climate given that the variety was bred at Oklahoma State University in the mid-20th Century.

A great deal of information about Rubaiyat grapes and wines remains to be scientifically evaluated; there have been no previous published scientific analyses of the properties of Rubaiyat grapes and wines before this research. This study not only serves as a reference for future evaluation of the Rubaiyat grape itself but also as a reference for color development during maceration.

It is important to note first of all that the Rubaiyat wine tested in this study was made from grapes grown in one season and harvested from a single location. This implies that Rubaiyat wines would need to be analyzed from additional growing years and from additional growing locations to refine these initial findings and conclusions.

That noted, overall the Rubaiyat grapes evaluated in this study did not produce wine that was substantially higher in red pigments than other commercially-available red wines made from Oklahoma-grown grapes. The Rubaiyat wine produced had a fairly typical red color, but it lacked the intense pigmentation that was originally hypothesized. Thus, based on this study, we cannot conclude that Rubaiyat grapes are bound to produce a wine that is necessarily suitable for enhancing the color of other red wines via blending. Given that Rubaiyat grapes are known to be capable of producing highly-pigmented fruit, further research is necessary to determine what factors influence the development of pigments during ripening in this grape variety.

In addition to the overall conclusion presented above, the specific objectives and results of this study related to the skin contact time, phenolic content, and color analysis are given below:

1. The color of red wine comes directly from the skin contact time during the maceration process of winemaking. There is scant research on the effect of grape skin contact time on color density. The data collected showed an average 34.5% increase to color density per day over the first two days of maceration, after which color density stabilized. Also, there was a significant difference seen in the extraction of free and polymerized anthocyanins which were measured by two different wavelengths at 420nm and 520nm. The extraction rate of the free anthocyanins was higher with an average increase of 44% per day than the polymerized anthocyanins with an average increase of 23% per day. This research may act as a reference for additional analyses of color compound extraction as it relates to color density. Also, this research could act as a reference for

winemakers who are trying to create a wine with specific characteristics or under specific conditions. For example, if a winemaker wished to produce a young red wine with vibrant red color, that is full-bodied, and limit the maceration time so that the wine may not need to age as long, the winemaker may want to consider grapes that have shown to have higher rates of unpolymerized anthocyanins extraction.

2. Total phenolic content affects the quality and potential health benefits of wine. The analysis of this characteristic showed that this harvest of Rubaiyat grapes produced a wine that was neither low nor high in the concentration of phenol/polyphenols to other red wines. However, perceived wine quality depends a great deal on the makeup of the phenolic compounds present in the wine moreso than the total concentration. Therefore, more research should be done on consumer acceptance of Rubaiyat wines. For example, a sensory panel would provide information to help understand the consumer's attitudes toward the quality characteristics affected by phenols/polyphenols.

3. Due to their red flesh and purple/blue skin, Rubaiyat grapes were originally hypothesized to work well as an alternative coloring agent for red wines. However, based on the colorimetric analyses performed, we could not conclude that the wine we produced from Rubaiyat grapes was suitable for this purpose. However, more research is required to determine whether or not Rubaiyat grapes are generally suitable as tenturier grapes when grown in Oklahoma. Also, sensory evaluation of Rubiyat wines would be useful to assess potential consumers' ratings of the color of the varietal wine produced from Rubaiyat grapes.

Professional panels could classify the wine relative to other wines on the market in a way that we are not capable of using objective colorimetric analyses.

We believe that this research represents a starting point for additional, important research on Rubaiyat grapes and wines. Due to the number of variables that inevitably accompany the winemaking process, it is difficult to draw sweeping conclusions from a single study. Further research on this hybrid grape variety that has the potential to grow well and survive in Oklahoma's climate with fewer inputs and less intense management in terms of pest control compared to the more common European varieties could potentially help Oklahoma become a well-known producer of high-quality wines.

Recommendations for Future Studies

As noted above, the most critical recommendation for future studies is to replicate this work using grapes from additional harvests and, ideally, from additional growing locations. It would also be interesting to produce wine from different varieties of grapes from the same vineyard as the Rubaiyat grapes and directly compare the wine that is produced from each variety. Terrain has a huge effect on the physical and chemical composition of the grapes themselves and thus the wine they produce. It can be difficult to compare a wine made in the lab with a commercial wine simply because the details of the winemaking process employed by a commercial winemaker may not be known. For example, winemakers are not necessarily required to label everything that they add to the wine, such as coloring agents. So, for an accurate comparison of Rubaiyat wine to wine made from other, more common grapes, wines should be produced from two or three common wine grape varieties and compared them to the wine made from Rubaiyat grapes.

Also, future studies that focus on color extraction and skin contact time would benefit from evaluating all of the samples taken during maceration for pH, titratable acidity, and alcohol content. This would allow the researchers to examine possible correlations among color and anthocyanin extraction with the above-mentioned characteristics. Finally, it is strongly recommend to perform colorimetric evaluations on samples immediately before and after pressing. With this data, it should be possible to conclusively characterize the impact of pressing on pigment concentration. If sample size permits, it would also be desirable to remove and press samples at various times during the maceration process, rather than simply removing and analyzing liquid samples from the fermentation vessels.

Final Thoughts

In summary, this research showed that wine produced from Rubaiyat grapes did not produce an intensely, deeply-pigmented wine as was expected. However, this research also demonstrated that it is possible to produce wine with typical basic quality parameters from Oklahoma-grown Rubaiyat grapes. This study serves as a baseline for continued research with the Rubaiyat grape and suggests future avenues of inquiry. With enough data similar to the data presented here, one day a catalog of qualitative and quantitative physical and chemical characteristics for various grape varieties may be readily available for Oklahoma and regional winemakers to use to create new and innovative high-quality wines.

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APPENDICES

The Figure 14 below represents the absorbance's values obtained via cheese cloth filtration as described in the "Methodology" chapter.

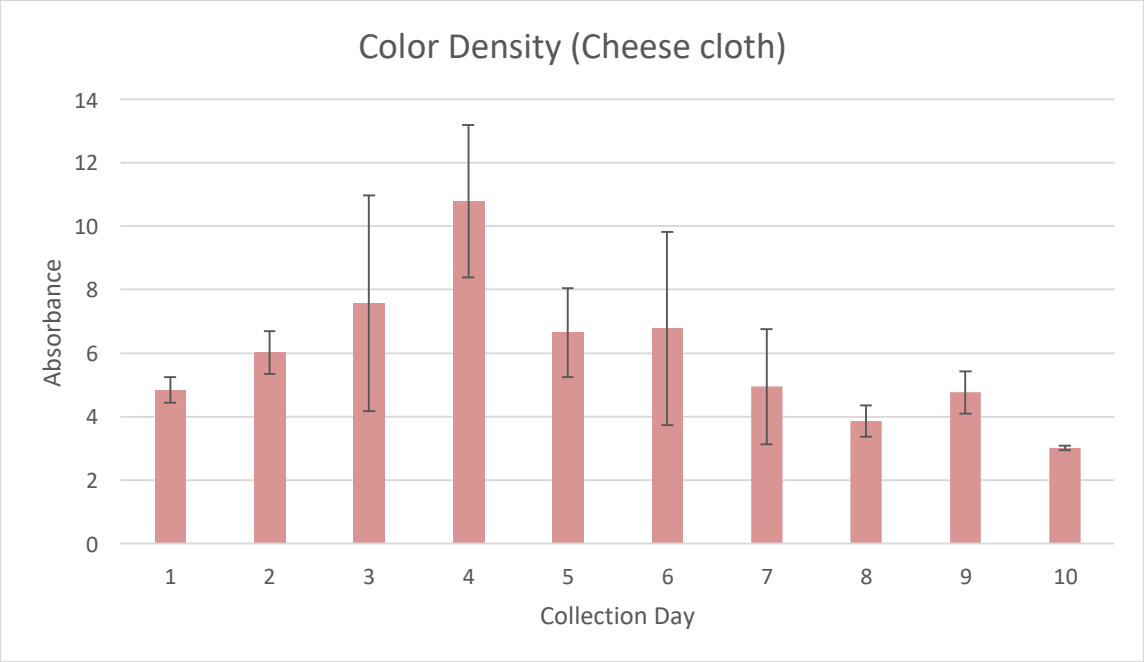

Table 13 below represents the data points obtained via cheese cloth filtration and centrifugation as described in the "Methodology" chapter (± standard deviation).

Collection Day	Day 0	Day 1	Day 2	Day 3	Day 4
Absorbance	4.84±0.41	6.02±0.67	7.57±3.39	10.8±2.4	6.65±1.4
Collection Day	Day 5	Day 6	Day 7	Day 8	Day 36
Absorbance	6.77±1.81	4.95±1.81	3.86±0.50	4.77±0.66	3.02±0.07

The Figure 15 below represents the absorbance's values obtained via cheese cloth filtration and centrifugation as described in the "Methodology" chapter (± standard deviation).

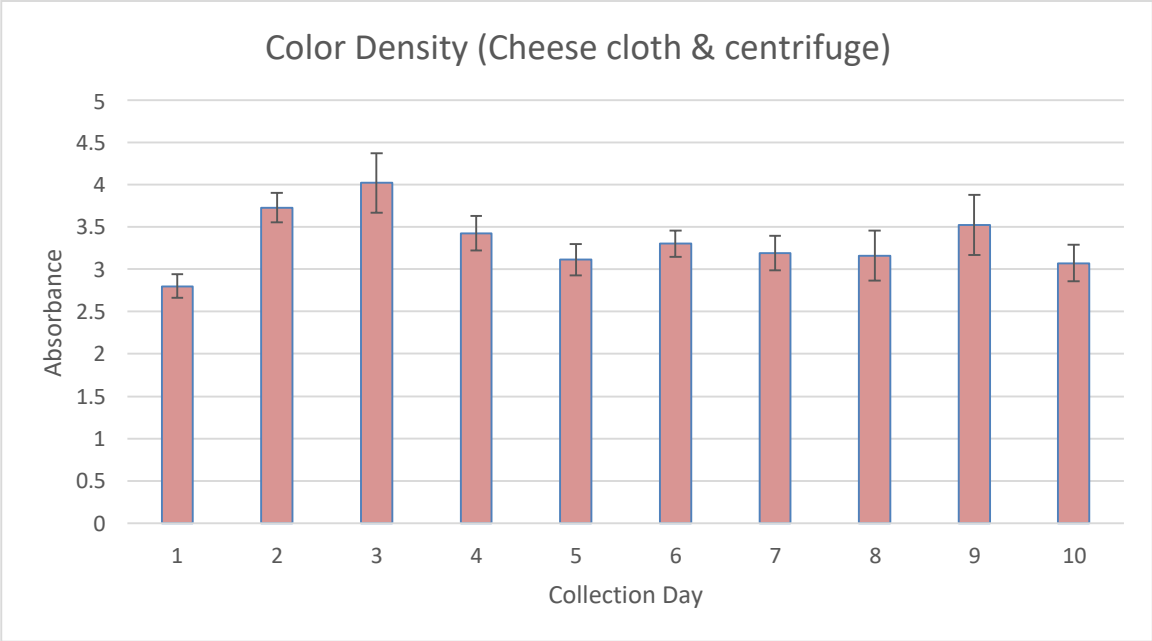

Table 14 below represents the data points obtained via cheese cloth filtration and centrifugation as described in the "Methodology" chapter.

Collection Day	Day 0	Day 1	Day 2	Day 3	Day 4
Absorbance	2.80±0.14	3.73±0.17	4.02±0.35	3.43±0.20	3.11±0.19
Collection Day	Day 5	Day 6	Day 7	Day 8	Day 36
Absorbance	3.30±0.16	3.19±0.020	3.16±0.30	3.53±0.35	3.07±0.21

VITA

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