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A Viticultural and Enological Evaluation of Four *Vitis Vinifera cv. Petite Sirah* (Durif) Clones During the 2021 Vintage

By

ANDREW MISIALEK
MASTER'S DEGREE - THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

VITICULTURE AND ENOLOGY

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

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2023

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Abstract

Viticulturists and winemakers utilize wine grape cultivar clones in order to achieve specific goals related to yield, quality, and wine characteristics. *Vitis vinifera cv.* Petite Sirah, also known as Durif, is an important wine grape cultivar in California in the production of varietal wines and as a means to improve the color/tannic structure of red wines through blending. Four Petite Sirah clones (FPS01, FPS03, FPS04, and FPS05) were evaluated to compare their agronomic performance and grape and wine characteristics over the course of the 2021 growing season. Eighty vines of each of the four clones were each organized into four biological replicates in a randomized block design at the Robert Mondavi Institute vineyard (Davis, CA). Five vines per biological replicate were evaluated for vine water status, canopy density, ripening parameters, veraison progression, harvest must chemistry, and cluster condition. Clones were harvested at similar ripeness levels, yields determined, and winemaking performed in triplicate. Both the grape and wine phenolic profiles were determined by the Adams-Harbertson Assay and reversed-phase high-performance liquid chromatography (RP-HPLC). Grape and wine aroma profiles were determined by used head space-solid phase micro extraction-gas chromatography-mass spectroscopy (HS-SPME-GC-MS). Wine phenolic extraction during fermentation was monitored daily by spectrophotometric measurements. Results indicate that clones FPS01 and FPS04 may ripen and accumulate sugar quicker than FPS03 and FPS05 depending on the growing season. Grape must from FPS05 contained higher level of malic acid while FPS03 contained the lowest. FPS03 wines had least amount of phenolic compounds in comparison to other clones as they contained the lowest amount of flavan-3-ols,

anthocyanins, and flavonols. Wines from FPS01 and FPS04 were higher in flavan-3-ols and flavonols respectively. Grapes and wines from each clone also showed differences in the composition of volatile compounds. Therefore, it can be stated that differences in chemical composition exist among Petite Sirah clones. In this specific year, these chemical differences did not translate to clear, significant differences in sensorial perception based on descriptive analysis.

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Section 1. Introduction

1.1 Genetic Variability in Wine Grape Cultivars

As in most living organisms and biological systems, genetic variability can be found in the grape species that is used to make wine in California and the rest of the world (Myles et al 2011). *Vitis vinifera*, or the species of grapevine most used in wine production, has developed genetic variability that has led to the rise of many different grape cultivars that are used to produce blended and varietal wines (Riaz et al 2018; Myles et al 2011). While genetic variability has occurred over time as grapevines have naturally crossed and reproduced, variability has also occurred due to human interaction in the form of the breeding and the selection of grapevines for their desired genetic traits to better meet wine production goals (Mullins et al 1992). These desired phenological traits derived from differences in genetics can be seen in the color of the grape skin, flavor/aroma, size of clusters/berries, ripening times, adaptability to adverse growing conditions, disease resistance, and many other observable characteristics (Dai et al 2011).

One accessible and distinct example of differences among cultivars is seen in a comparison of two of California's most widely planted wine grapes: *Vitis vinifera cv* Cabernet Sauvignon and *Vitis vinifera cv* Chardonnay. These two wine grape varieties belong to the same species but, due to relatively small differences within their genetic code, are used to produce vastly different wines. Cabernet Sauvignon is a red skinned cultivar that is typically utilized by producers to make wines that have aromas reminiscent of red or dark fruit, are deeply colored,

and have strong, tannic structures. Chardonnay is a white grape that is more aromatically subtle and delicate in comparison to Cabernet Sauvignon and used throughout the world to produce white wines (Bettiga 2013).

1.2 Clonal Propagation and Selection

While small differences in genetic code have given rise to different grape cultivars, genetic differences can occur within a cultivar, producing clones that have their own variations that may include, but not be limited to, berry chemical composition, environmental adaptability, external stress tolerance, and yield (Dai et al 2011). These important differences may lead to eventual variation in fruit and the subsequent wine quality perceived by wine producers and consumers.

Removing a cutting from a parent plant to produce a genetically identical plant (also known as a clone) through propagation is a practice that has been performed in agriculture for centuries, if not millennia (Zhao 2011). In fact, the term “clone” has its origins in the ancient Greek word “klon”, which translates to “twig used for propagation” (Diamandopoulos and Goudas 2000). This propagation method allows for the production of a new plant from a carefully chosen parent that meets phenotypical characteristics a farmer or producer may desire in terms of yield, fruit quality, disease resistance, and more. Unsurprisingly, this practice is critical to the success in modern day agriculture, with the wine industry being no exception.

The utilization and selection of different wine grape varieties and their respective clones is a long-standing practice to achieve the desired wine styles of producers (Myles et al 2011).

There is a long, established history of producers choosing varietal clones and extensive research performed on clonal selection and breeding of popular, widely planted varieties in California such as the Chardonnay and Cabernet Sauvignon cultivars (Bettiga 2003; Gatti 2022). Dr. Harold Olmo's work is an example of extensive clonal research in California as he worked with Chardonnay and other varieties to increase yield and help establish the state as a prolific wine producing region in the world during the post-prohibition period in the mid 20th century United States (Sweet 2007). Due to its importance to the economic success of many countries' wine industries, there have been numerous clonal studies performed throughout the world to better understand cultivar clone differences in order to provide industry members with information that may aid them in selection decisions. These clonal studies have often evaluated a wide range of different grape and wine characteristics that are relevant to producers.

Studies have included results related to ampelographic, viticultural, and agronomical performance of different clones. Understanding clonal variability of a cultivar is important as differences may influence and inform viticulture practices, economic planning, and the eventual wine styles made by a producer (Bettiga 2013). Clonal variability can result in differences in yield. Clonal comparisons for yield have been performed by evaluating a clone's total yield, yield per vine, cluster size, berry size, berries per cluster and more (Benz et al 2005; Benz et al 2007; Rühl et al 2004; Atak et al 2014; Mercado-Martin et al 2006; Fidelibus et al 2006; Anderson et al 2008). Other important factors related to viticultural performance that have been investigated are vegetative vigor and vine balance using pruning weights, water use efficiency and drought tolerance using plant water potentials or stomatal conductance,

resistance to different diseases, and more (Benz et al 2005; Benz et al 2006; Tortosa et al 2016; van Leeuwen et al 2013).

Another common method of clonal comparison is the characterization of phenolic compounds found in the grapes and respective wines. Whether by using instrumentation such as high-performance liquid chromatography (HPLC) (Peng et al 2002) or spectrophotometric assays such as Adams-Harbertson (Harbertson et al 2015), Folin-Ciocalteu (Slinkard and Singleton 1977), or more, these investigations are critical to producers as phenolic compounds are directly responsible for the color and mouthfeel of grapes and wines and are commonly used as markers of wine quality (Merkytė et al 2020). Pantelić et al (2016) used the Folin-Ciocalteu and pH differential methods to compare the phenolic compounds of Merlot and Cabernet Franc clones. Kupe et al (2021) used similar methods with the addition of using HPLC to compare grape skin, pulp, and seed phenolic compounds found in nine "Karaerik" clones, a Turkish grape cultivar. Giannini et al (2016) also used the Folin-Ciocalteu method and HPLC with mass spectrometry to determine the phenolic content of the seeds of different wine grape cultivars and clones.

The aroma profiles of a *Vitis vinifera* wine can be dependent on several aspects, including clonal variability (Šuklje et al 2016; Gómez-Plaza et al 1999). Different methods of gas chromatography are commonly used to characterize the aroma profiles of grapes and wines (Rapp 1988; Vilanova and Oliviera 2012). Botelho et al (2008) used gas chromatography-mass spectroscopy (GC-MS) and gas chromatography-olfactometry (GC-O) to find differences between Trincadeira clonal wines over two vintages. Duchêne et al (2009) also used GC-MS to compare differences in terpenes in different cultivars and clones. Ziegler et al (2020) used head

space-solid phase micro extraction-gas chromatography-mass spectroscopy (HS-SPME-GC-MS) to show that there was significant clonal impact on the production of specific aroma compounds in Riesling clonal wines.

Another means of characterizing the aroma and sensorial profile of a wine is using descriptive analysis (DA) by a trained panel of experts. This method has been a commonly accepted practice for many years as this process is able to produce data that is helpful in the sensorial characterization and differentiation of wines based on a set of determined terms (Lawless and Heymann 2010; Heymann and Noble 1987; Guinard et al 1987). Descriptive analysis performed by Visser (2003) was able to show differences in wines produced from Merlot and Cabernet Sauvignon clones. While the publications referenced above in this section have evaluated the clonal differences of a wide range of different *Vitis vinifera* cultivars, little to no clonal research has been performed on Petite Sirah (Bettiga 2013).

1.3 Vitis vinifera cv. Petite Sirah

Vitis Vinifera cv. Petite Sirah, also known as Durif in other wine producing regions of the world, is currently an important component in California blended and varietal red wines as it contains a large amount of deeply colored pigment and good tannin structure (Meredith et al. 1999). This cultivar arose after the botanist Francois Durif crossed *Vitis vinifera cv.* Syrah and *Vitis vinifera cv.* Peloursin c.1880 in France (Comiskey 2016). This cross resulted in a medium-clustered (often winged or double clustered), mid-late ripening cultivar that produces wines that are deeply colored, full-bodied, and suitable for aging (Bettiga 2003).

There have been no published studies that have investigated the differences among Petite Sirah clones. In fact, there is very little published compositional data on Petite Sirah in general. One of the only studies that could be found that was performed specifically on Petite Sirah was done by Wiid (2016) in Paarl, South Africa. This study investigated the effects of sequential harvesting on Petite Sirah berry and wine composition and sought to create a sugar loading model for this cultivar. While the primary goals of their research were not entirely relevant to clonal performance and variability, it produced data that is helpful for generally characterizing Petite Sirah within the context of other grape varieties. They found that the spectrophotometric absorbance of anthocyanins in their Petite Sirah wines was higher than a previous study investigating anthocyanin absorbance of four hundred Cabernet Sauvignon and Syrah samples (Wiid 2016; Somers and Ziemelis 1985). They also found that the overall color intensity of the Petite Sirah wines was very high compared to literature, as their wines produced absorbance units (AUs) ranging from 1.3-2.1 whereas other sources showed that the typical range for wines to be 0.8-1.3 AU (Ribéreau-Gayon et al 2006). Another paper by Sommer and Cohen (2008) investigated different anthocyanin extraction methods for analysis utilizing the grapes of eleven different, common grape species and cultivars, which included Petite Sirah. This study also included six other *Vitis vinifera* cultivars: Cabernet Sauvignon, Cabernet Franc, Merlot, Barbera, Mourvèdre, and Syrah. The researchers found that the Petite Sirah wines contained 1224 mg/L tannins on average, which was only less than two other vinifera cultivars evaluated (Merlot and Cabernet Sauvignon). Petite Sirah grape skins contained the highest amounts of extractable polyphenols and anthocyanins of all the vinifera species evaluated. In the finished wines, Petite Sirah contained the highest amounts of anthocyanins

(340 mg/L) of all vinifera cultivars and was second in total phenols with 1430 mg/L, which trailed only Cabernet Sauvignon (1460 mg/L). Somewhat expectedly, the performers of this study also found that their Petite Sirah wines had the highest total color absorbance of all the *V. vinifera* wines. These findings are more evidence of Petite Sirah containing relative high levels of phenolic compounds, which routinely leads to wines that are more deeply colored and well-structured. It is these characteristics that make this cultivar desirable to California grape growers and winemakers.

1.4 Petite Sirah in California

Petite Sirah has a long history in California viticulture, dating back to the emergence of the state's north coast as a wine-producing region in the late 1800s. Shortly after its creation, it was brought to California and was found to be a suitable fit for the climate (Comiskey 2016). For over a century, its identity was uncertain due to a convoluted history of the naming of this cultivar. Some believed it got its name for producing wines reminiscent of Syrah, some felt that this variety was a mix of inter-planted cultivars, and others were convinced of several other theories (Comiskey 2016). It was not until the late 1990s that Dr. Carole Meredith at the University of California, Davis determined its genetic lineage and that most vines planted in California as Petite Sirah were genetically identical to the Rhône variety Durif (Meredith et al 1999). Regardless of the ambiguity of its identity, this variety has long shown the aptitude to thrive and produce quality wines in California.

While Petite Sirah certainly has had a place in California's wine growing history dating back to the late 19th century, it still has relevance as it is currently one of the top red wine cultivars used in the state. It has consistently been approximately 4.5% of all the red wine grapes crushed by volume over recent years according to California crush reports from 2018-2022 (https://www.nass.usda.gov). As of 2021, approximately 11,000 acres of Petite Sirah is planted throughout California, mostly in the San Luis Obispo, Yolo, Napa, San Joaquin, and Sonoma counties (https://www.nass.usda.gov). The crush volume and planting acreage totals both rank in the top six of all red wine grape varieties in California. Therefore, achieving a better understanding of Petite Sirah clones could potentially have significant financial impacts on the state's wine industry.

Foundation Plant Services at the University of California, Davis has 10 different Petite Sirah clones available for commercial nurseries but little information is available about clonal differences in grape composition and subsequent sensory differences in wines. Grapevine cultivar clones can contain genetic variability that may affect several aspects of grape ripening and finished wine attributes. This includes, but is not limited to, berry sugar and phenolic compound composition and accumulation, aroma compound content, berry weight, and overall yield (Bettiga 2013). One example of genetic differences impacting grape phenotype is the expression of genes such as VvMYBA1 which is associated with the biosynthesis pathway for anthocyanins and other phenolic compounds (Dai et al. 2011; Fernandino et al. 2010). Information on how grapevine cultivar clones differ in phenology may help producers gain the ability to select specific clones for their desired yield, berry quality, and wine characteristics, which could allow them to meet their specific goals in a more efficient, cost-effective manner.

Having a greater understanding of the clones of a deeply colored, well-structured variety such as Petite Sirah may allow producers to find success and maintain wine quality when faced with an ever-evolving wine consumer base and the external pressures caused by a changing, unpredictable climate (Wolkovich et al 2017). Given its well-known tannin and color content, this grape variety can potentially be used by producers to maintain or achieve their desired phenolic profiles in their wines by blending when faced with reduced phenolic concentration of color or tannins in other grapes due to abiotic and biotic stressors. Some possible external influences in California could include, but not be limited to, being forced to pick late season varieties (such as Cabernet Sauvignon or Zinfandel) before their desired phenolic/color profile due to threat of wildfires, a rise in widespread grape vine viruses that cause a decrease in phenolics due to uneven or incomplete ripening, and decreasing water availability and associated metabolic deficiencies that may reduce the accumulation of secondary metabolites like phenolic compounds throughout the growing season (Girardello et al 2020; Gambetta et al 2020).

Another important factor to note is the effect of decreasing water availability in California and its subsequent impact on viticultural practices. One such method to cope with lack of water is the utilization of dry farming to reduce water usage in the vineyard (Pagay et al 2022). An increasingly popular practice, several varieties of grapes seem to adapt well to this method of water management, and Petite Sirah is being commonly utilized in dry farming systems (agwaterstewards.org). Producers having additional clonal information about Petite Sirah may allow them to select a clone for their purposes more confidently and mitigate fears in switching to the potentially more risky and lower-yielding dry-farming method.

1.5 Research Notes and Objectives

As previously mentioned, there have been no published clonal studies performed on Petite Sirah. Therefore, a major goal of this study was to investigate any differences that exist between the grapes and wines produced by Petite Sirah clones. This thesis covers the third year of a three-year study, with this project only covering data and analyses gathered and performed during the 2021-2022 season. Another major goal of this work is culminating the three-year experiment and to provide substantial data intended for the publication of a larger body of work.

The methods used in this work were established during the 2019 and 2020 seasons. It is also important to note that this project evaluates both the grapes and the eventual wines produced by the four Petite Sirah clones separately. Grape characteristics were produced from biological replicates from each clone in the vineyard. These biological replicates were then combined during harvest to produce the clonal wines. Due to this experimental design, it may be best to view these projects as two, distinct sections: grapes and wine.

The main purpose of this study is to provide growers and wine producers with information that may aid them in selecting a Petite Sirah clone that allows them to better, and more efficiently, produce the grapes and wines that meet their desired wine style goals and expectations.

Section 2. Materials and Methods

2.1 Petite Sirah Experimental Vineyard:

This study was performed at the University of California, Davis in the Robert Mondavi Institute experimental vineyard. Four clones of Petite Sirah (Durif FPS01, Durif FPS03, Durif FPS04, and Durif FPS05) were grafted onto 420A rootstock and planted in 2010 in a randomized block design to evaluate grapevine agronomic traits as well as sensorial and chemical composition of their grapes and respective wines. The grapevines were trained on a bi-lateral cordon training system with a four-wire, T-top trellis and a vine spacing of 2 m x 2.4 m between vines and rows respectively. In the dormant season, grapevines were pruned to two-bud spurs and shoot density was adjusted to 1 shoot per spur to homogenize canopy density and to one cluster per shoot to ensure the grapes reached commercial ripeness of approximately 25 Brix. The vineyard was drip irrigated by a micro-irrigation system that delivered 4 L/h per plant. The vineyard is monitored by a surface renewal system and weekly pressure chamber readings during the summer, with 65% of actual crop evapotranspiration replaced weekly as the mid-day stem leaf water potential reached -1.0MPa or 10 bars.

2.2 Viticultural Evaluation

For each clone, four biological replicates (n=4) composed of five “data” grapevines each, were monitored and used for the following agronomical measurements at harvest on September 1st, 2021: number of clusters/vines, cluster weights, number of berries per cluster, berry weights, and yield. The clusters’ conditions were also evaluated as means to establish

quality. At the time of harvest, the clusters were evaluated visually and sorted into different categories that included “Good”, “Rotten”, “Sunburnt”, and “Green”. If approximately 75% of berries were healthy/ripe and not green, sunburnt, or rotten, then the cluster were deemed “good”. If less than 75% of the berries on a cluster were healthy and ripe, these specific clusters were then described depending on their condition. It was possible for clusters to fall into more than one “bad” category if it contained significant amounts of either green, sunburnt, or rotten berries. These results were used to calculate the percentage of clusters affected in different ways. Biological replicates were combined at harvest and measured for total yield in kilograms prior to processing for wine production.

Vine nutrient status was determined to help rectify any deficiencies in order to eliminate vine nutrition as a source of variability in subsequent analyses. This was performed by petiole sampling and subsequent analysis. Fifty petioles of young, yet mature “count” leaves were sampled per biological replicate on May 17th, 2021. Nutritional analysis was performed by Dallavalle Laboratory Inc. (Davis, CA) and included Nitrogen (%), Phosphorus (%), Potassium (%), Zinc (mg/kg), Manganese (mg/kg), Sodium (%), Boron (mg/kg), Calcium (%), Magnesium (%), Iron (mg/kg), and Copper (mg/kg). According to Dallavalle Laboratory, the resulting levels were then categorized into “Deficient”, “Low to Adequate”, “Normal/Optimal”, or “High to Excessive” as seen in Table 1. Using this information, the biological replicates received the appropriate fertilizer treatment (in the form of potassium thiosulfate (KTS) at 37.42 L/ha) in order to mitigate potassium deficiencies. Kaolin was applied by spraying Surround® WP crop protectant (Tessenderlo Kerley Inc., Phoenix, AZ, USA) on June 30th, 2021, as means of UV protection against possible heat wave events and excessive sun exposure.

Vine water status were monitored weekly beginning June 3rd, 2021, until August 26th, 2021. Veraison rate and timing were evaluated by observing the vineyard every 3-5 days after the first sign of veraison and noting the percentage of berries per cluster that have gone through color change on each of the five established "data" vines per biological replicate. These measurements occurred from July 6th, 2021, through July 28th, 2021, which is when veraison was observed as complete.

Potential vegetative growth and vigor differences among clones were evaluated by measuring canopy density by photovoltaic cell, known colloquially as a "Paso Panel" (County of San Luis Obispo). This was performed by placing a photovoltaic cell, attached to a voltmeter, under direct sunlight to measure a baseline voltage produced by the unimpeded light at solar noon. Then the panel was placed under the vine to be measured, once on both side of the canopy and the two voltages are averaged. Due to the vineyard alignment, the measurements were performed on the eastern and western sides of the vines. The produced voltage was then compared to the baseline, producing a percentage of voltage created under the vine. Theoretically, the higher this percentage is, the more light is passing through the vine. It can be said that a lower percentage of light passing through the canopy will indicate a denser canopy and more vegetative growth, whereas higher percentages will indicate a sparser canopy and, therefore, less vegetative vigor. These numbers were then compared amongst the clones. The grapevines were pruned in January and their pruning weights were measured by a HEETA Fish Scale and used to compare vegetative vigor. This pruning weights were used to calculate the RAVAZ Index of the clones by dividing yield per vine by its eventual pruning weight to monitor overall vine balance regarding vegetative growth and total fruit production (Ravaz 1903).

During ripening, grape berries were collected from data vines weekly from veraison until harvest for berry sugar accumulation in the form of total soluble solids measured as degrees Brix (grams of soluble solid per 100 g of solution) with a refractometer RFM110 (Bellingham + Stanley Ltd, Tunbridge Wells, UK), pH with an Orion-5-Star pH meter (Thermo Fisher Scientific Inc, Waltham, MA, USA) and titratable acidity (TA) with a DL50 Graphix titrator (Metter-Tolledo Inc, Columbus, OH, USA).

Grapes collected at harvest were analyzed for the basic chemical composition relevant to winemaking (Brix, pH, TA, malic acid, yeast nitrogen assimilable) as well as phenolic profile (individual and total flavan-3-ols, hydroxycinnamic acids, individual and total flavonols, and anthocyanins) by Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) (Peng, et al, 2002) and total phenols and tannins were determined by the Adams-Harbertson protein precipitation assay in order to predict potential sensorial effects of tannins (Harbertson et al 2015). Grape aroma volatile compounds were analyzed by an automated Headspace Solid-Phase Microextraction-Gas Chromatography-Mass Spectrometry (HS-SPME-GC-MS) as described in Hendrickson et al (2016).

2.3 Winemaking

For each clone, approximately 900 kg of grapes (except for FPS05 due to lower yields) from 80 grapevines were harvested when grapes reached optimal maturity. Wines were made at the UC Davis LEED Platinum Teaching and Research Winery (Davis, CA, USA). Grapes were destemmed and crushed using a Bucher Vaslin Delta E2 (Santa Rosa, CA, USA)

destemmer/crusher, and approximately 127 L of must was placed into 200 L stainless steel research fermentors. Fermentations were carried out in triplicate (n=3). Fermentation conditions were controlled by the Integrated Fermentation Control System (IFCS) units (Cypress Semiconductor San Jose, CA, USA) (Lerno et al. 2015).

Similar to the grape analysis, must chemistry was measured at the beginning of fermentation with a refractometer RFM110 (Bellingham + Stanley Ltd, Tunbridge Wells, UK). Must titratable acidity, pH, free SO_2, and total SO_2 was measured with an OMNIS Auto Titrator produced by Metrohm (Herisau, Switzerland). Must yeast assimilable nitrogen (YAN) was measured as a means of controlling yeast nutrition as a variable. This was performed using a Gallery Enzyme Master by Thermo Fisher (Waltham, MA, USA). An Anton Paar (Los Angeles, CA) portable density meter (DMA 35 Standard) was also used to monitor Brix and temperature daily until the completion of fermentation.

Prior to yeast inoculation, 50 mg/L of sulfur dioxide was added as a 15% potassium metabisulfite solution. Diammonium phosphate (DAP) (Omnisal GmBH, Lutherstadt Wittenberg, Germany) and tartaric acid (American Tartaric Products, Windsor, CA, USA) was used to respectively adjust the YAN and TA of the must to 250 mg/L and 6 g/L. Must was inoculated with *Saccharomyces cerevisiae* strain EC-1118 (Lallemand, Montreal, Canada) following their manufacturer's rehydration procedure. Fermentations temperature was controlled at 28°C with one tank volume pump-over twice a day, and wine samples were collected daily for anthocyanin and tannin extraction evaluation using models based on the protein precipitation assay (Harbertson et al. 2015) developed by Wine X Ray LLC (https://www.winexray.com/, Napa, CA, USA) using a Genesys10S UV-Vis Spectrophotometer (Thermo Fisher Scientific,

Madison, WI, USA). Fermentations were considered finished when wines are dry (<2 g/L residual sugar). The must was pressed on the eighth day of maceration using a basket press, and then wines were inoculated with *Oenococcus oeni* to initiate malolactic fermentation (MLF). Malic acid content of fermentation replicates was tracked using a Gallery Enzyme Master by Thermo Fisher Scientific (Waltham, MA, USA). After MLF was completed, wine free SO_2 was adjusted to 35 mg/L prior to bottling in Bordeaux style bottles with Saranex screw caps (Saranex/Transcendia, Franklin Park, IL, USA) in November of 2021 and stored at 15°C until chemical and sensory analyses.

2.4 Wine Chemical Composition

At the completion of MLF and prior to bottling, the wines were analyzed for basic chemical composition. The metrics considered were alcohol percentage, pH, TA, volatile acidity (VA), and free and total SO_2. Alcohol content was determined using an Alcolyzer by Anton Paar (Los Angeles, CA, USA). TA, pH, and SO_2 measurements were performed using an OMNIS Auto Titrator from Metrohm (Herisau, Switzerland). VA was measured using a Gallery Enzyme Master by Thermo Fisher Scientific (Waltham, MA, USA). The phenolic profiles of the wines were determined by RP-HPLC and the Adams-Harbertson Assay. Volatile aroma compounds were measured by HS-SPME-GC-MS. Wine phenolic profile analysis by RP-HPLC and protein precipitation as well as volatile aroma compounds analysis by HS-SPME-GC-MS were performed as described below.

2.5 Grape Phenolic Composition Sample Preparation

For each biological replicate composed of five data vines, three sets of 15 berries (approximately 20 g of grape tissue) were randomly selected from clusters at harvest. Phenolic compounds were extracted from whole berries as described by Girardello et al (2019). Grape berries were homogenized for 3 minutes at 1,355 x g using an IKA ULTRA-TURRAX®T18 basic homogenizer (IKA® Works, Inc., NC, USA). The homogenate was then combined with a solution of 1:1 ethanol (Sigma-Aldrich, St. Louis, MO, USA): water containing 0.1% hydrochloric acid (HCl) (Sigma-Aldrich, St. Louis, MO, USA) and 0.1% of ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA) at a ratio of 1 ml of solvent to 0.1 g of tissue and extracted overnight for 22 hours at 4°C. The samples were then centrifuged at 3,200 x g at 4°C for 15 minutes, and the subsequent supernatant liquid was decanted off the pellet and stored at -20°C. The pellet of the remaining tissue was then extracted with a solution of 70:30 acetone (Sigma-Aldrich, St. Louis, MO, USA): water containing 0.1% ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA) at the same ratio as the ethanol/water solution (1 ml/0.1 g of tissue) and stored overnight at 4°C for an additional 22 hours. Following this extraction period, the homogenate mixture was centrifuged at 3,200 x g at 4°C for 15 minutes. The two supernatants were combined and then concentrated under reduced pressure to approximately 5 ml at 35°C and transferred quantitatively to a 10 mL volumetric flask.

2.6 Grape and Wine Phenolic Profile Analysis

The phenolic profiles of the grape extracts and subsequent, finished wines were analyzed by RP-HPLC using an Agilent 1260 Infinity equipped with a PLRP-S 100A 3µM 150x4.6 mm column (Agilent Technologies, Santa Clara, CA, USA) at 35°C, an autosampler with temperature control at 8°C, and a diode array detector. Two mobile phases separate phenolic compounds: mobile phase A (water containing 1.5% (v/v) phosphoric acid (Sigma-Aldrich, St. Louis, MO, USA) and mobile phase B (80% acetonitrile (Sigma-Aldrich, St. Louis, MO, USA) and 20% mobile phase A). Twenty µl of the sample was injected with the mobile phase flow rate set at 1 ml/min. The separation gradient used in this analysis is outlined by Peng, et al (2002). The eluted compounds were identified by spectral and retention time comparisons to authentic standards at four different wavelengths: 280 nm (gallic acid, (+)-catechin, dimer B1, (-)-epicatechin, dimer B2, epicatechin gallate, and polymeric phenols), 320 nm (caftaric acid, caffeic acid, coutaric acid, *p*-coumaric acid), 360 nm (quercetin-3-galactoside, quercetin-3-glucuronide, quercetin-3-glucoside, and quercetin-3-rhamnoside) and 520 nm (anthocyanins and polymeric pigments). The identified compounds were quantified by external calibration curves. All data processing was performed with Agilent® CDS ChemStation software version D.04 (Agilent Technologies, Santa Clara, CA, USA). Calibration curves were constructed for gallic acid, (+)-catechin, (−)-epicatechin, caffeic acid, quercetin, *p*-coumaric acid, purchased from Sigma-Aldrich (St. Louis, MO, USA), and quercetin-rhamnoside and malvidin-3-*O*-glucoside chloride purchased from Extrasynthese (Genay, France). These compounds were quantified by themselves while other compounds were quantified as the following: B1, B2, epicatechin gallate, and polymeric phenols as (+)-catechin equivalents; caftaric acid as caffeic acid equivalents; coutaric acid as *p*-coumaric acid equivalents; quercetin-3-galactoside, quercetin-3-

glucuronide, quercetin-3-glucoside as quercetin-3-rhamnoside equivalents; and anthocyanins and polymeric pigments as malvidin-3-*O*-glucoside chloride equivalents. The results were calculated and are reported as “Gallic Acid”, “Hydroxycinnamic Acids”, “Total Flavonols “, “Total Flavan-3-ols”, “Total Glycosylated Anthocyanins”, “Total Acetylated Anthocyanins”, and “Total *p*-Coumarylated Anthocyanins”.

2.7 Grape and Wine Aroma Compound Analysis

Grape and wine volatile compounds were determined by HS-SPME-GC-MS. The analysis was carried out using an Agilent 7890A gas chromatography coupled to a 5975C inert XL EI MSD with a triple-axis detector (Agilent Technologies, Santa Clara, CA, USA) and controlled by Maestro (ver. 1.2.3.1, Gerstel Inc, Linthicum, MD, USA) using a method described by Girardello et al (2020b).

Grape samples were prepared for volatile compound analysis from four sets of 15 berries (approximately 20 g of tissue) collected at harvest as described in Hendrickson et al (2016), with few adaptations. The relative responses were normalized to berry tissue weight. Regarding the wine samples, two bottle replicates of each wine fermentation replicate were analyzed in triplicate. Ten ml of the sample was transferred to a 20 ml amber glass headspace vial (Agilent Technologies, Santa Clara, CA, USA) containing 3 g of NaCl (Sigma-Aldrich, St. Louis, MO, USA) and 50 µl of an internal standard (IS) solution of 2-undecanone (Sigma-Aldrich, St. Louis, MO, USA) (10 mg/L prepared in 100% ethanol). Each sample was analyzed in a random order using the following parameters: five minutes agitation at 500 rpm after reaching 30°C

followed by sample exposure to a 1 cm polydimethylsiloxane/divinylbenzene/carboxen (PDMS/DVB/CAR) (Supelco Analytical, Bellefonte, PA, USA), 23-gauge SPME fiber for 45 minutes. The initial oven temperature was kept at 40°C, while gas helium was used as carrier gas at a flow of 0.8636 ml/min, in a DB-Wax 231 ETR capillary column (30 m, 0.25 mm, 0.25 µm film thickness) (J&W Scientific, Folsom, CA) column, with constant pressure at 5.5311 psi. The oven temperature was kept at 40°C for 5 min and increased in increments of 3°C/min were performed until 180°C, with another increase of 30°C/min until 260°C was reached, which was then kept stable for 7.67 min. The SPME fiber was desorbed in a splitless mode for grape samples and split mode with a 10:1 split ratio for wine samples and held in the inlet for 10 min to prevent carryover effects. The method was retention time-locked to the internal standard, 2-undecanone. The total run time was 61.67 min and electron ionization was performed with a source temperature of 230°C and the quadrupole at 150°C. The samples were measured using synchronous scan and selected ion monitoring (SIM mode). The scan ranged from 40 m/z to 300 m/z, and compounds were detected using between two and six selected ions with a scan rate of 5.80 scans/sec.

Data analysis was performed using MassHunter Qualitative Analysis Software Version B.07.00 (Agilent Technologies, Santa Clara, CA, USA). Results were expressed as peak areas and were determined after normalization with 2-octanol (Sigma-Aldrich, St. Louis, MO, USA) for grape samples and 2-undecanone (Sigma-Aldrich, St. Louis, MO, USA) as internal standard for wine samples. Compounds were identified by their molecular and product ions and retention times in comparison with the National Institute of Standards and Technology database (NIST) (https://www.nist.gov). Specific aroma compound odors were identified and characterized for

discussion with the help of The Good Scents Company Information System database (www.thegoodscentscompany.com).

2.8 Wine Sensory Evaluation

Due to the restrictions imposed by the University of California, Davis (UCD) during the Covid-19 pandemic, sensory evaluation of the wines made in the first two years of this study were outsourced to Applied Sensory, LLC (http://www.appliedsensory.com). Thus, to keep sensory evaluation methodology consistent over the three years of the study, the sensory evaluation of wines made during the season under discussion was also performed by Applied Sensory, LLC.

Wines were evaluated by descriptive analysis (DA) over two sessions by six trained panelists. A red wine descriptive analysis scorecard containing aroma, mouthfeel, and taste attributes as well as color scores were developed. Bench tasting identified the following aroma descriptors: Ethyl Acetate/Volatile Acidity Aroma, Sulfide Aroma, Specific Chemical Aromas (Rubber, Onion), Red Fruit Aroma, Specific Red Fruit Aromas (Cassis, Strawberry, and Raspberry), Dark Fruit Aroma, Specific Dark Fruit Aromas (Plum, Blackberry, and Blueberry), Jam/Dried Fruit Aroma, Specific Jam/Dried Fruit Aromas (Berry Jam, Raisin, and Prune), Herbal Aroma, Specific Herbal Aromas (Menthol and Stemmy), Vanilla Aroma, and Chocolate Aroma. Panelists were refreshed on recognition of these attributes with reference standards prior to evaluations.

A new bottle of wine per fermentation replicate was opened. A modified Latin square design was used to randomize the wine presentation to the panelists. The wines were served in clear, tulip-shaped wine glasses of 220-ml capacity and coded with 3-digit random number codes. A 60-ml (at 20°-24° C) wine sample was added to each glass and covered with a 5.7 cm diameter plastic Petri dish for at least 15 minutes prior to the two evaluations. The tests were conducted in a room with “daylight” fluorescent lighting and daylight lamps in each booth. The panelists were separated by dividers and not allowed to communicate while the session took place. Panelists expectorated the wines and rinsed their mouths with bottled water between tastings. Each judge rated the intensity of the attributes for the different wines using structured 10-point (0 to 9) scales. These scales were anchored at the ends with terms “weak” and “strong”, “low” and “high”, “thinner” and “thicker”, or “short” and “long” depending on the attributes rated.

2.9 Data Analysis

The resulting, raw data produced from the mentioned analyses were analyzed using a combination of Microsoft Excel for Mac (version 16.66.1) and XLSTAT (version 24.2.1). The base package XLSTAT was used to run analyses of variation (ANOVAs) on all the data to establish any statistical, significant differences at an alpha level of 0.05 (95% confidence). This involved the production of Least Squared Means when appropriate and ANOVAs that used the Tukey Test to establish significant differences. All Principal Component Analysis (PCA) figures were performed

using the XLSTAT base package. Multiple Factor Analysis (MFA) was performed by using the premium package of XLSTAT that includes software to perform sensory data analysis.

Section 3. Results and Discussion

3.1 Viticultural Evaluations

3.1.1 Vine Nutrient Status

Table 1. Vine nutrient status in May of 2021 (n=5).

Clone	Bio Replicate	N (%)	P (%)	K (%)	Zn (mg/kg)	Mn (mg/kg)	Na (%)	B (mg/kg)	Ca (%)	Mg (%)	Fe (mg/kg)	Cu (mg/kg)
1	1.1	0.91	0.47	1.08	39.7	79.8	0.01	34.8	1.33	1.31	49.8	16.2
1	2.3	0.86	0.56	0.87	113	140	0.01	35.9	1.74	1.48	31.6	23.7
1	3.3	0.94	0.54	1.30	43.1	116	0.01	36.9	1.44	1.39	29.1	22.7
1	4.3	0.98	0.49	1.02	36.1	87.1	0.01	37.0	1.31	1.36	30.5	23.2
3	1.3	0.86	0.57	1.13	65.8	105	0.01	35.2	1.73	1.45	30.6	24.0
3	2.1	0.89	0.53	1.03	68.0	101	0.01	34.6	1.53	1.55	30.3	19.4
3	3.1	0.87	0.48	1.06	38.6	84.4	0.01	34.8	1.51	1.42	33.5	21.5
3	4.4	0.85	0.43	1.37	30.7	81.0	0.01	42.3	1.55	1.50	33.1	35.6
4	1.2	0.88	0.53	1.28	39.0	82.3	0.01	35.3	1.57	1.24	32.2	20.7
4	2.4	0.94	0.54	1.03	44.3	111	0.01	37.9	1.60	1.46	27.2	21.3
4	3.2	0.89	0.49	0.97	35.1	100	0.01	34.2	1.51	1.37	28.1	21.1
4	4.2	0.97	0.42	1.03	39.7	73.6	0.01	37.4	1.26	1.37	30.2	24.3
5	1.4	0.82	0.55	0.93	45.8	101	0.01	33.6	1.82	1.48	30.8	26.6
5	2.2	0.80	0.56	0.95	76.8	111	0.01	37.3	1.64	1.42	31.4	24.4
5	3.4	1.01	0.43	1.30	42.6	93.6	0.01	37.2	1.41	1.34	28.3	22.7
5	4.1	0.92	0.41	0.79	37.6	87.5	0.01	35.3	1.23	1.36	28.9	19.1

* Red denotes excessive levels, black denotes normal/optimal levels, green denotes low levels, and blue denotes deficient levels

Vine nutrient status was evaluated to control nutrition as a variable amongst clones, with Table 1 showing the resulting nutrient concentrations. While these numbers were not evaluated with the intention of identifying potential clonal differences, an ANOVA was still performed to see if there were any significant differences among the clones. However, no significant differences were observed. Another reason this data is included in this thesis is to

note the excessive levels of magnesium and deficient levels of potassium found in all the grapevines and throughout the vineyard. It can be hypothesized that the high levels of magnesium in the UC Davis RMI vineyard could have potentially affected the vines' potassium content as excessive magnesium in the soil can interrupt the plants' root cation exchange mechanism, leading to deficiencies in other cations such as calcium and potassium (Hannan 2011). It is also possible that the RMI vineyard contains low levels of potassium, but soil analysis was not performed in this study. Potassium deficiency in grapevines has been shown to produce plants with stunted shoot growth and, therefore, a less dense canopy, which can adversely affect fruit set and ripening (Nadeem et al 2018). In order to prevent this, potassium was applied both through the drip irrigation system and as a foliar spray. While this helped rectify any vegetative vigor issues, this may have contributed, in part, to the higher pH levels found in the wines as potassium can precipitate with acids, such as tartaric acid and increase the pH and decrease titratable acidity during different stages of the vinification process (Morris et al 1980).

3.1.2 Vine Water Status

Figure 1 shows a linear plot of the four clones and their respective water potentials throughout 13 weeks of the summer growing season in 2021, beginning June 3rd and concluding August 26th. It is worth noting that a heatwave event occurred between the 2nd and 3rd weeks of sampling and an associated, corresponding spike in negative water potentials can be seen in Figure 2. Like the vine's nutrient statuses, these measurements were mainly focused on

controlling vine water status as a variable. As in the vine nutrition evaluations, performing an ANOVA on each weekly sample indicated no significant differences among clones. This indicates

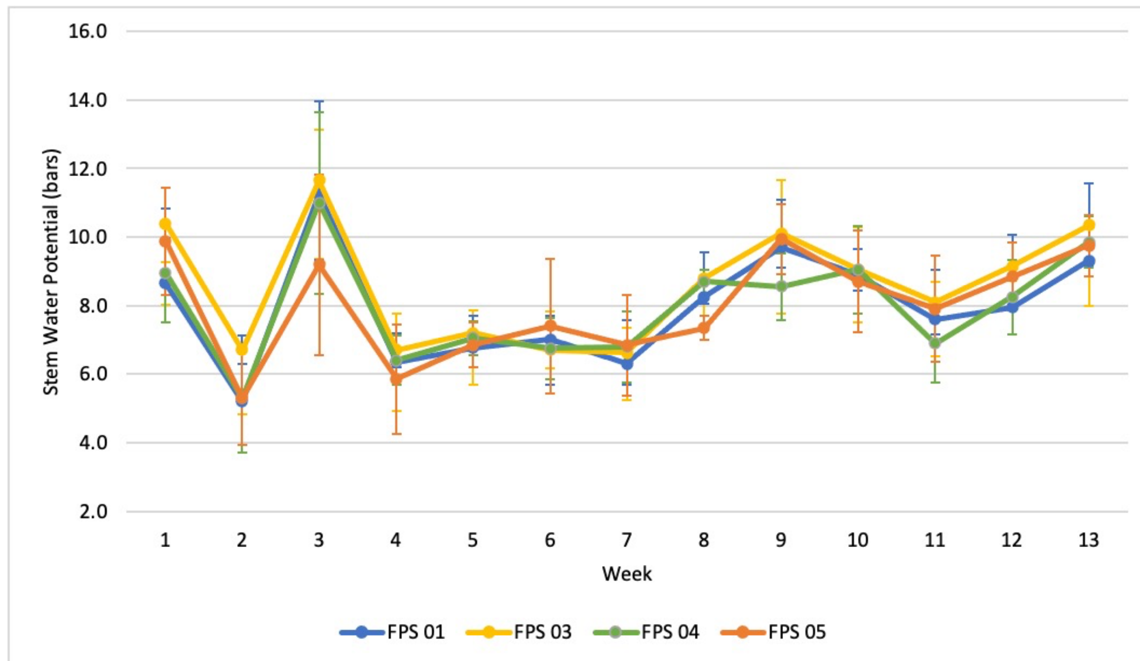

Figure 1. Stem water potential measurements during the 2021 growing season (n=4) with significance determined by ANOVA at $p < 0.05$.

that there are no statistically significant impacts of selecting one clone over another in terms of water status and, likely, drought resistance or sensitivity. Another interesting evaluation would be to investigate different scion/rootstock interactions to see if there were differences amongst the clones when different rootstocks were used. It is well known that different wine grape cultivar scions and rootstock interactions play an important role in overall vine performance. It is also possible for specific clonal differences when it comes to clone/rootstock interactions as there has been research that has shown how different clone and rootstock combinations can give rise to different levels of graft efficacy, vegetative vigor, environmental adaptability (e.g., cold hardiness), and other phenological traits related to agronomical performance (Tedesco et al 2020; Hébert-Haché et al 2021;). However, effects of rootstock interactions were outside the scope of this study.

3.1.3 Veraison Rate

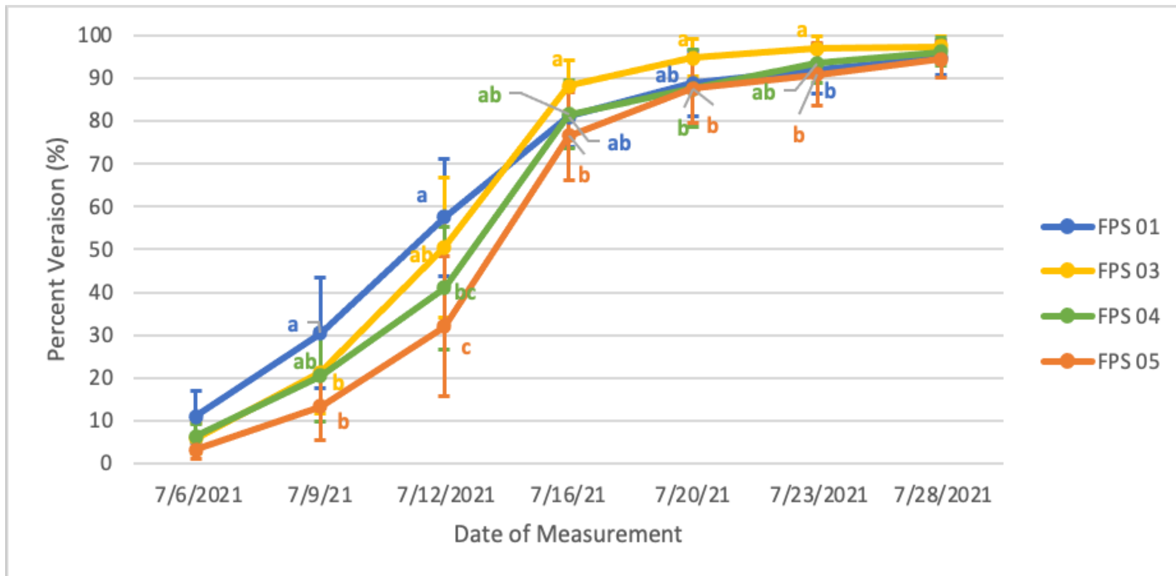

Figure 2. Veraison tracking of clones beginning at the first sign of color change until completion of veraison (n=4).
* Significant terms determined by ANOVA at $p < 0.05$

While there were some significant differences among clones throughout veraison, it is worth noting that all four clones had no statistically significant differences between both the beginning and final observations made (Figure 2). In other words, the clones did not exhibit differences in both the timing of the start or end of veraison which could indicate little differences in how the onset of ripening is regulated hormonally. Based on this year's data, clones may have similar genetic makeups related to the expression and generation of specific plant hormones responsible for regulating ripening such as abscisic acid (ABA) (Pilati et al 2017).

3.1.4 Vine Vegetative Vigor and Balance

Table 2. Vine vigor and balance measurements (n=4).

Clone	% of Voltage From Light Through Canopy	Weight of Pruned Canes (kg)	RAVAZ Index (kg of grapes/kg pruning weight)
FPS 01	62.66 ± 10.16	0.711 ± 0.103	9.28 ± 0.71
FPS 03	67.24 ± 9.03	0.565 ± 0.178	11.56 ± 2.51
FPS 04	62.51 ± 11.18	0.658 ± 0.107	10.36 ± 0.70
FPS 05	66.98 ± 10.71	0.630 ± 0.146	10.04 ± 0.71

* No significant differences by ANOVA at $p < 0.05$

Vine vegetative vigor was evaluated in two ways: canopy density and pruning weights. Canopy density was measured using a “Paso Panel” in July of 2021 to evaluate how much sunlight passed through the canopy at solar noon. An increase in light passing through the canopy can be interpreted as a sparser canopy and lower vegetative vigor. The results are shown in Table 2.

Data analysis showed that there were no significant differences among clones regarding canopy density, with all clones allowing similar amounts of light to pass through their canopies. In the same table, the results for pruning weights performed in January of 2022 can be seen. Again, there were no significant differences found in pruning weights among clones. These pruning weights were also used to calculate RAVAZ Index, a commonly used metric to evaluate vine vegetative and fruitfulness balance. Research suggests that a “healthy” RAVAZ Index value

can fall between 5-10 (Bravdo et al 1985). Given this information, it seems as though FPS01 falls within that range, which suggests that this clone could be the most “balanced” of all the clones.

However, at 95% confidence, there were no significant differences among clones. If this interval was expanded to 90%, it could be said that FPS01 had the lowest RAVAZ Index (less fruit per vegetative growth mass) and FPS03 would have the highest (more fruit per vegetative growth mass). However, at the 90% level, this should be interpreted as a possible trend, not a confirmed finding. Based on this information, there are no real recommendations that can be made for clone selection when considering vegetative vigor.

3.1.5 Ripening Chemistry

Figures 3A, B, C, D show the four metrics that were measured during grape ripening. Brix (A), pH (B), titratable acidity (C), and malic acid content (D) were all found to have no significant differences among clones during ripening. Therefore, no real recommendations in terms of clonal selection can be given when concerning basic, wine chemical compound ripening rates.

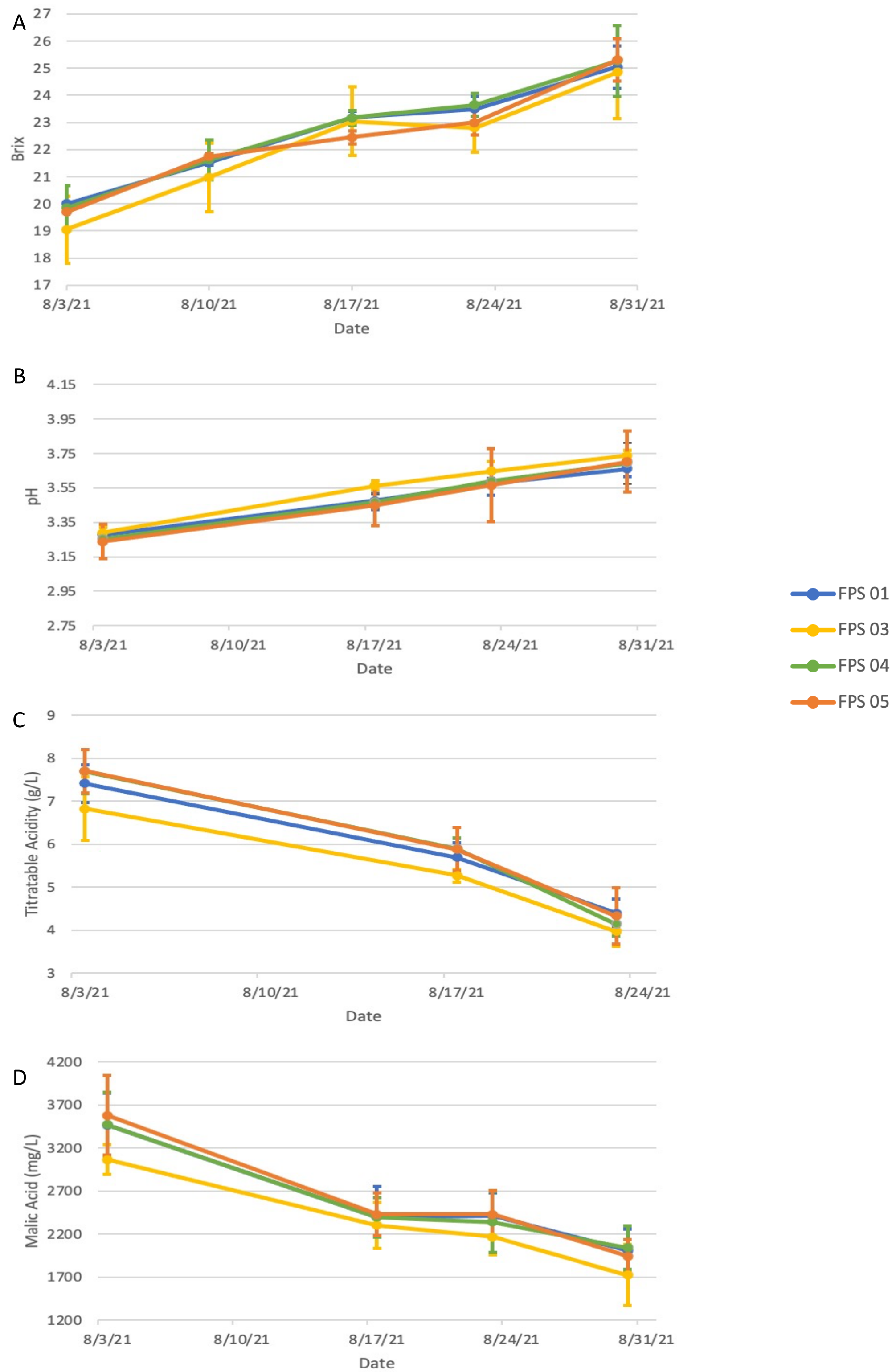

Figure 3. Ripening parameters including Brix (A), pH (B), Titratable Acidity (C), and Malic Acid (D) sampled post-veraison until harvest (n=4).
* Significant differences and significant groups determined by ANOVA at $p < 0.05$

3.2 Post-Harvest Grape Evaluations

3.2.1 Yield Components and Cluster Condition

Table 3. Yield components and cluster condition evaluated at harvest (n=4).

Clone	FPS 01	FPS 03	FPS 04	FPS 05
	Yield Components			
Cluster/vine*	15 ± 1 b	17 ± 2 a	16 ± 1 ab	16 ± 2 ab
Yield/vine (kg)	6.24 ± 0.69	6.15 ± 2.16	6.40 ± 0.65	5.99 ± 0.36
Cluster mass (g)	599.71 ± 154.38	532.68 ± 182.43	569.87 ± 137.45	533.40 ± 119.49
Mass of 20 berries (g)	25.94 ± 1.96	24.99 ± 4.53	25.47 ± 1.97	24.77 ± 2.84
Berry Mass (g)	1.30 ± 0.1	1.25 ± 0.2	1.27 ± 0.1	1.24 ± 0.14
Berries/Cluster	465 ± 106	412 ± 111	448 ± 91	422 ± 56
Total Yield (kg)	928	884	880	714
	Cluster Condition			
Good Clusters (%)	74.7 ± 7.1	74.9 ± 9.0	75.5 ± 7.8	73.1 ± 17.8
Sunburnt Clusters (%)	20.4 ± 4.9	18.9 ± 7.9	18.9 ± 6.5	21.4 ± 19.6
Rotten Clusters (%)	0.5 ± 0.9 b	4.4 ± 3.55 a	1.5 ± 2.1 b	0.8 ± 0.8 b
Green Clusters (%)	4.9 ± 2.39	4.3 ± 4.9	5.3 ± 4.0	7.8 ± 3.0

* Significant differences and significant groups determined by ANOVA at $p < 0.05$

Table 3 shows the results from the evaluations of yield and cluster condition. There were no significant differences at 95% confidence outside of number of clusters per vine with FPS01 having the least and FPS03 the most. It is important to note that this may have been affected by vineyard practices.

As in the previous years of this study, vines were thinned to one shoot per spur and one cluster per shoot. This means that there should be as many clusters per vine as spurs per vine.

Considering that FPS01 was observed as belonging to its own significant group for clusters per vine and FPS03 was observed as belonging to its own significant group for having more clusters per vine, these results seem to indicate that FPS01 may have had one less spur on average than the other clones and that FPS03 possibly had one more. FPS01 may have had shorter cordons or could have been missing one spur position in comparison to the other clones. Given this discussion, further studies may be required before definitively stating that there were significant differences among clone yield components.

It is interesting to compare these results with vine balance results from the RAVAZ index. FPS01 produced one less cluster on average and was the clone that produced vines that were potentially the most balanced. FPS03 produced the most clusters and results indicated that this clone could be the most unbalanced. This could suggest that Petite Sirah could benefit from extensive cluster thinning to ensure a balance between vegetative growth and fruit production. Although this could be site specific, this cultivars clusters are very large and sugar and phenolic accumulation could be reduced if it is allowed to over-fruit.

There were no significant differences among the clones when cluster condition was considered. However, there was a possible trend of clone FPS03 containing more rotten clusters, but further study would need to be performed to confirm this. The total yield from FPS05 was an outlier compared to other clones using a 90% confidence interval. Additional study over the course of multiple vintages may show that this clone could be lower yielding.

3.2.2 Grape Phenolic Content

Table 4. Grape phenolic compounds quantified by reverse phase- high performance liquid chromatography (n=4).

	Clone			
Compounds (In mg/g Grape)	**FPS 01**	**FPS 03**	**FPS 04**	**FPS 05**
Gallic Acid	0.010 ± 0.003	0.005 ± 0.001	0.008 ± 0.001	0.010 ± 0.003
Flavan-3-ols[a]	0.158 ± 0.029 a	0.098 ± 0.012 b	0.172 ± 0.014 a	0.163 ± 0.012 a
Hydroxycinnamic Acids	0.014 ± 0.001 b	0.012 ± 0.002 b	0.017 ± 0.003 a	0.012 ± 0.001 b
Total Flavonols	0.096 ± 0.014 b	0.076 ± 0.023 b	0.135 ± 0.034 a	0.097 ± 0.011 b
Flavonol Glycosides	0.092 ± 0.014 b	0.074 ± 0.022 b	0.125 ± 0.032 a	0.090 ± 0.011 b
Flavonol Non-Glycosides	0.004 ± 0.001 c	0.002 ± 0.001 d	0.011 ± 0.001 a	0.006 ± 0.0003 b
Total Monomeric Anthocyanins	0.942 ± 0.188 a	0.852 ± 0.108 a	0.352 ± 0.116 b	0.548 ± 0.191 b
Anthocyanin-glucosides	0.534 ± 0.121 a	0.512 ± 0.081 a	0.209 ± 0.077 b	0.317 ± 0.125 b
Anthocyanin-acetylglucosides	0.184 ± 0.037 a	0.175 ± 0.026 a	0.065 ± 0.022 b	0.108 ± 0.034 b
Anthocyanin-*p*-coumarylglucosides	0.220 ± 0.032 a	0.218 ± 0.015 a	0.077 ± 0.017 b	0.112 ± 0.026 b
Polymeric Pigment	0.053 ± 0.005 a	0.043 ± 0.009 ab	0.035 ± 0.005 b	0.049 ± 0.010 a

* Significant differences and significant groups determined by ANOVA at $p < 0.05$

[a] The flavan-3-ol class is sum of concentrations of gallocatechin, epigallocatechin, (+)-catechin, (-)-epicatechin, B1, and B2

Two different methods were used to quantify the phenolic content in both the grapes and wines. This was done because different, accepted methods of phenolic measurement have higher correlation and accuracy when measuring the different phenolic compounds found in samples (De Beer et al 2004). Reverse phase-high performance liquid chromatography (RP-HPLC) was deemed appropriate for almost all the classes of phenolic compounds as published literature has shown this method to be successful in accurately measuring total phenols, monomeric flavan-3-ols, monomeric anthocyanins, and other color monomers (De Beer et al

2004). On the other hand, protein tannin assays (such as Adams-Harbertson (AH) assay used in this study) have been found to be better at measuring polymerized flavan-3-ols, the main component of wine tannins. The results of these assays can be highly correlated with the perceived astringency of a wine as it can measure the tannins that are precipitable by a protein solution that is comparable to salivary proteins (Kennedy et al 2006). As winemakers care a great deal about the perceived astringency of their grapes and wine, this method is an appropriate inclusion to this study.

Except for gallic acid, the phenolic content of the different clones showed differences in all phenolic categories and compound classes evaluated by RP-HPLC (Table4). It is noteworthy that significant differences were observed given the fact that some of the results exhibited relatively high amounts of variability among clonal bio-replicates, which can often be seen due to vineyard variability. Even with this variability, clones could be separated from one another.

Table 4 shows how clones FPS01, FPS04, and FPS05 all had similar levels of flavan-3-ols with FPS03 exhibiting significantly lower levels. Like other flavonoids, flavan-3-ols play an important role in plant stress responses to biotic and abiotic stressors (Treutter 2005; Ullah et al 2017). This could potentially explain FPS03's possible trend of producing more rotten clusters as this clone has significantly lower levels of flavan-3-ols in comparison to other clones. This clone could have had decreased chemical protection against biotic stressors.

There are also important implications of flavan-3-ols in grapes as their concentration could have possible effects on wine. When monomers of flavan-3-ols polymerize, they produce proanthocyanidins which are the essential building blocks for tannins which can be perceived by consumers and affect the sensory experience of a wine (Aron and Kennedy 2008). Also,

these compounds can react with anthocyanins during wine aging, which increases the color stability of a wine while reducing astringency (Vrhovsek et al 2002). Therefore, the number of flavan-3-ols in grape tissue may ultimately influence the color, age-ability, and overall quality of a wine. It is also important to remember that the sample preparation for these measurements in this study involved the pulverization of both skins and seeds, which may release more seed tannins than otherwise expected to be extracted during winemaking. This practice may release more of the short-chain flavan-3-ol polymers that can be found in the seed endosperm, which can lead to wines that have excessive bitterness and astringency (Pavez et al 2022). Thus, this measurement may be most useful for understanding the maximum number of tannins that could be extracted from grapes produced by the clones.

Despite differences in flavan-3-ols found by RP-HPLC, the AH assay for tannins (Table 5) showed no statistically significant differences among clones. As mentioned above, the AH-assay is dependent upon the precipitation of tannins with a protein solution. Therefore, this method will only measure the tannins that are precipitable, which means this method is also the highest correlated with perceived astringency of a sample. The reaction responsible for the measurement of tannin during the assay is like the one which occurs between tannins and the salivary proteins in one's mouth (Gawel 1998). Longer polymers of tannins are more easily precipitable (both with the protein serum used in this study and with the components that make up saliva), with the efficacy of precipitation increasing as polymers increase in size (Harbertson et al 2014). RP-HPLC measured flavan-3-ols are a group containing monomers and short oligomers whereas the AH assay measured the longer polymers that interact with proteins. While RP-HPLC showed differences in grape tannin components, there would likely be

little to no differences in perceivable astringency among clonal grape tissue. Producers may care only for the levels of perceptible astringency, making the AH results for tannins useful. That said, the monomeric flavan-3-ols could polymerize over time in an active fermentation or finished wine medium, eventually affecting the astringency of a wine.

Table 5. Phenolic content of grapes determined by Adams-Harbertson Assay (n=4).

Clone	Total Phenolics (mg/g Grape CE)	Tannins (mg/g Grape CE)	Anthocyanins (mg/g Grape M3G)
FPS 01	5.68 ± 0.75	4.90 ± 0.41	1.85 ± 0.46 a
FPS 03	5.89 ± 1.18	5.02 ± 0.81	1.81 ± 0.15 a
FPS 04	6.05 ± 0.82	4.80 ± 0.45	0.81 ± 0.23 b
FPS 05	6.80 ± 0.37	5.12 ± 0.39	1.17 ± 0.32 ab

* Significant differences and significant groups determined by ANOVA at $p < 0.05$

In terms of flavonol content, FPS04 contained significantly more than all the other clones, which were not significantly different from each other. This was true for both the glycosylated and non-glycosylated forms. The role of flavonols in wine are not as well understood as tannins or anthocyanins. However, they are involved with color copigmentation and stabilization through reactions with anthocyanins, meaning that wines and fruit juices that contain more of these compounds will have more stable color that is less susceptible to loss through oxidation and changes in temperature (Boulton 2001; Cao Y et al 2023). Based on these results, grapes from FPS04 would seem to have the potential for more copigmentation and its young wines could be more intensely colored. In plants, flavonol synthesis is stimulated by UV light exposure and plays the role of “sunscreen” in fruit to prevent excessive sun burning and damage (Sternad-Lemut 2013). FPS04 had canopy densities and pruning weights that were

similar to the other clones and was not overcropped. This implies that this clone's clusters were exposed to similar levels of sunlight in comparison to the other clones. Given this, it seems that FPS04 may be able to accumulate these compounds more readily than the other clones evaluated. This could potentially imply that FPS04 may be more resistant to sunburn. However, cluster condition results did not seem to indicate this as all clones had similar levels of sun burnt clusters.

There was a clear distinction of two separate significant groups when it came to anthocyanin content, the compounds that are primarily responsible for color in grapes and wine. FPS01 and FPS03 contained higher levels and FPS04 and FPS05 contained lower levels of anthocyanins. These relationships persist throughout the different forms (glycosylated, *p*-coumarylated, and acetylated) of anthocyanins as well. It is worth noting that these results were somewhat correlated with the results found in the AH assay; FPS01 and FPS03 had higher levels of anthocyanins and FPS04 had significantly lower levels. While the HPLC results for anthocyanins is considered more accurate and precise for this compound class, it was reassuring to see that the AH-assay produced similar results.

It was interesting to see that FPS04 had very high levels of flavonols, and the least anthocyanins based on the RP-HPLC results. This could be due to how the phenolic synthesis pathways behave in this clone. Flavonols and anthocyanins are both different termini in this pathway and it is possible that FPS04 is predisposed for flavonol production in lieu of anthocyanins (Šikuten et al 2020). Additional years of study and data would be required to confirm this.

The hydroxycinnamic acid content of the clones was also found to be significantly different. FPS04 was found to contain the highest amount of these acids, with FPS03 and FPS05 were found to contain the least amount, and FPS01 fell in the middle of all the clones. Hydroxycinnamic acids play a role in potential off-aroma compounds typically associated with spoilage caused by microbes, namely *Brettanomyces bruxellensis* (Schopp et al 2013). Perhaps more relevant, these compounds are a principal enzymatic oxidation component as well as a strong cofactor for copigmentation (Bimpilas et al 2016). White wines with higher levels of hydroxycinnamic acids have been shown to brown more easily (Fernández-Zurbano et al 1998). Conversely, their role as a copigmentation cofactor suggests that grapes and wines containing more of these compounds may show the associated increased color intensity. Considering this, FPS04 grapes may produce wines that have the potential to show more oxidative browning but also increased color intensity through copigmentation.

3.2.3 Grape Volatile Aroma Compounds

Of the 54 aroma compounds measured by HS-SPME-GC-MS, 26 aroma compounds were found to show significant differences among the clones. In Figure 4, a PCA of the significant aroma compounds are shown.

The PCA indicates that clonal, biological replicates were clustered together with FPS01, FPS03, and FPS04 being relatively close together and FPS05 positioned on the opposite side of the biplot. The biological replicates being positioned together is encouraging as this suggests

that differences in aroma profile are more likely due to clonal differences and not vineyard or sample preparation variability.

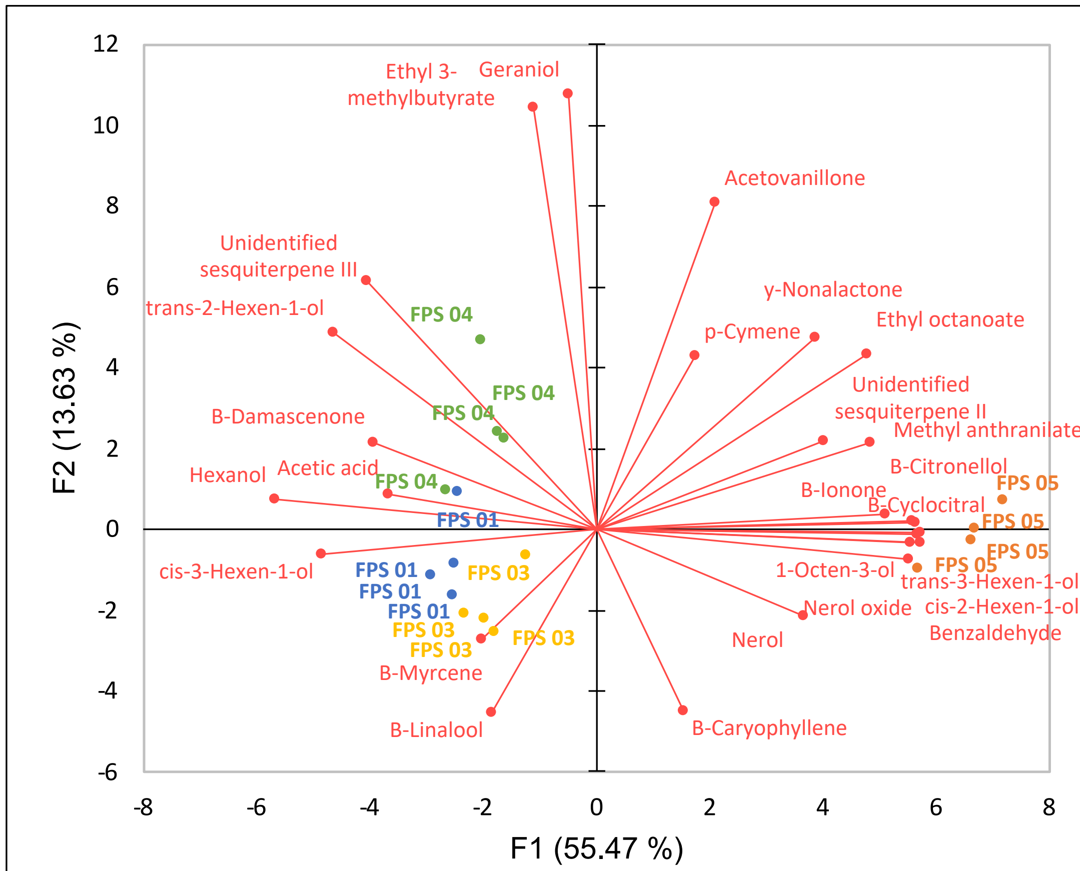

Figure 4. Principal component analysis (PCA) of significant aroma compounds in Petite Sirah clone grapes and biological replicates (n=4).

* Significant aroma compounds determined by ANOVA at $p < 0.05$

Results indicate that FPS01, FPS03 and FPS04 were more like one another in regard to aroma composition in comparison to FPS05, which can be viewed as the most different. This shows that there are clonal differences in grape aroma compound content. A closer examination of the PCA based on the relative responses for each significantly different aroma compound illustrates the major differences among clones.

The fermentation replicates of FPS05 are located on the right side of the biplot, on the x-axis, and far from the other clones. A look into the aroma compounds in close vicinity to the FPS05 replicates shows that this clone was highly correlated with 1-octen-3-ol (earthy; mushroom, green, fungal), benzaldehyde (fruity; almond, sweet, cherry), *cis*-2-hexen-1-ol (green; leafy, green bean), *trans*-3-hexen-1-ol (green; leafy, oily, petal) β-cyclocitral (tropical; saffron, herbal, rose, sweet, tobacco), β-Ionone (floral; woody, sweet, berry), β-Citronellol (floral; leathery, waxy, rose) and methyl anthranilate (fruity; grape, orange flower).

Given that the other three clones were positioned opposite FPS05, this implies that FPS01, FPS03, and FPS04 were inversely correlated with all the compounds that were shown to be correlated with. In contrast, the compounds correlated with the other three clones were inversely correlated with FPS05.

FPS04 showed high correlation with hexanol (herbal; ethereal, fusel oil, green), acetic acid (acidic; vinegar, sour), β-damascenone (floral; rose, plum, raspberry, grape), and *trans*-2-hexen-1-ol (fruity; leafy, unripe banana, fresh). FPS04 seemed to differ slightly from FPS01 and FPS03, primarily in its higher levels of β-damascenone and *trans*-2-hexen-1-ol, which can mean that FPS04 will be perceived as being fruitier than FPS01 and FPS03.

FPS01 and FPS03 seemed to be the most like one another in terms of aroma compound content. FPS01 was correlated with β-myrcene (spicy; peppery, terpenic, balsam), *cis*-3-hexen-1-ol (green; fresh, grassy, foliage), hexanol (herbal; ethereal, fusel oil, sweet), and acetic acid (acidic; vinegar, sour). In FPS01's case, this clone differed itself from FPS04 with higher levels of β-myrcene and *cis*-3-hexen-1-ol, potentially having a more spicy/peppery aroma due to the β-myrcene.

FPS03 grapes were like FPS01 as it contained similar levels of β-myrcene and *cis*-3-hexen-ol. However, it seemed that this clone may have a had slightly higher levels of β-linalool, which may add a floral or citrus element to the aroma of FPS03 grapes in comparison to the others.

While there were seemingly subtle differences among clones FPS01, FPS03, and FPS04, the larger differences of aroma compound profile could be found between these three clones and FPS05. FPS05 grapes contain compounds that could potentially impart more earthy, nutty, leathery, and general tropical overall aroma in comparison to the other clones. The other three clones could be a bit more fruity, fresh, and grassy by comparison.

It was interesting to see that some of the aroma compound differences among the clones were found in different isomers of the same compounds, specifically 2-hexen-1-ol and 3-hexen-1-ol. For example, FPS01, FPS03, and FPS04 were correlated with *cis*-3-hexen-1-ol and *trans*-2-hexen-1-ol. In comparison, FPS05 was inversely correlated with these and correlated with *trans*-3-hexen-1-ol and *cis*-2-hexen-1-ol. It has been shown that different samples of the same *Vitis vinifera* cultivar grapes can have different ratios of isomer composition of aroma compounds (Río Segade et al 2022). These results seem to suggest that this may be possible among the clones of a single cultivar as well.

Overall, these results could also suggest to producers that when deciding which clone to select, grapes from FPS01, FPS03, and FPS04 may show little difference in terms of aroma whereas FPS05 may be different. However, the winemaking process has the potential to alter the aroma profile found in grapes prior to fermentation and aging (Ferreira and Lopez 2019). Given this, while the grape aroma profile may consist of odors and other organoleptic

properties, the finished wines made from these clones may not maintain those same, specific properties and differences.

3.2.4 Must Chemistry

Table 6. Harvest must basic wine chemistry analysis (n=3).

Clone	Brix*	pH	TA* (g/L)	YAN* (mg/L)	Malic Acid* (mg/L)
FPS 01	26.4 ± 0.1 a	3.60 ± 0.02	5.08 ± 0.07 b	241 ± 4 a	1958 ± 16 c
FPS 03	25.3 ± 0.4 b	3.59 ± 0.02	4.96 ± 0.06 b	201 ± 9 c	1881 ± 21 c
FPS 04	26.4 ± 0.1 a	3.56 ± 0.04	5.04 ± 0.09 b	225 ± 5 b	2150 ± 35 b
FPS 05	25.7 ± 0.2 b	3.65 ± 0.06	6.60 ± 0.44 a	196 ± 3 c	2483 ± 107 a

* Significant groups determined at $p < 0.05$

At the time of harvest, the musts produced by each clone was measured for basic chemical parameters relevant to winemaking (Brix, pH, titratable acidity, yeast assimilable nitrogen (YAN), and malic acid content) as shown in Table 6. With this information, growers and winemakers may be able to better select clones based on their specific sites/growing regions and their desired wine characteristics. Outside of pH, the clones showed significant differences in all other metrics.

Clones FPS01 and FPS04 showed similar levels of Brix, whereas clones FPS03 and FPS05 contained lower levels. While significant, the differences in Brix level among clones was small and additional years of study will be necessary to confirm if these differences are consistent. There are also a few possible reasons for this disparity in sugar accumulation. It has been established that FPS03 was potentially imbalanced, with too much fruit, based on its RAVAZ

index measurements. Having too little vegetative growth to support the amount of fruit on FPS03 vines may have resulted in a dilution of the final sugar amounts in the berries.

Assuming that this was not the cause of the differences, and any differences were indeed based on clone, this information potentially allow producers to select clones that may be slower and/or faster to ripen given their specific needs. For example, producers primarily focused on sugar accumulation in their grapes and located in warmer climates may elect to choose clones FPS03 and FPS05 as they were slower to accumulate sugar and will not "overripen" in comparison to FPS01 and FPS04. On the contrary, producers located in cooler climates (or those who desire to pick early due to wildfire season, etc.) may elect to choose FPS01 or FPS04 as they will ripen earlier and still produce wines with desired alcohol levels and/or mature fruit aroma characteristics.

Titratable acidity (TA) analysis produced two distinct significance groups and FPS05 had higher levels of acidity in comparison to the other clones. This is especially interesting considering FPS05 also had a similar pH value in comparison to other clones. Logic would dictate that having a higher significantly higher TA would correlate with a lower pH, but this did not occur. A closer look at how tartaric acid dissociates at different pH values may present potential explanations of this phenomenon. Tartaric acid is a weak, diprotic acid and dissociates at a range of pH values. At approximately pH 3.65 a large shift in dissociation takes place, with the tartrate and bitartrate forms now both favored over the acid form (Rajkovic et al 2007). At this pH, tartaric acid additions as a winemaking practice have a different outcome. Under this value, tartaric acid additions result in a decrease in pH and additions at > 3.65 pH can potentially decrease TA while increasing pH (AWRI 2018). In both cases, cations (typically

potassium) are involved with precipitation reactions with bitartrate and tartrate anions, which further affects the acid equilibrium, either releasing or capturing hydrogen ions (AWRI 2018). This is all relevant due to FPS05's pH at harvest: 3.65. At this value, the dissociation and equilibrium involving tartaric acid, the predominant acid in grape must, is in flux and could have affected TA measurements. This could be supported by the standard deviation of FPS05's TA values, which is almost an order of magnitude higher than the other clones. This decreased precision may be the result of altered TA values due to different ratios of dissociation occurring around this pH. Unfortunately, without a deeper investigation into the must chemistry, there is no way to confirm this. If the results in differences in TA are to be believed, this may indicate that FPS05 may be a better selection if a producer desires a clone that maintains acidity and ripens later.

However, the higher TA value does seem to agree with malic acid results as FPS05 also has higher levels. Three significant groups were determined, with FPS05 containing the highest levels followed by FPS04. FPS01 and FPS03 were found to have the lowest concentration of malic acid present. FPS05 still had a bit of imprecision as it again had a higher standard deviation. This could support the theory that TA results were accurate, and imprecision was simply affected by a lack of homogeneity in FPS05 replicates.

Post-veraison, grape vines utilize malic acid as a fuel source for respiration, meaning that these results could also imply that this clone has a slower or more efficient malic acid metabolism that may be able to maintain its level of malic acid in comparison to the other clones when faced with higher temperatures (Sweetman et al 2014). While FPS05's malic acid implies higher acidity of this specific clone, it also suggests that malolactic fermentation

products (creamier aroma associated with ethyl lactate, diacetyl, etc.) in this wine could be potentially higher than other clones (Gil-Sanchez et al 2019).

An interesting result here is that FPS03 had lower levels of Brix but is in the lowest group when malic acid was considered. This is somewhat surprising as malic acid levels typically decrease with grape maturity. Assuming that clonal differences are responsible, this could suggest that FPS03 may have a metabolism that is less efficient at accumulating and/or maintaining malic acid, potentially using more of it during respiration post-veraison in comparison to the other clones. Based on this result, it may be better to select a clone other than FPS03 if higher malic acid content and its subsequent malolactic fermentation products (such as the creamy, buttery aroma from diacetyl) are desired in a finished wine.

3.2.5 Multiple Factor Analysis of Clonal Grape Data

A Multiple Factor Analysis (MFA) was run to assess relationships of overall grape characteristics with individual clones (Figure 5A and B). The MFA included: must chemistry, phenolic content from AH-assay, phenolic content from RP-HPLC, and aroma compounds. The separation of the clones demonstrates the existence of clonal differences. The MFA accounts for ~82% of observed variability, which is relatively good given the number of terms evaluated.

It appears must chemistry played a large part in the separation of the clones. FPS05 seemed to be separated by its higher levels of titratable acidity and malic acid. FPS01 and

FPS04 were positioned opposite to FPS05, which makes sense given their lower levels of TA and

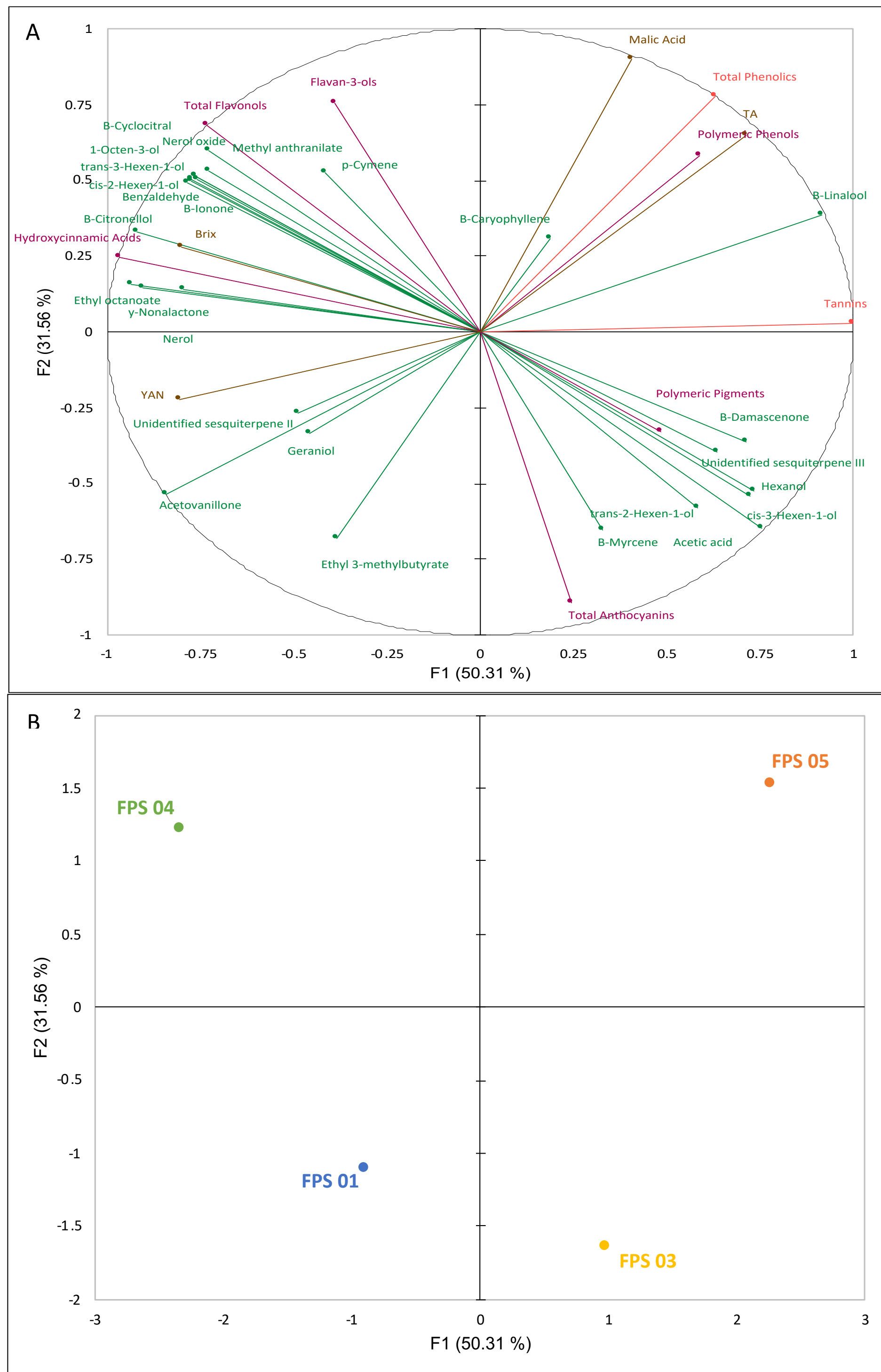

Figure 5. Multiple Factor Analysis that considers aroma compounds, phenolic profile, and basic wine chemistry of grape data with the variables (A) and observations and positioning of clones (B).

* Significant terms determined by ANOVA at $p < 0.05$

* Variables included in Figure 14A: AH-assay phenolics (red), HPLC phenolics (pink), aroma compounds (green), and wine chemistry (brown)

malic acid. These two clones also had the highest brix and YAN levels, which is also demonstrated by their position on the biplot. FPS03 exists far from all the wine chemistry terms, which agrees with its results as it contained the lowest levels of Brix, YAN, TA, and malic acid.

HPLC phenolics also played a considerable role in demonstrating the differences among the clones. FPS01 is in the bottom-left of the biplot which is relatively close to the position of total anthocyanins. This location agrees with the results as this clone had higher levels of this compound class. FPS03 was also located close to the anthocyanin term, which is not a surprise given it was found to contain the most along with FPS01. It also contained the least amount of the flavan-3-ols and flavonols, evidenced by it being positioned nearly opposite to these terms. FPS04 had the highest levels of hydroxycinnamic acids and total flavonols and its position in the MFA agrees with this. It also had the lowest amount of polymeric pigment, which is located opposite of its position on the MFA. It is also located far from total anthocyanins and polymeric phenols, which makes sense given that FPS04 had low levels of these compound classes. The MFA shows FPS05 highly correlated to polymeric phenols which is in agreement with the HPLC results. This clone seemed to mostly fall in the intermediate for phenolic compounds with the exception of polymeric phenols and flavan-3-ols. It had a great deal more of the polymeric phenols than the other clones, which may explain why this (along with TA and malic) is the main “separator” for this clone.

It appears that aroma compound profile was less efficient in separating the clones from one another in comparison to the wine chemistry and phenolic results. This could potentially be due to the large number of significantly different terms and the fact that, although the clones

were different, the differences were not that large in comparison to the other measurement considered in the MFA. One example of this is the position of FPS04 on the MFA. Its position would seem to indicate that it was correlated with several compounds more than any other clone. After observing the previous PCA of aroma compounds (Figure 4), it seems like this is not the case. In fact, based on the PCA, FPS04 was more correlated with β-damascenone, acetic acid, hexanol, and *trans*-2-hexen-1-ol. The MFA seems to indicate the exact opposite. One (or potentially both) of the figures could be incorrect due problems inherent with these sorts of visual statistical representations. Both PCAs and MFAs are useful in that they can visually represent complex, multi-variable data in order to show how samples are similar or different. The issue is that these methods essentially take a three-dimensional spread of significant terms and samples and reduce them to two dimensions, which can lead to misrepresented results (Lawless and Heymann 2010). An evaluation of the relative returns of different aroma compounds, it appears as though the PCA is a more accurate representation of the aroma profile of clones. Generally, clones had intermediate or higher responses for the compounds they were found to be correlated with on the PCA. Therefore, the MFA may prove to be less useful in describing a clone's aroma profile.

Considering all the metrics evaluated, it seems that the MFA illustrated a few important factors that were responsible for the "separation" or differentiation of the clones. FPS01 had higher levels of Brix, YAN, and total anthocyanins and lower overall acidity. FPS03 had lower Brix, YAN, acidity, flavonols, and flavan-3-ols but higher levels of total anthocyanins. FPS04 had higher levels of Brix, YAN, hydroxycinnamic acids, and flavan-3-ols but lower levels of

anthocyanins and acidity. FPS05 grapes had higher levels of TA, malic acid, and polymeric phenols but lower to intermediate levels of all other phenolics.

Based on this MFA, clones FPS01, FPS03, and FPS04 were more similar to each other and FPS05 was more different than the other clones. This was also the case when the aroma compound PCA was produced. In addition to aroma profile differences, FPS05 seemed to exhibit differences related to higher acidity and overall phenolic compound profile.

While it seems that the first three clones were similar, further investigation shows that the similarities and differences among the clones are more complex. FPS01 and FPS04 were higher in levels of Brix, YAN, and hydroxycinnamic acids and lower in levels of acidity. Although FPS03 was similar to these clones, it was lower in most of these attributes except for acidity. FPS03 and FPS05 were more similar in that they were lower in Brix and YAN at harvest. It is important to note that there may be different observations among clones which may disagree with the results produced by the MFA. For example, FPS01/FPS03 and FPS04/FPS05 were more like each other if anthocyanins were the only thing considered. This MFA, while helpful to gain a better understanding of the overall trend of the clonal differences and similarities, can oversimplify results.

This MFA is meant as only one possible way to interpret and generalize the previous collected data. A grower or wine producer may elect to focus on only one or two metrics specific to their desired wine/grape characteristics as opposed to evaluating the grape results holistically. One such example of this is that producers may have little interest in the differences in aroma compounds in grapes if their intention is to use Petite Sirah as a color or structure enhancer for a blended wine. In this case, they may have more interest in the results

produced for anthocyanin, polymeric pigment, and/or tannin content in the grapes. Similarly, if producers feel that all Petite Sirah clones meet their phenolic standards but are more concerned with sugar accumulation and acidity, they may elect to focus on must chemistry.

3.3 Wine Evaluations

3.3.1 Primary Fermentation Curves

Daily Brix measurements were performed after a morning pumpover to determine if any differences existed in the rate with which the clones were fermenting. Figure 8 shows these results in the form of fermentation curves. It is no surprise that the first data point, the beginning of fermentation, showed significant differences as this starting Brix point was already established as significantly different in the starting must chemistry (Table 6). What was interesting is that FPS05 may have “soaked up” releasing more sugar than determined in must analysis to increase its Brix level to be more in line with clones FPS01 and FPS04. This clone could have had more dried berries in its clusters resulting in additional sugar release with prolonged extraction time, increasing its overall Brix value. The cluster condition results (Table 3) showed that FPS05 did have a higher percentage of sunburnt berries, but this result was not statistically significant. While the destemmer used for fruit processing is designed to remove these shriveled and/or underdeveloped berries, it is possible that some of these dry berries

made it into the must. FPS03 still remained the lowest level of Brix when the measurements began.

There were no significant differences in Brix level throughout the fermentation process until the last day of measurements. While clone FPS03 contained the lowest amount of Brix on the final day of measurement (which is not entirely surprising given its lower level at the start

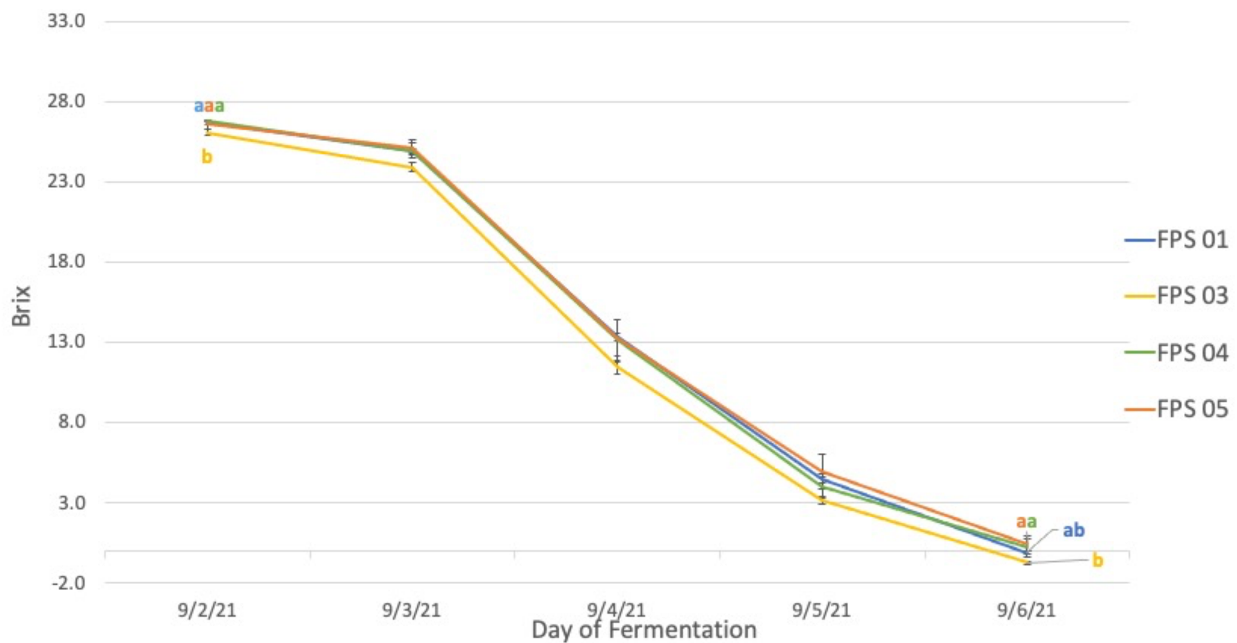

Figure 6. The primary fermentation curves with daily measurements of the four Petite Sirah clones (n=3).
* Significant groups determined by ANOVA at $p < 0.05$

of fermentation) clones FPS04 and FPS05 contained higher levels and were slower to finish primary fermentation. FPS01 belonged to both significant groups as its final Brix level was in the middle of both the high and low levels found in the clones. However, Brix readings become increasingly inaccurate as soluble solid levels from sugars approach zero as fermentation nears completion due to instrument and method constraints. Thus, though the clones were significantly different in Brix level at the end of fermentation, it is worth noting that these

differences were relatively small and may go unnoticed by winemakers as all the clones seemed to produce healthy, consistent fermentations at similar rates.

3.2.2 Malolactic Fermentation Curves

The malolactic acid fermentation curves elicited similar results as alcoholic fermentation in that the differences in malic acid concentration at the beginning of fermentation resulted in the different significant groups. Figure 9 shows these fermentation curves. Clone FPS05 had the highest levels at the beginning of fermentation followed by FPS04 which belonged to both

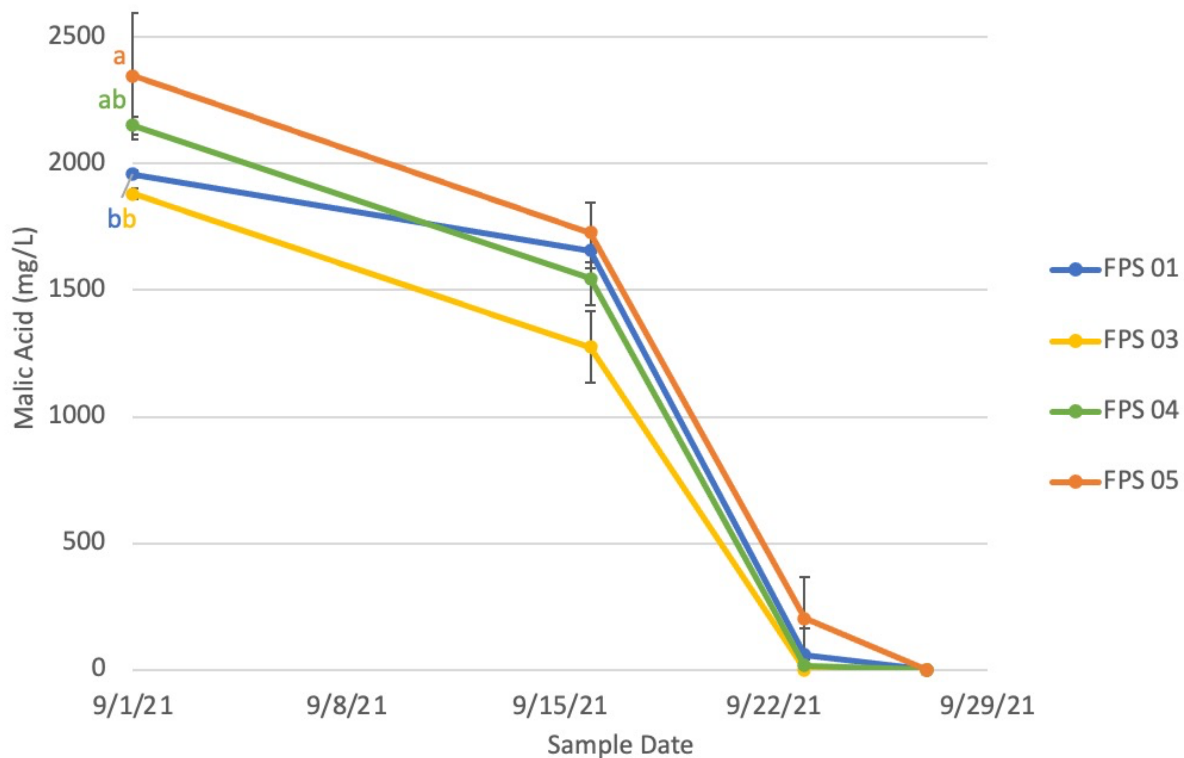

Figure 7. The malolactic fermentation curve with weekly measurements of the four Petite Sirah clones (n=3).

* Significant groups determined by ANOVA at $p < 0.05$

significance groups. Finally, FPS01 and FPS03 had the least amount of malic acid in their must.

All the malolactic acid fermentations were complete within a month, with no differences in levels during the fermentation itself.

3.3.3 Finished Wine Chemistry

Table 7. Basic wine chemistry measured upon dryness (n=3).

Clone	**Alcohol (%v/v)***	**pH****	**TA (g/L) ***	**Residual Sugar (g/L)**	**Acetic Acid (g/L)**	**Free SO2 (mg/L)**	**Total SO2 (mg/L)**
FPS 01	15.13 ± 0.30 **a**	3.89 ± 0.01 **a**	5.76 ± 0.03 **ab**	0.41 ± 0.01	0.48 ± 0.07	24 ± 3	33 ± 3
FPS 03	14.68 ± 0.04 **b**	3.80 ± 0.02 **b**	5.95 ± 0.12 **a**	0.39 ± 0.03	0.43 ± 0.05	23 ± 1	32 ± 1
FPS 04	15.40 ± 0.16 **a**	3.86 ± 0.03 **a**	5.63 ± 0.07 **b**	0.45 ± 0.05	0.49 ± 0.04	22 ± 2	34 ± 1
FPS 05	15.20 ± 0.21 **a**	3.77 ± 0.03 **b**	5.95 ± 0.16 **a**	0.42 ± 0.02	0.43 ± 0.09	21 ± 2	32 ± 0

*- Denotes significance by ANOVA at $p < 0.05$
**- Denotes significance by ANOVA at $p < 0.01$

The final wine chemistry was analyzed when the wine was determined to be "dry" or when residual sugar (RS) content was below 2.0 g/L. The measurements analyzed were alcohol % (v/v), pH, TA, RS, acetic acid content, free SO2, and total SO2 (Table 7). Some of these parameters were manipulated by winemaking practices that are widely accepted by industry (e.g., TA adjusted to 6.0 g/L and SO_2 additions). Therefore, these results should be viewed with less emphasis as they may not be indicators of clonal differences but potential variability during the winemaking process.

It was found that clones FPS01, FPS04, and FPS05 produced wines with higher alcohol and FPS03 produced wines with the lowest. It was expected that FPS01 and FPS04 would have the highest levels as they were observed as having the highest Brix level at the start of fermentation. While FPS05's must was lower in Brix, it is possible that dried and/or raisined berries could have influenced the overall sugar content available to the fermenting yeast, leading to an elevated alcohol level. FPS03 wines containing less alcohol was more expected given its lower Brix level in its must at harvest. This also reemphasizes previous recommendations that FPS03 may be a good clone selection if later ripening and/or less sugar is desired.

While the pH of the clones was not significantly different at harvest, the finished wine pH results were found to be significantly different. While these results were significant, these differences were relatively small and any real-world impacts on winemaking practices or wine quality may be minor and unnoticeable. Again, it must be stated that tartaric acid adjustments were made with the goal of producing wines with 6.0 g/L TA and this may have impacted the pH. The pH results of the wine agree with the commonly accepted belief that more mature grapes will produce wines with higher pH values as the grapes consume malic acid during respiration. This seemed to be the case based on the starting must results, with the "riper" grapes (FPS01 and FPS04) producing higher pH wines and the "less ripe" grapes (FPS03 and FPS05) producing wines with lower pH values.

Titratable acidity showed significant differences among clones. However, these differences were relatively small and should have little impact on the sensorial perception of the finished wines.

3.3.4 Phenolic Extraction During Fermentation

Wine Xray was used as a quick method to monitor and evaluate the extraction of phenolic compounds during fermentation and maceration. Total anthocyanins and tannins were measured beginning on the day of crush until pressing, with a measurement performed both before and after pressing. Wine Xray uses spectrophotometric data and a predictive model to calculate the amount of phenolics in a sample. Given this fact, it may be less accurate than other widely accepted, direct methods of measurement such as RP-HPLC. However, due to number of measurements and the need to measure the samples quickly, this method is deemed fit for purpose and useful for determining and evaluating any possible extraction trends among the clones.

Total anthocyanin content of the clones during fermentation can be seen in Figure 10A. Total anthocyanins were measured beginning the day of crushing, which established low, baseline levels prior to any significant extraction associated with fermentation and/or maceration. The extraction of anthocyanins accelerated between "day zero", which was the day of yeast inoculation, and day one. An increase in anthocyanin content at this timepoint could be due to an increase in temperature and ethanol concentration as the fermentation began. Along with the longer extraction time, these conditions are known to increase anthocyanin extraction. However, anthocyanins tend to extract quickly and are soluble in water, meaning that an increase in ethanol may not have contributed. The extraction curve for all four clones continued to increase until day five and six, when anthocyanin concentration plateaued and then gradually began to decrease. The shape of the extraction curve of the clones and their relative

initiation and deceleration seems to be typical and observed in other studies (Lerno et al 2017; Ribéreau-Gayon et al 2006). The anthocyanin extraction curve showed that FPS05 had higher anthocyanins on the day of crush compared to the other clones. The following day showed no significant differences as the other clones' anthocyanin concentration seemed to catch up to FPS05. However, throughout the rest of fermentation, FPS05 maintained higher levels of total anthocyanins, especially as fermentation created a more alcoholic wine matrix around day 3 or day 4. FPS04 was found to have the second most anthocyanin, followed by FPS01 and FPS03 respectively.

These results were somewhat unexpected as this did not relate to the grape anthocyanin content data. RP-HPLC of the grapes found that FPS01 and FPS03 had the highest number of anthocyanins and were not significantly different from one another. FPS04 and FPS05 also shared a significance group, with FPS04 containing the least anthocyanins. There may be a few possible explanations for the disparity between the grape tissue and the fermenting wine anthocyanin content. These differences may be due to differences in overall grape phenolic profile and the overall extractability of anthocyanins from grape cell walls, which has been seen in published research (Medina-Plaza et al 2019). Regarding FPS04 having higher than anticipated anthocyanins, this clone's grape phenolics showed higher levels of flavonols. This compound class can complex (copigmentation) with anthocyanins which may shift the chemical equilibrium of the fermenting wine to favor the production of the colored flavylium form of the anthocyanins and increase the color absorbance of a wine by a significant amount (Boulton 2001). Given that this method of anthocyanin measurement depends on a spectrophotometer and absorbance of the wine sample, this could explain the higher-than-

expected anthocyanins for FPS04. This may also explain the lower-than-expected anthocyanins for FPS03 as the opposite may have occurred.

FPS03 could have also shown lower anthocyanins during fermentation due the relatively high percentage of anthocyanins that existed in the acylated state compared to the glycosylated state. The former has been shown to more easily bind with yeast cells which were removed through centrifugation prior to analysis (Morata et al 2003). While the other clones' acylated anthocyanins made up approximately 40% of its total anthocyanins, FPS03's anthocyanins accounted for approximately 46% of its total anthocyanins. While this difference is small, it may have contributed to this clones' lower number of anthocyanins during fermentation.

Another possible explanation of differences in anthocyanin content could be the overall extractability of anthocyanins due to cell wall composition differences. Other research has indicated that phenolic extraction, which is predicated on the compounds desorbing or adsorbing to cell wall solids, can be dependent upon the composition of cell wall compounds such as pectin, lignin, cellulose, and other macromolecules (Medina-Plaza 2019). It is possible that FPS04 and FPS05's cell wall composition or condition allowed for more and faster extraction of anthocyanins compared to FPS01 and FPS03. This can only be speculated as cell wall chemical composition was not analyzed during this study.

The early, significantly higher extraction of anthocyanins on the day of crushing may also indicate that FPS05 grape skin condition could have been different from the other clones. This suggests that the crushing/destemming process itself may have mechanically released more anthocyanins. Research has shown that softer and smaller berries may release more

anthocyanins into solution (Zouid et al 2013). In terms of size and weight, harvest data seems to indicate that FPS05 had berries with the least mass, albeit not significantly different. The lower overall yield of FPS05 may also supports this. However, firmness and geometric size of berries as a source of increased extractability can only be speculated about as these were not analyzed during this study.

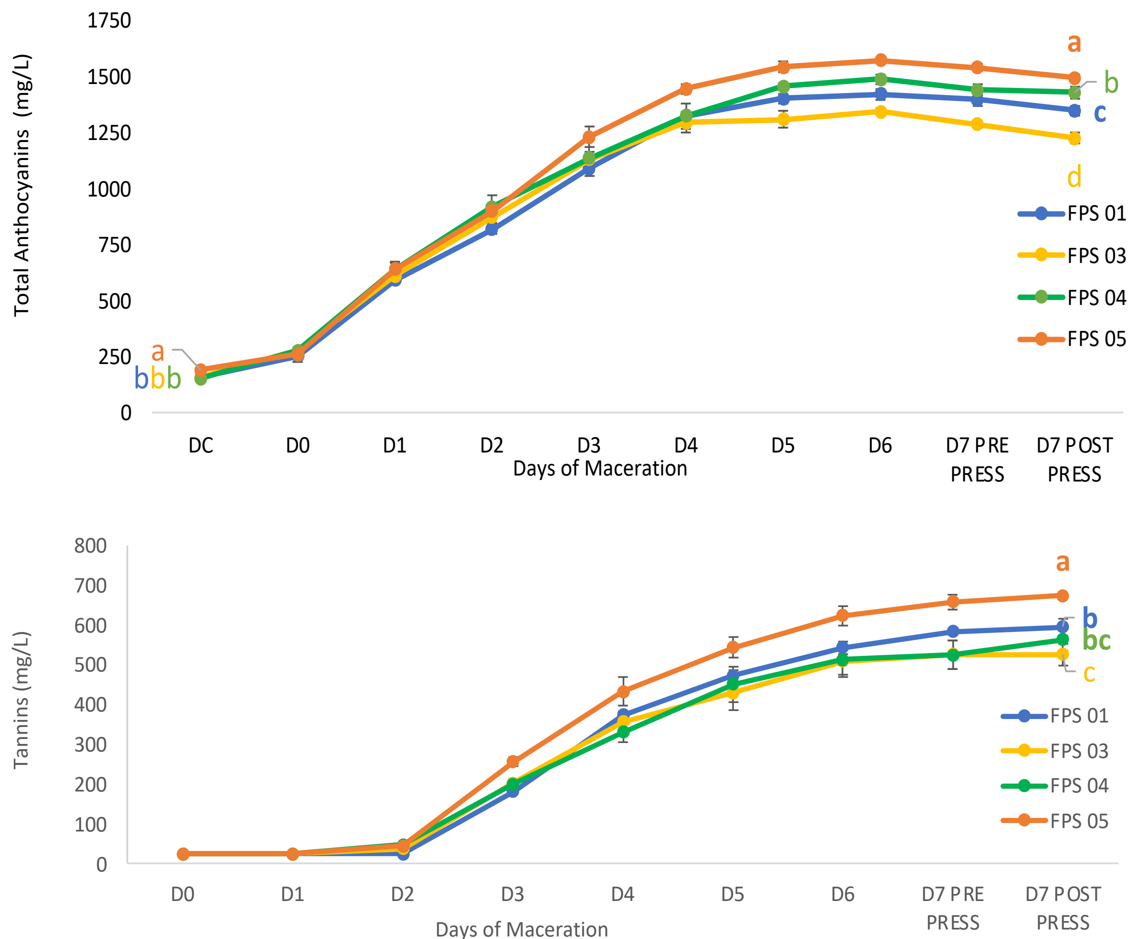

Figure 8. The phenolic extraction during fermentation beginning at the day of crushing (DC) until day seven of fermentation after pressing (D7 Post Press), including total anthocyanins (A) and tannins (B)

* Significant groups determined by ANOVA at $p < 0.05$

Tannin extraction became measurable when alcohol production started (Figure 10B) on day two of fermentation. Prior to this point, there was not enough ethanol present in the fermentation solution to quickly extract these compounds in large amounts and their concentrations were below the limit of detection of the method (<10 mg/L) (Lerno et al 2017). Once enough ethanol was accumulated, measurements were able to be made. FPS05 consistently had the highest number of tannins, followed by FPS01, FPS04, and FPS03 respectively. This result was somewhat correlated to the grape HPLC analysis in that FPS05 belonged in the significance group that had the most tannin components and FPS03 had the fewest. The Wine Xray results for anthocyanins and tannins produced similar results in terms of clonal rank. FPS05 contained the highest amounts of tannins and anthocyanins post-pressing and FPS03 had the lowest. FPS01 and FPS04 both fell into the intermediary for both phenolic categories, but FPS04 contained more anthocyanins and similar levels of tannins to FPS01.

3.3.5 Wine Phenolic Content

Like the grape results, all the phenolic compounds measured by RP-HPLC were found to be significantly different among clones, except for gallic acid. The wine results can be found in Table 8. FPS01 contained the highest amount of flavan-3-ols, and FPS03 contained the lowest. Clones FPS04 and FPS05 contained similar amounts of flavan-3-ols. These results are somewhat like the grape results, with FPS03 containing the lowest amount of these compounds. As stated previously, monomeric flavan-3-ols make up proanthocyanidins, or condensed tannins, and impact the sensorial perception of astringency and bitterness in a wine. These structures can

also cross-link with anthocyanins during wine aging, protecting them from oxidation and the associated loss of color (He et al 2012).

Table 8. Phenolic content of Petite Sirah clonal wines as determined by RP-HPLC (n=3).

	Clone			
Compounds (in mg/L)	**FPS 01**	**FPS 03**	**FPS 04**	**FPS 05**
Gallic Acid	11.80 ± 0.51	11.17 ± 0.52	11.55 ± 0.24	11.63 ± 0.30
Flavan-3-ols [a]	130.73 ± 7.16 a	108.71 ± 3.97 c	117.94 ± 1.03 b	121.00 ± 4.93 b
Hydroxycinnamic Acids	39.52 ± 2.21 a	31.33 ± 0.93 b	33.92 ± 2.03 b	34.97 ± 2.86 b
Total Flavonols	92.86 ± 8.01 b	100.63 ± 6.28 ab	109.81 ± 3.10 a	102.05 ± 7.45 ab
Flavonol Glycosides	91.50 ± 8.01 b	99.29 ± 6.28 ab	108.45 ± 3.09 a	100.71 ± 7.44 ab
Flavonol Non-Glycosides	1.37 ± 0.01 a	1.34 ± 0.01 b	1.36 ± 0.01 a	1.34 ± 0.01 b
Total Monomeric Anthocyanins	403.33 ± 30.25 bc	376.16 ± 5.59 c	442.11 ± 3.95 a	427.44 ± 18.92 ab
Anthocyanin-glucosides	252.18 ± 18.55 bc	236.95 ± 4.31 c	280.92 ± 4.91 a	267.85 ± 11.71 ab
Anthocyanin-acetylglucosides	115.09 ± 7.94 a	102.44 ± 1.31 b	119.49 ± 1.99 a	119.79 ± 5.30 a
Anthocyanin-*p*-coumarylglucosides	32.14 ± 3.79 a	32.81 ± 0.97 a	36.89 ± 0.14 a	35.32 ± 3.61 a
Polymeric Pigment	22.11 ± 3.60 b	26.42 ± 1.63 ab	31.05 ± 2.21 a	30.40 ± 4.05 a

* Significant groups determined by ANOVA at $p < 0.05$

[a] The flavan-3-ol class is sum of concentrations of gallocatechin, epigallocatechin, (+)-catechin, (-)-epicatechin, B1, and B2

Based on these results, one may be able to predict that FPS01 may produce more astringent wines that have the potential for a more stable color throughout aging. The opposite may be true for FPS03 wines, which may produce wines that have less complexation between flavan-3-ols and anthocyanins. Therefore, FPS03 wines could see color intensity diminish quicker than other clones. FPS03 containing the lowest number of anthocyanins among the clones could also support this. However, additional aging studies evaluating the clonal wines over several years would need to be performed in order to confirm this.

Different from the grape RP-HPLC results, FPS01 wine contained the highest amount of hydroxycinnamic acids, with the other clones all being similar and containing less. As previously mentioned, these phenolic acids are a main component of enzymatic oxidation. Juices and wines that contain more of these compounds could contain more oxidation products and associated brow color. While red wines may be more resistant to oxidation due to higher levels of total phenols and any color changes associated with oxidation may be less obvious than white wines. However, the difference in hydroxycinnamic acids observed among clones is small and any difference in the accumulation of oxidation products will likely be unnoticeable. It is also possible that differences in hydroxycinnamic acids in these young wines may have been impacted by winemaking practices. When the grapes were analyzed, FPS04 contained the most hydroxycinnamic acids and the other three clones were similar to one another. In the wines, FPS01 had the most and the other three clones were similar. Given that these compounds are easily oxidizable, it is possible that different exposure levels of oxygen during the winemaking process may affect the concentration of these phenols. For example, FPS04 fermentations may have been exposed to more oxygen in comparison the FPS01. While no specific oxygen exposure events were noted during the making of these wines, it could be possible. Hydroxycinnamic acids are also known to be a cofactor for copigmentation. Wines with more of these compounds may appear to be more colorful than otherwise expected based strictly on anthocyanin and flavonol content (Bimpilas et al 2016). Given this, FPS01 may see increased levels of copigmentation and the associated color intensity in its young wines.

FPS04 contained the highest amount of total flavonols and FPS01 the least, with FPS03 and FPS05 sharing significance groups with each. These results relate to the grape flavonol

content as FPS04 grapes also contained the greatest concentration of these phenols and the other three clones contained lower levels that were more like one another. While the differences in wine flavonols were not drastic, they were still statistically significant. These phenolic compounds are strong copigments as they can complex with anthocyanins and increase the overall color intensity of a wine (Boulton 2001). While the results of flavonol concentration are small, they may imply that FPS04 wines have a greater capacity for copigmentation and the resulting increase in color intensity in younger wines in comparison to the other clones. Given that the differences among clones are small, the differences in color appearance may go unnoticed by producers.

The difference in anthocyanin content of the clones was small but found to be statistically significant. FPS04 contained the highest concentration of anthocyanins, followed by FPS05, FPS01, and FPS03 respectively. This differed from the grape anthocyanin results that demonstrated FPS04 and FPS05 as the clones containing the least amount of anthocyanins and FPS01 and FPS03 contained almost double. As previously stated, anthocyanin extractability and stability are dependent on several factors that include, but are not limited to, temperature, time, grape cell wall composition, and overall wine chemical composition. In the grape phenolic analysis, an exhaustive extraction is performed by mechanical homogenization of grape tissue and exposure to concentrated organic solvents. This process is designed to extract all the phenolic compounds present in the grape tissue. Anthocyanin extraction during winemaking is dependent upon contact time of grape solids with water, ethanol, and elevated temperatures caused by yeast fermentation. While grape anthocyanin and phenolic profiles can correlate with wine phenolic profiles, this will not necessarily occur due to differences in extraction

media. It is possible that the clones that had higher anthocyanins in the grapes (FPS01 and FPS03) possess a cell wall composition that favors the adsorption or retention of anthocyanins whereas the opposite could be true for clones that had lower levels of anthocyanins in the grapes. Also, lower levels of flavonols and other compounds in the grapes to act as copigments and shift the anthocyanin concentration gradient may see reduced anthocyanin extraction. FPS04 and FPS05 grapes could potentially have cell walls or produce a fermentation chemical matrix that allows for higher levels of anthocyanin extractability. FPS04 wines contained the highest level of alcohol concentration, which could have resulted in higher levels of anthocyanin extraction. FPS04 also contained the largest amount of stable, polymeric pigment, followed by FPS05.

Table 9. Phenolic content of Petite Sirah clonal wines determined by Adams-Harbertson Assay (n=3).

Clone	Total Phenolics (mg/L CE)	Tannins (mg/L CE)	Anthocyanins (mg/L M3G)
FPS 01	1276.30 ± 14.37 a	268.00 ± 22.01 a	679.05 ± 22.62 a
FPS 03	1164.46 ± 54.05 b	292.75 ± 34.67 a	623.99 ± 12.66 c
FPS 04	1226.08 ± 34.34 ab	218.24 ± 16.47 b	660.30 ± 12.91 b
FPS 05	1257.32 ± 85.10 a	256.78 ± 46.73 ab	660.14 ± 7.29 b

* Significant groups determined by ANOVA at $p < 0.05$

Based on AH-assay results (Table 9), FPS01 and FPS05 contained the highest amount of total phenolics, while tannin analysis indicated that FPS01 and FPS03 contained the most, and FPS04 the least. FPS01 and FPS03 wines may be the most astringent and “drying” of all the

clonal wines. Anthocyanin analysis showed significant differences among clones with FPS01 having the most and FPS03 having the least. Although there were differences, the difference in concentration between FPS01 and FPS04/FPS05 were relatively small. This could suggest that FPS01, FPS04, and FPS05 wines would be the most colorful and FPS03 would be the least. The wine AH-assay results differed from grape AH-assay results, most likely due to differences related to grape phenolic composition and overall phenolic extractability.

As discussed previously, the AH-assay methodology for tannins relies upon the precipitation of flavan-3-ols with bovine serum albumin (BSA). Monomers and dimers are not reactive with BSA whereas as polymers get more reactive as they increase in length. The grape AH-assay data showed no differences among clones, but it is possible that different rates of polymerization during fermentation and bottle-aging led to different proportions of oligomers in clonal wines. These differences could have resulted in differences in protein precipitation and, therefore, measured tannins in wines.

AH-assay results for anthocyanins also produced a somewhat unexpected result. RP-HPLC results for total anthocyanins indicated that FPS04 and FPS05 wines contained the greatest amount followed by FPS01 and FPS03 respectively. The AH-assay related with these results except for FPS01, which showed the most anthocyanins. This could potentially be due to FPS01's higher level of hydroxycinnamic acids in solution which can act as a cofactor for copigmentation, increasing the coloration of a wine (Bimpilas et al 2016). As this assay relies upon spectral measures, it is possible that this led to a higher absorption of the light source in FPS01 wines.

3.3.6 Wine Volatile Aroma Compounds

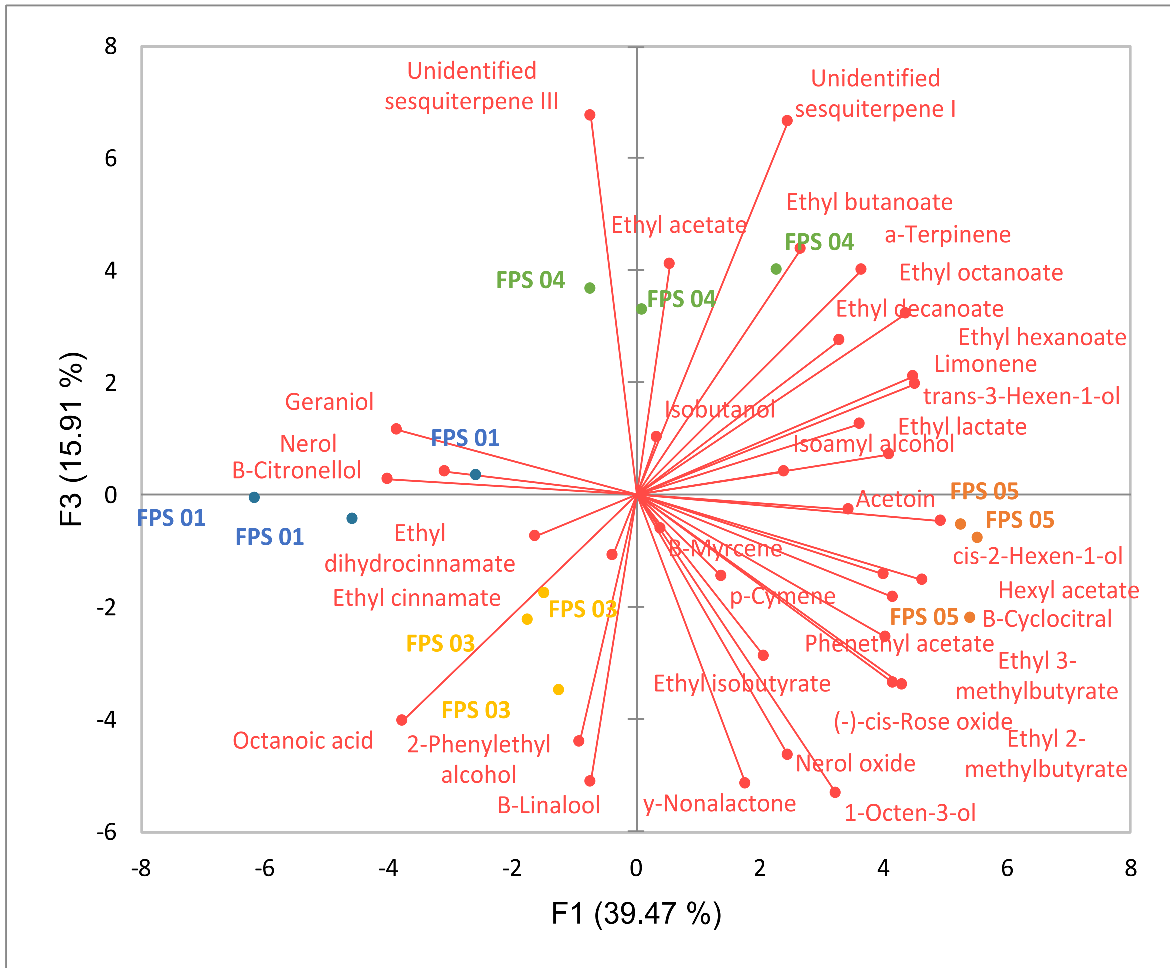

Figure 9. Principal component analysis of aroma compounds as variables and clonal wine replicates as observations (n=3).

* Significant aroma compounds determined by ANOVA at $p < 0.05$, aroma compounds are colored red and clones are colored blue (FPS01), yellow (FPS03), green (FPS04), and orange (FPS05)

HS-SPME-GC-MS was used to determine any differences among aroma compounds in the wines made from the different Petite Sirah clones. Of the 48 measured compounds, 35 were found to be significantly different among the clonal treatments. Figure 9 shows a PCA of the significantly different aroma compounds and the fermentation replicates of all the clones. The first and second dimensions explained only 58% of the overall variability. While the first

and third dimensions accounted for approximately 55% of the overall variability. This means that only 58% of the variance among the samples were explained by the PCA which is low. While there does not seem to be any obvious trends with compound classes, the important conclusion to be drawn is that there were clonal differences and clone fermentation replicates were grouped among themselves which indicates there were no large differences due to fermentation variability. This PCA was produced in order to distinguish clones from one another based on different levels of aroma compounds. Clones may not be highly correlated with certain compounds, but these compounds can still be found in the wines and contribute to the overall aroma profile.

The chemical process and results of fermentation leads to differences in aroma compounds among grapes and certain aroma compounds in grapes are consumed/augmented in reactions or volatize (Romano et al 2022). Similarly, there are aroma compounds that only exist in wine as they rely upon chemical reactions performed by the fermenting yeast or require suitable chemical/physical properties found in a fermenting wine, such as increased temperatures, concentration of inoculum, available yeast nutrients, specific pH, or the presence of a specific compound like ethanol (Molina et al 2007; Molina et al 2009; Carrau et al 2010). For example, the compound geraniol originates in grapes but can undergo acid-catalyzed reactions to produce α-terpineol and linalool and yeast enzymatic reactions to produce β-citronellol (Vaudano et al 2004). The resulting combination of geraniol and its associated products after fermentation can all be found in wine and contribute to wine aroma character. There are some aroma compounds, like geraniol, measured in this study that can be found in both before and after fermentation.

FPS01 wine results did not show high levels of correlation with compounds that were found in FPS01 grapes. Some of this was likely due to some of the compounds (like hexanol), that were observed as highly correlated in the grapes, being present in very low quantities in the finished wine. However, there were compounds that FPS01 was inversely correlated with in both grapes and wine, such as *cis*-2-hexen-1-ol, β-cyclocitral, and *trans*-3-hexen-1-ol. FPS01 showed a high correlation with geraniol, nerol, β-citronellol, and ethyl dihydrocinnamate. FPS01 was correlated with both geraniol and β-citronellol as the former is converted into the latter by yeast enzymatic reactions. Both these (and nerol) are monoterpenes and can contribute a floral, citrus, and terpenic aroma to a wine. It is possible that FPS01 may produce wines that have more of these qualities than the other clones. It was also interesting to see that this clone had some correlation with ethyl dihydrocinnamate and, to a lesser extent, ethyl cinnamate considering this clone's higher relative level of hydroxycinnamic acids. It is possible that the higher levels of these acids reacted with ethanol to produce these compounds. Ethyl dihydrocinnamate can smell of honey, flowers, and rum whereas ethyl cinnamate is more of a spiced, balsamic aroma. Based on these results, FPS01 could have more of these qualities than the other clonal wines.

FPS03 wine aroma was similar to its grape aroma in that there was also a high correlation with β-linalool and an inverse correlation with ethyl octanoate. Like FPS01, FPS03 wines showed a correlation with ethyl dihydrocinnamate and ethyl cinnamate. FPS03 wines were also correlated with octanoic acid and 2-phenethyl alcohol, which are both yeast metabolites. Octanoic acid can add a fruity aroma but its accumulation during fermentation and final concentration in wine can be dependent upon yeast species and strain as well as

fermentation conditions (Torija et al 2003; Tronchoni et al 2012). Therefore, the concentration of this compound in a finished wine may vary given the circumstances. As yeast species and fermentation conditions were controlled, FPS03 grapes could potentially contain more precursors for this compound but this cannot be known for certain. Octanoic acid was inversely correlated with ethyl octanoate (and other fatty acid esters). FPS03 wines contained the lowest alcohol percentage by volume. One can speculate the lower levels of ethanol in the fermentation led to a decreased amount of fatty acid esters in comparison to the other clones.

Phenethyl alcohol is another yeast metabolite that can contribute a rose-like aroma to a wine. This compound can accumulate in several ways, one mode being the utilization of L-phenylalanine as a nitrogen source in the *Saccharomyces cerevisiae* and other yeasts' metabolism (Kim et al 2014). Therefore, differences in nitrogen sources in the grape must could be a possible explanation into FPS03 containing more of this compound in comparison to other clones. FPS03 grape must belonged to the significance group that contained the least amount of YAN. While this level was adjusted prior to fermentation with additions of yeast nutrients, it is possible that the proportions of the nitrogen sources were different. Having different levels of phenethyl alcohol precursors could impact its concentration at the end of fermentation. FPS05 also had lower levels of YAN. However, without knowing more about the specific protein and amino acid composition of the original grape must before and after adjustment, this is speculation.

FPS04 wines were correlated with two unidentified sesquiterpenes, which is similar to the grape results. Unfortunately, as evidenced by their names, these compounds were not identified in this method. Sesquiterpenes are typically less volatile but strong in odor (Buckle

2015). Grapes contain several types of sesquiterpenes which can include β-elemene, β-bisabolene, β-caryophyllene, germacrene D, β-farnesene, α-humulene, farnesol, and α-bisabolol (Durán et al 2018). These unidentified sesquiterpenes could be one of the above apart from farnesol and β-caryophyllene, which were both identified and analyzed with this specific method. It is possible that FPS04 wines may contain some of these compounds which would add to its overall aroma.

FPS04 was also correlated with ethyl acetate, ethyl butanoate, α-terpinene, and somewhat correlated with several other fatty acid esters. This clone's wines contained the highest alcohol percentage of all clonal wines produced, which may have led to the higher correlation of this clone with fatty acid ester compounds as the fatty acids could have been more likely to react and esterify with the ethanol. These compounds can contribute a fruity aroma when they are shorter in length (e.g. ethyl butanoate) which transitions to a fruity/waxy aroma as these esters increase in size (e.g. ethyl decanoate). The increased alcohol concentration in this clone's wines may have also led to a higher level of ethyl acetate. This compound can contribute a solvent, nail-polish like aroma to a wine. This could have also been the result of FPS04 grapes having a higher amount of acetic acid prior to fermentation. Having more of this compound and, therefore, more acetate ions, may have led to a higher amount of ethyl acetate in these wines. As acetic acid is a metabolite of acetic acid bacteria, the elevated levels of acetic acid in the grapes prior to fermentation could indicate that this clone had a higher amount of a microbial infection in comparison to the other clones. Another potential piece of supporting evidence of microbial pressure on FPS04 could be the correlation of this clone's wines with isobutanol. The presence of this compound has been associated with

spontaneous fermentations (Phillip et al 2021). This could potentially indicate microbial issues in the grapes and, eventually, the finished wines. Regardless of the source, isobutanol can provide an alcoholic, brandy-like aroma. Based on the results in this study, this aroma may be found more in FPS04 wines than the other clonal wines.

FPS05 wines showed similar correlations to their grapes, specifically *cis*-2-hexen-1-ol, *trans*-3-hexen-1-ol, and β-cyclocitral. It seems that these compounds, which were highly correlated with FPS05 grapes, maintained their differentiating presence in FPS05 wines. FPS05 showed a higher correlation with the most aroma compounds, which is also a similar result to the grapes.

FPS05 showed high levels of acetoin, which smells buttery. This was an interesting result as this compound can originate from wine yeast and lactic acid bacteria during malolactic fermentation (Romano and Suzzi 1996). This clone had the highest levels of malic acid in its must prior to fermentation. It is possible that these high levels were eventually turned into acetoin by microbe metabolisms, leading to the high correlation of this compound and FPS05 wines. Another piece of supporting evidence that FPS05's grape malic acid content affected wine aroma compounds is FPS05 wines showing correlation with ethyl lactate. This compound is also a product of malolactic fermentation and, like acetoin, can contribute a creamy/buttery aroma to a wine (Lasik-Kurdys et al 2018).

FPS05 was also correlated with hexyl acetate which can smell of fresh, green apples. There is some merit in investigating this compound as its production during fermentation is dependent upon the composition of its precursors in the starting must (Dennis et al 2012). Therefore, the concentration of this compound and its associated aromatic characteristics could

be related to any differences found among clonal grape tissue. FPS05 wines may differentiate itself from other clonal wine aromas due the presence of this compound and the other compounds that are characteristic of the products of malolactic fermentation.

This PCA suggests that aroma compounds of wines made from FPS01 were the most different from the others, followed by FPS05, with FPS03 and FPS04 being the most similar to one another. This may show that, based strictly on aroma compound concentration, FPS01 may produce wines that are noticeably different from FPS03 and FPS04. While these differences exist, these differences may not necessarily be perceived by wine producers and consumers given the complex wine chemical matrix (Villamor and Ross 2013).

The important takeaway from this analysis is that the clones were in fact different, their fermentation replicates were similar, and may produce wines with different aroma characters dependent upon winemaking practices. While there were differences based on aroma compound concentrations, further investigation in the form of sensory evaluations were performed to establish any distinct, noticeable differences in the perceivable aroma of the clonal wines.

3.3.7 Descriptive Analysis of Clonal Wines

Sensory evaluations were performed by a trained panel by Applied Sensory Inc (Napa, CA). Six judges evaluated all 12 wines produced (three fermentation replicates per clone). Of all the terms that were evaluated, there were seven wine attributes that had *p*-values less than 0.32. A PCA was produced using these terms and can be seen in Figure 13. Based on this PCA, it

seems that the panel was unable to discern differences among clones as the different clonal fermentation reps were dispersed amongst each other. Also, while the AH-assay was able to

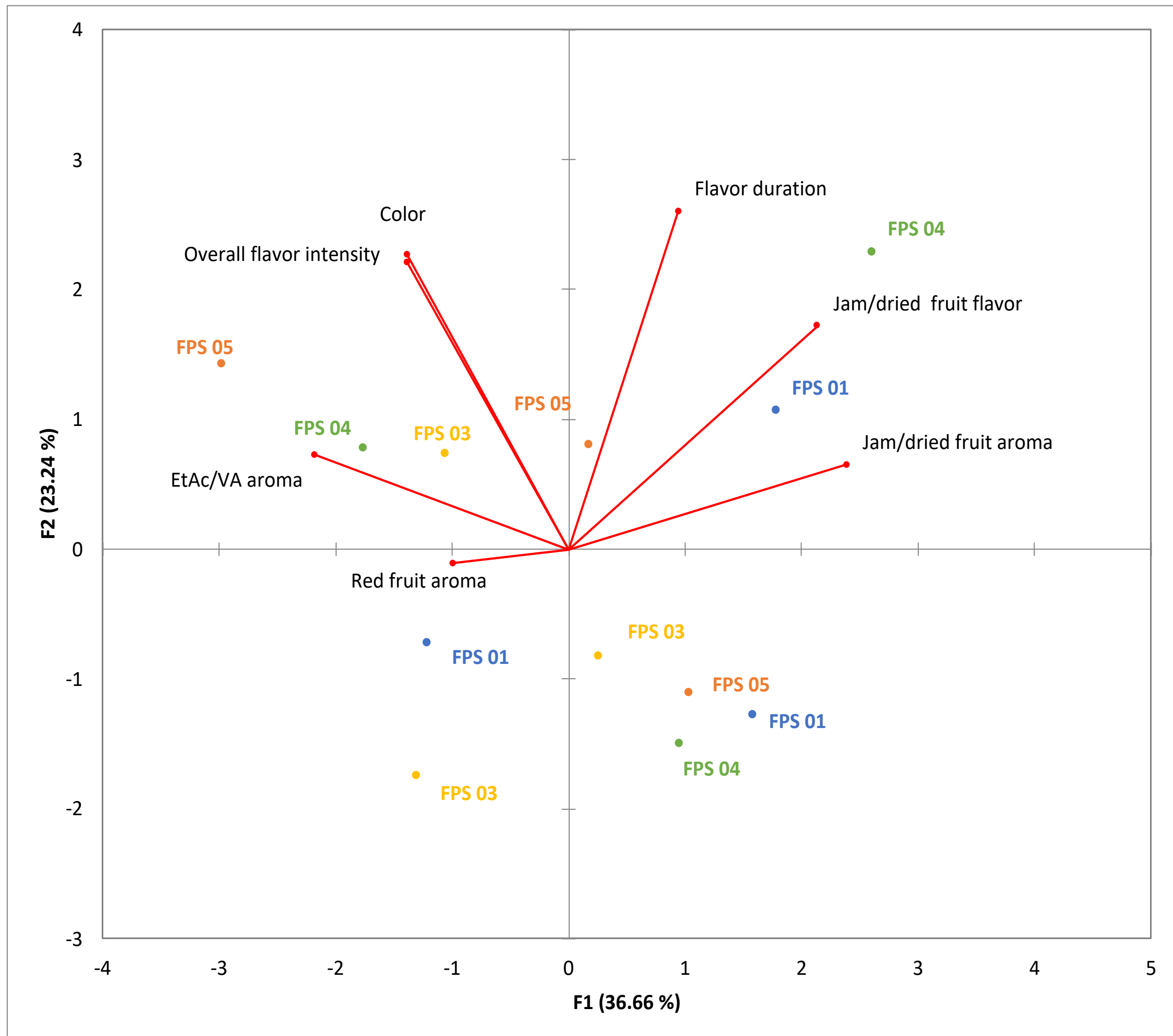

Figure 10. Principal component analysis sensory significant attributes of different clonal wines (n=2).
* Sensory attributes are colored black and clones are colored blue (FPS01), yellow (FPS03), green (FPS04), and orange (FPS05)

produce significant differences for tannin content, this panel determined that there were no significant differences in perceptible wine astringency. It could be that the chemical differences found in AH, while significant, were too small to be perceived by judges. Also, Petite Sirah is a cultivar that contains a very high level of anthocyanins. Some research has suggested that high levels of anthocyanins may reduce the overall perceived astringency of a wine (Villamor et al

2009). It can be concluded that although there were differences in the chemical profiles of the clonal wines, these differences were small and that the judges were unable to distinguish wines based on sensory characteristics.

3.3.8 Multiple Factor Analysis of Clonal Wine Data

An MFA (Figure 14A and B) was performed that included the finished basic wine chemistry, tannin/total phenolic content from the AH-Assay, phenolic compound content from RP-HPLC analysis, aroma compound composition, and descriptive analysis results. The variables included in this biplot were selected from all the analyses performed on the clonal wines and used to establish a general understanding of how the clonal wines may or may not be different. The MFA produced results that accounted for approximately 77% of observed variability. The averaged fermentation reps of all the clones were in distinct areas of the biplot, with clones FPS01 and FPS04 sharing the same quadrant. This implies that FPS01 and FPS04 were more similar to each other and FPS03 and FPS05 were more similar, albeit at a lesser degree than FPS01 and FPS04.

However, clonal selection is done to achieve specific goals and producers may find their desired traits evaluated during this research may be stronger or weaker in clones that share are in close proximity to each other on the MFA. A deeper investigation into the MFA can lead to a more detailed understanding of how the clones are different. It seems that though aroma compound profiles had a significant effect on the positioning of clones and attributes as these compounds and clones are similarly positioned on their PCA (Figure 11).

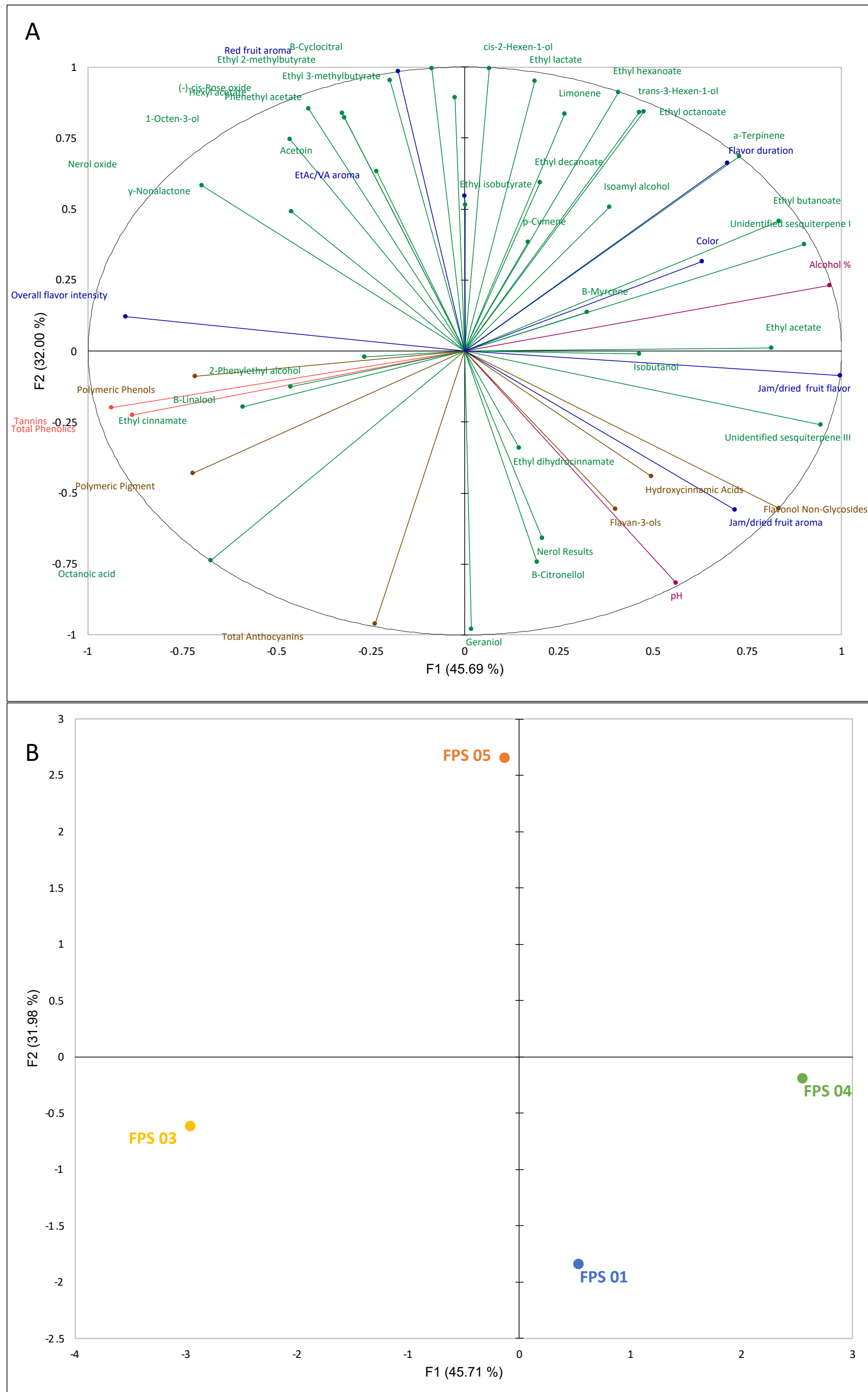

Figure 11. Multiple Factor Analysis that considers AH-assay phenolics, aroma compounds, HPLC phenolic profile, and basic wine chemistry, and sensory results with the variables (A) and observations and positioning of clonal wines (B) (n=3).

* Significant terms determined by ANOVA at $p < 0.05$

* Variables included in Figure 14A: AH-assay phenolics (red), HPLC phenolics (brown), aroma compounds (green), descriptive analysis terms (blue), and wine chemistry (pink)

For example, this MFA showed FPS01 being correlated with geraniol and nerol and negatively correlated with compounds like acetoin. FPS05 was the opposite; it showed high correlation with acetoin and ethyl lactate and negative correlation with nerol and geraniol (among other compounds). It was also correlated with the fatty acid ethyl esters, which was the same in Figure 11. These types of relationships between clone and aroma compounds exist for all clones.

Clone FPS01 and FPS04 shared several attributes that could explain their similar positioning on the biplot. Regarding wine chemistry, these two clones were higher in pH which can be seen on the biplot. The sensory panel found that these clone wines were typically higher in jam/dried fruit aroma and flavor. This makes sense as these clones had higher Brix levels at harvest and this elevated "ripeness" could lead to higher levels of jam or mature fruit aroma in a wine (Antalick et al 2021). Alcohol percentage also seemed to have a strong influence on clonal position. FPS04 was the highest, followed by FPS05 and FPS01 respectively.

Section 4. Conclusions

The primary goal of this study was to establish any potential differences between four Petite Sirah clones and to provide producers with information that may help them select a clone to better achieve their desired goals both in the vineyard and in the winery. The data produced during this study was able to illustrate statistically significant differences in both the grapes and wines produced from Petite Sirah (Durif) FPS01, FPS03, FPS04, and FPS05 clones.

These results could also be helpful in aiding vineyard managers and winemakers to meet their production goals more efficiently.

Vineyard, yield, and cluster condition analysis produced no significantly different results at 95% confidence. However, there was a possible trend in the data that may suggest clone FPS03 could be more prone to rot and that FPS05 could have overall lower yields. Producers may want to consider FPS03's rot potential when deciding their spray and harvest strategies. FPS05's lower yield potential may inform economic and wine style strategies. Both findings will need additional years of research to confirm. Based on the RAVAZ index, FPS01 seemed to be the most balanced between vegetative growth and fruitfulness. FPS03 was the most imbalanced which may suggest this clone may be prone to over-fruiting. This can affect FPS03's ability to accumulate sugar, acid, and secondary metabolites.

Harvest must chemistry produced from the grapes showed high levels of Brix and pH values in clones FPS01 and FPS04, implying that these varieties may be faster to ripen and may be good clonal selections for producers who want earlier ripening dates for various reasons. These reasons could include their desire to produce wines with mature fruit aroma or wanting to harvest sooner as a solution to external pressures such as wildfires or labor shortages. FPS03 and FPS05 seemed slower to ripen, containing lower amounts of Brix, lower pH values, and higher amounts of titratable acidity. These clones may be desirable if, for example, a producer is located in a warmer region and wants to maintain acidity and prevent excessive alcohol levels in their wines.

FPS03 grapes contained lower levels of malic acid. This may indicate that this clone's grape metabolism may be different enough that it produces or consumes malic acid at different

levels, but additional research would need to be performed to confirm this. FPS05 grapes showed higher levels of malic acid. Wines produced from FPS05 grapes also showed a higher correlation with aroma compounds that are known to be malolactic fermentation products such as acetoin and ethyl lactate. This could suggest that FPS05 would be the preferable selection for producers who want to make Petite Sirah wines containing higher levels of the creamy or buttery characteristics associated with malolactic fermentation.

All clonal grapes were similar in terms of flavan-3-ols, apart from FPS03, which had less. This may imply that FPS03 may have the potential to produce wines with reduced astringency/bitterness in comparison to other clones. Lower levels of the monomer flavan-3-ols could result in a wine that is less bitter and/or astringent due to a decreased capacity for polymerization. This also could result in FPS03 producing wines with less stable polymeric pigment. The AH-assay results indicated that there were no significant differences in grape tannins, which are polymerized flavan-3-ols. This indicates that there were differences in flavan-3-ol monomers and dimers but not for any oligomers and the overall impact may thus be small.

FPS01 and FPS03 grapes contained higher levels of anthocyanins in comparison to the other two clones. If a winemaker wants to maximize the color in their young, Petite Sirah wines, grapes from these clones may be the better selections. Given its higher level of anthocyanins and flavan-3-ols, FPS01 may also have a higher capacity to form polymeric pigment which could be critical to winemakers who use Petite Sirah as a means of adding color to blended wines designed for aging. When this information is combined with harvest must results, it may indicate that FPS01 may be even more desirable as it achieved higher levels of phenolic content

(flavan-3-ols, anthocyanins, precipitable tannin, polymeric pigment, etc.) at the same harvest point as the other clones. FPS01 was able to ripen fully in terms of sugar content and it was able to accumulate more, or comparable, phenolic compounds that are sought after by producers and associated with wine quality.

Aroma compound analysis by HS-SPME-GC-MS demonstrated that grapes from clones FPS01, FPS03, and FPS04 were generally more similar whereas FPS05 was different. This means that producers may find that aromas from the first three clones may be indistinguishable from one another, and they may find FPS05 to be different. However, multi-year data is required to confirm or dismiss this conclusion.

An MFA suggested that FPS01, FPS03, and FPS04 were more like each other and FPS05 was different. Thus, wines made from the grapes of clones FPS01, FPS03, and FPS04 could be similar or indistinguishable from each other. However, producers may choose to select a clone for a specific characteristic to meet their vineyard production or winemaking goals.

Regarding wine phenolic content, FPS01 exhibited the highest amount of flavan-3-ols, while FPS03 contained the lowest. Wines produced by the FPS04 contained the most anthocyanins and flavonols of all the clones, with FPS03 containing the least. This suggests that young wines produced by FPS04 have the potential to have deeper color, especially if significant flavonol related copigmentation occurs. FPS01 wines seemed to have the highest overall concentration of phenolic compounds, including the protein-precipitable tannins correlated with perceived astringency. It also contained the most total anthocyanins according to the AH-assay and an intermediate amount according to the RP-HPLC results. Considering all the phenolic results for FPS01, this specific clone could produce wines that are highly structured,

less oxidizable, all while having the potential to be similarly colored in comparison to the other clones' wines.

A PCA showed that the aroma compounds found in the clonal wines were different. FPS01 was the most dissimilar of all the clones and FPS03 and FPS04 were most like each other in terms of aroma compounds. However, descriptive analysis by a trained panel showed that the judges were unable to distinguish clones form one another as there were no large, perceptible differences among the different clonal wines.

An MFA of wine phenolics, aroma compounds, chemistry, and descriptive analysis results demonstrated that, in general, wines produced from FPS01 and FPS04 were more similar to each other, and wines produced from FPS03 and FPS05 were more similar to each other.

The results of this study may help producers select a clone to achieve their vineyard production and wine style goals. The grapes from the different clones showed differences in aroma profiles, phenolic profiles, and general chemical composition which carried over into the wines they produced. These differences could be utilized to produce grapes and wines that achieve stylistic goals more efficiently, whether it be through the production of varietal Petite Sirah wines or by using specific clones to blend for increased color, structure, or other sensory characteristics to wines that may be lacking desired qualities.

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