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Title: The Influence of Vineyard and Winery Nitrogen Supplementation on Chardonnay and Pinot Noir Winemaking and Wine Quality

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Nitrogen is a crucial nutrient required by yeast to successfully complete alcoholic fermentation. In particular, the concentration of yeast assimilable nitrogen (YAN) influences fermentation kinetics and the production of a range of volatile aroma compounds, both desirable and undesirable. YAN is naturally present in the grape, but producers can boost YAN concentration in the vineyard and/or the winery if concentrations are deemed insufficient. In the vineyard, nitrogen can be boosted through the incorporation of organic matter into the soil and/or through the use of fertilizers. In the winery, additions of commercial products containing inorganic and organic nitrogen can be made. While many studies point out the importance of YAN in relation to fermentation kinetics, little is understood about how YAN impacts the sensory properties of wine. In this study, the goal of this was to investigate the impact of boosting YAN in the vineyard or winery on fruit and wine chemistry, fermentation kinetics, and wine sensory properties of Chardonnay (CH) and Pinot noir (PN). Five treatments, including no N addition (Control), winery N addition using either diammonium phosphate (+DAP) or organic N (+Nutriferm), and vineyard N addition either to the soil (+Soil N) or through foliar spray (+Foliar N), were established with four replicates for each variety. CH and PN wines were produced following standard winemaking practices and sensory evaluations were

completed using triangle testing and Napping® and Ultraflash profiling (UFP) for aroma and mouthfeel.

Both winery and vineyard supplements raised YAN to sufficient levels for successful fermentation in both the CH and PN. There was variation in the efficiency of the fertilizer applications to raise YAN in CH compared to PN where application through the soil raised YAN more so than foliar application. In the winery, DAP boosted YAN through ammonia N while Nutriferm Arom Plus boosted free amino acid content. No significant differences in time to completion were noted between PN and CH treatments. To investigate how yeast strain and nitrogen source interact to alter fermentation rate and volatile aroma an additional experiment was conducted in CH juice. The same juice from the Control, +DAP, + Nutriferm, and +SoilN treatments were used, but this time there were three different strains of *S. cerevisiae* (D47, DV10, and CVW5). Nitrogen source had a significant effect on fermentation rate and there was also a significant interaction effect between yeast strain and nitrogen source on fermentation rate.

In addition to increasing YAN, boosting nitrogen in the winery and/or vineyard also altered the concentrations of certain free amino acids. For Chardonnay, the addition of nitrogen in the vineyard consistently boosted arginine, alanine, serine, and glutamine while the addition of Nutriferm Arom Plus consistently boosted threonine compared to other treatments. Additional volatile aroma analysis will be needed to determine if differences in total YAN as well as individual amino acids will impact yeast derived volatile aroma compounds. However, sensory analysis did reveal differences between these wines that could be due to initial differences in juice/must YAN concentration and composition. Nitrogen treatments affected Chardonnay and Pinot noir wine properties, but the extent of impact was dependent on varietal. Panelists could tell apart the CH treatments based on aroma and described these differences using unique terminology. In contrast, panelists could separate the PN treatments based off aroma, but could not successfully describe the differences. These findings suggest that CH producers should carefully consider where and how

much YAN they add as fermentation rate and final wine aroma can be altered. However, PN producers have more flexibility in where they add nitrogen as any changes to aroma were very subtle, meaning they can chose both where (vineyard or winery) and how (DAP or organic product) to boost nitrogen based on cost and/or production efficiency rather than worry about potential changes in wine quality.

The Influence of Vineyard and Winery Nitrogen Supplementation on Chardonnay and Pinot Noir Winemaking and Wine Quality

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Meghan L. Ruppel, Author

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Chapter 1

Literature Review

The general practice of converting grapes into wine has long been established, but as our understanding of the scientific principles underlying this process improves, so do opportunities to improve the consistency and quality of wines produced. One key area recently studied is how grape composition impacts wine quality. In this review, the influence of nitrogen, a key component of grapes, on wine quality will be examined. Specifically, the impact of nitrogen supplementation in the vineyard vs. nitrogen supplementation in the winery will be examined.

Red and White Winemaking

Winemaking begins in the vineyard with the production of grapes. Various practices are employed throughout the grape growing season to produce a target yield of grapes that meet certain quality parameters. Grapes are harvested primarily based on a set of easily measurable maturity indices such as sugar (measured as °Brix) and acidity (measured as pH and titratable acidity) (Jackson 2000). The acid/sugar ratio is one of the most heavily relied on parameters to determine grape maturity. This is because sugar content will dictate how much alcohol will be produced during fermentation, and acid is an important component for balancing wine taste and mouthfeel (Boulton et al. 2013). Other parameters can also be examined such as, yeast assimilable nitrogen (YAN), tannins, and anthocyanin's, although they are usually secondary to acid and sugar (Jackson 2000).

After grapes are harvested the processing of turning them into either white or red wine is very similar. The key difference between the two procedures is that white grapes are pressed prior to fermentation to separate the solids (skins and seeds) from the juice while red grapes are fermented in contact with the skins and seeds and pressed at the completion of alcoholic fermentation. Therefore, in white winemaking the first step is to immediately de-stem and press the grapes after harvest (Boulton et

al. 2013). After pressing, the juice is settled and racked off to help clarify it prior to fermentation. However, in red winemaking grapes are only crushed and destemmed prior to fermentation. The red wine fermentation process is referred to as on-skin fermentation (Jackson 2000) and results in the extraction of anthocyanins and other phenolics such as flavonols into the wine.

Once the grape juice or must is ready to ferment it is usually inoculated with a commercial yeast strain (Boulton et al. 2013) to induce alcoholic fermentation. Most fermentations are inoculated with a commercial strain of *Saccharomyces cerevisiae* (Gao and Fleet 1988, Pretorius 2000). The high reliability and predictability of *S. cerevisiae* is due to its ability to tolerate high sugar, high acid, and high alcohol environments (Arroyo-López et al. 2010, Querol and Fleet 2006). While a large number of commercial strains are available, many producers still rely on naturally present yeast to perform alcoholic fermentation. These yeast strains may be present on equipment in the winery or on the grapes from the vineyard (Jackson 2000).

During alcoholic fermentation, yeast metabolize glucose and fructose via glycolysis (Fleet 1993, Ribereau-Gayon et al. 2006). The glycolysis pathway is mediated by many enzymes that convert glucose into ethanol (Waterhouse et al. 2016). In addition to ethanol, energy, in the form of ATP, and carbon dioxide is also produced. However, there are many other byproducts that can also be formed during alcoholic fermentation (Ribereau-Gayon et al. 2006, Waterhouse et al. 2016). Glycerol and organic acids are examples of secondary byproducts that can be indirectly produced from glycolysis. There are many different yeast strains available for winemakers to purchase and use. Strains are typically advertised for different fermentation environments, grape varieties, and wine styles. Thus, yeast selection plays a crucial role in determining wine quality.

Fermentations can be performed in a variety of vessels although the most common are stainless steel tanks and oak barrels. Fermentation in stainless steel will help preserve varietal aromas (González-Marco et al. 2008, Ibern-Gómez et al. 2001) and

not impart any oak derived characteristics, while fermenting and/or aging in oak can result in the addition of oak based flavor and phenolic compounds. These oak compounds can impart an oaky, buttery, or vanilla aroma into the wine (Gutiérrez Afonso 2002, Jackson 2000). Typically red wines are aged in oak barrels while most white wines are not. Another point of variation in the standard winemaking processes of red and white wine is the temperature of primary alcoholic fermentation. The fermentation of white wine is frequently conducted between 13-15°C, while red wine fermentations are commonly performed between 25-30°C. The progression of fermentation is monitored by daily Brix (soluble solids/sugar) measurements (Robinson and Beazley 1992). Fermentation is generally considered complete when there is less than 0.5 g/L sugar remaining (Jackson 2000).

In red wine processing there are often additional steps taken by winemakers to increase the extraction of certain compounds, like on-skin fermentation (Jackson 2000). Typically winemakers are looking to increase the extraction of phenolic compounds (Jackson 2000). Phenolic compounds primarily come from the skin, seed, and stem of the grape cluster (Waterhouse 2002). Phenolics are broken down into two categories; flavonoids and non-flavonoids (Waterhouse 2002). Non-flavonoids consist of the following compound subgroups: hydroxycinnaminc acids, benzoic acids, hydrolysable tannins, and stillbenes (Waterhouse 2002). Benzoic acids and hydrolysable tannins are mainly derived from oak and not the grape cluster (Ribereau-Gayon et al. 2006). The major non-flavonoid component not derived from oak is hydroxycinnamic acid, which plays a bigger role in white wines than in red wines due to the overall lower phenolic content of white wines (Fernández-Zurbano et al. 1998).

Flavonoids are divided into flavonols and anythocyains (Waterhouse 2002). This group of phenolic compounds is derived primarily from the grape skins and seeds, and plays a large role in the color, taste, and mouthfeel of red wine (Ribereau-Gayon et al. 2006, Sacchi et al. 2005). This means phenolic compounds are a key aspect of red wine. Therefore, in addition to looking at acid/sugar levels in grapes, vineyard

managers and winemakers may try to examine the phenolic content of the grapes to determine grape quality (Jackson 2000). However, the correlation between the phenolic content of berries and wine quality has yet to be well established (Jackson 2000). This is most likely because the extraction of phenolics mainly comes from the skin and seed of the grape, and is heavily dependent on processing techniques to be extracted into the wine (Sacchi et al. 2005, Suriano et al. 2015). Besides fermenting on-skin, there are other steps implemented by producers during fermentation to increase the extraction of phenolic compounds. Common extraction practices include punch downs, pump overs, extended maceration, and enzyme additions (Ivanova et al. 2012, Ribereau-Gayon et al. 2006). These practices vary among producers, but are usually carried out through the majority of primary alcoholic fermentation (Jackson 2000).

After alcoholic fermentation, wines may undergo a secondary fermentation called malolactic fermentation (MLF) (Jackson 2000, Lasik 2013). Classically, MLF begins after primary alcoholic fermentation, although sometimes the grape must can be co-inoculated for alcoholic fermentation and malolactic fermentation at the same time (Abrahamse and Bartowsky 2012). MLF is typically induced by the inoculation of a commercial strain of *Oenococcus oeni* (Liu 2002). During MLF malic acid is decarboxylated to lactic acid (Waterhouse et al. 2016). This conversion reduces the acidity of the wine leading to an increase in pH (Boulton et al. 2013, Jackson 2000). Since MLF raises the pH of wines it is widely used in cold or cool climates where grapes tend to have higher amounts of acid than sugar (Robinson 2006). In warm or hot winegrowing climates, wines that undergo MLF must be carefully monitored to avoid over de-acidifying the wine (Davis et al. 1985).

After all fermentations are complete, wine will undergo stabilization and clarification, to ensure protein and tartrate stability, and to eliminate sulfide aromas and any other potential faults (Jackson 2000). Wine is usually cold settled and racked, or centrifuged to remove most large solids (Malfeito-Ferreira 2011, Ribereau-Gayon et al. 2006). Fining agents like gelatin, bentonite, PVPP, and enzymes are also used to

help clarify the wine and decrease haze (Lomolino and Curioni 2007, Puig-Deu et al. 1996, Waters et al. 2005). In addition, it is common practice for winemakers to add sulfur dioxide (Fleet 1993) to prevent oxidation, and inhibit or kill any microbes that could cause spoilage in the wine (Bartowsky 2009). Once all fining and stabilization steps are completed, wine can be filtered for clarification and/or for the removal of any microorganisms prior to bottling (Fleet 1993, Malfeito-Ferreira 2011).

Grape Composition, Nitrogen, and Wine Quality

The winemaking processes outlined above are the basic steps followed by most producers. However, differences in grape composition can lead to significantly different wines, even when following the exact same winemaking procedures. In the same manner, wine made from the same grapes can be significantly different from each other due to changes in a range of winemaking practices. A key component of grapes that can affect the production of wine is nitrogen. Nitrogen is a key nutrient for yeast metabolism and can impact fermentation kinetics and wine sensory attributes (Bell and Henschke 2008a). The type and concentration of nitrogen present during fermentation is of particular interest as not all nitrogen can be utilized by *S. cerevisiae* during alcoholic fermentation. Nitrogen that can be utilized by *S. cerevisiae* during alcoholic fermentation is referred to as yeast assimilable nitrogen (YAN). Yeast assimilable nitrogen is comprised of two types of nitrogen, ammonia and primary amino acids (Bell and Henschke 2008a). Primary amino acids are those amino acids that have a primary amine group. The concentration of ammonia or primary amino acids in a grape is dependent on a number of different factors including grape variety, climate, and water availability (Bell and Henschke 2005). It is important to understand that not all amino acids found in wine grapes contribute to YAN. For example, proline is an amino acid often found in high concentrations in Chardonnay, but it is a secondary amino acid (Huang and Ough 1991). Since proline is a secondary amino acid it cannot be utilized by yeast, and thus does not contribute to the amount of YAN (Ribereau-Gayon et al. 2006). Sources of nitrogen can be naturally present in the vineyard soil, but producers can boost nitrogen levels in the vineyard through the incorporation of organic material into the soil and/or through the use of fertilizers (Bell and Henschke 2008a).

YAN and Fermentation Kinetics

It is well established that yeasts require nitrogen to successfully complete alcoholic fermentation. However, there is some dispute over how much YAN is required. A minimum of 140 mg N/L is frequently cited as the necessary amount for fermentation (Bell and Henschke 2008a). However, studies that have investigated YAN requirements for alcoholic fermentation often use concentrations much higher than 140 mg N/L (Bell and Henschke 2008a, Beltran et al. 2005). Often times, fermentation kinetic studies boost YAN levels up to 400 mg N/L (Vilanova et al. 2007). While these high concentrations are successful at preventing slow and problematic fermentations (Bisson 1999, Ugliano et al. 2010), others have reported that concentrations less than 100 mg N/L can result in complete fermentations (Bell and Henschke 2008a, Schreiner et al. 2018). There is also concern that excessive nitrogen can cause overly vigorous fermentations, off-aroma production (Ugliano et al. 2011), and stability issues during aging and storage (Jackson 2000).

The main concern of having insufficient YAN is a slow or problematic ferment referred to as stuck or sluggish (Bisson 1999, Gobert et al. 2019). A stuck ferment refers to one that has stopped fermenting before reaching a desired sugar concentration and a sluggish ferment is one that has slowed down to an inefficient rate (Bisson and Butzke 2000). The concern of stuck and sluggish ferments in the wine industry has resulted in many producers adding high and occasionally excessive amounts of nitrogen at the start of fermentation (Fleet 1993, Ribereau-Gayon et al. 2006). However, if there is more than enough nitrogen present during fermentation the yeast will not utilize all of it, resulting in ammonia and/or primary amino acids to be leftover in the wine (Fleet 1993). Leftover nitrogen could be problematic because it can serve as a potential nutrient source for other spoilage bacteria or yeasts after primary alcoholic fermentation (Fleet 1993). Not only do stuck and sluggish ferments cause wine production issues, but they are also known to cause negative wine sensory characteristics (Swiegers and Pretorius 2007).

YAN and Hydrogen Sulfide

The concentration and composition of YAN present during fermentation can also impact the production of hydrogen sulfide (H_2S) by yeast (Jiranek et al. 1995a). Hydrogen sulfide is a volatile sulfur compound, with an undesirable aroma, naturally produced by yeast during fermentation (Rauhut et al. 1996). When H_2S is produced above its threshold level, it imparts a rotten egg, decaying seaweed, or rubbery aroma on wine (Fleet 1993). In the production of sulfur containing amino acids, yeasts naturally produce H_2S via the sulfate reduction pathway (Swiegers and Pretorius 2007, Rauhut et al. 1996). Yeast assimilable nitrogen concentration is reported to influence H_2S production because, as nitrogen is required for the formation of the sulfur containing amino acids methionine and cysteine. A number of studies have reported that having inadequate levels of YAN during fermentation can lead to H_2S production (Jiranek et al., 1995b; Park et al., 2000; Vos and Gray, 1979). Jiranek et al. 1995 reported that high H_2S production under low YAN conditions was due to the ongoing reduction of sulfite to sulfide by sulfite reductase even though the nitrogen-containing precursors that react with sulfide to form methionine and/or cysteine were exhausted. However, others have reported the opposite trend, reporting that juice/musts with low YAN produce less H_2S than those with higher YAN concentrations (Mendes-Ferreira et al. 2009, Ugliano et al. 2009, Yuan et al. 2018).

While there is still uncertainty regarding what the ideal concentration of YAN is to minimize H_2S formation, wine producers are typically more concerned about low YAN leading to an increase in H_2S. Therefore, boosting YAN concentration in a juice or grape must to prevent excessive H_2S production is a common winemaking practice (Bell and Henschke 2008a). Furthermore, the link between low YAN and stuck fermentations provides further motivation to ensure one has sufficient YAN during fermentation. YAN can be increased either in the vineyard or in the winery. In the vineyard, the concentration of nitrogen in the grapes can be boosted through a variety of fertilizers and application techniques. A few types of fertilizers have been studied including urea, ammonium nitrate, and compost (Delgado et al. 2004, Spayd et al. 1994). Fertilizers can be added to the vineyard through either a foliar spray, or

through the irrigation system into the soil (Lasa et al. 2012). Regardless of the application method, some researchers caution against boosting nitrogen in the vineyard due to the potential increase in the productive capacity of the grapevine, which can increase berry size (Keller and Hrazdina 1998). This is problematic because an increase in berry size is often associated with an increase in the pulp/skin ratio, and an increase in the pulp/skin ratio can cause the dilution of important phenolic compounds (Delgado et al. 2004, Gutiérrez-Gamboa et al. 2017). Excessive nitrogen can also lead to excessive vine vigor resulting in large canopies, shading of fruit, and increased disease pressure (Conradie and Saayman 1989, Keller et al. 1999). In contrast, other researchers suggest that boosting nitrogen in the vineyard might be beneficial due to enhanced secondary metabolism of certain compounds, allowing for an increase in the synthesis of flavonoid compounds (Bell and Henschke 2008a).

If grapes contain insufficient YAN at harvest, then adjustments can be made in the winery through the addition of nitrogen containing supplements. Supplement choice in the winery can vary as there are many different commercial products containing organic and/or inorganic nitrogen (Gobert et al. 2019a, Torrea et al. 2011). The most commonly used inorganic nitrogen supplement is diammonium phosphate (DAP) (Bell and Henschke 2008a). DAP is easily dissolved in grape must and provides a comparatively large increase in YAN compared to most other products. While ammonia supplements, like DAP, have been proven to effectively raise YAN levels, there are also amino acid based supplements that are available to boost YAN. It has been suggested that a balance of amino acid and ammonia supplements might be ideal, for both fermentation kinetics and aroma (Torrea et al 2011, Villanova et al 2015).

Nitrogen Metabolism

Yeast utilize nitrogen in a variety of ways. Primarily, nitrogen is used in the formation of proteins that perform key cellular roles. When yeast cells take up nitrogen, different nitrogenous compounds proceed down various metabolic pathways. For example, the amino acids asparagine, glutamine, and glutamate

produce a strong nitrogen catabolic repression effect (Waterhouse et al. 2016). When these amino acids are metabolized in the yeast cell, they serve as major donors for amino acid biosynthesis and can also be regenerated from α-ketoglutarate and glutamate (Bell and Henschke 2008, Beltran et al. 2004). Other primary amino acids can act as nitrogen donors to regenerate the intermediates, α-ketoglutarate and glutamate, for amino acid and nucleotide synthesis (Styger et al. 2011, Waterhouse et al. 2016). This means that the uptake and metabolism of ammonia and amino acids can be a continuous process that allows yeast to utilize nearly any primary amino acid (Beltran et al. 2004).

Nitrogen can also be used in the formation of volatile aroma compounds. When nitrogen compounds are processed by yeast cells they can be transformed into volatile aroma compounds like higher alcohols, esters, and acids (Styger et al. 2011, Waterhouse et al. 2016). Higher alcohols can be formed through anabolic or catabolic pathways where amino acids are first combined with glucose and pyruvate to form α-keto acids (Waterhouse et al. 2016). These α-keto acids, often referred to as carbon skeletons, can be decarboxylated to aldehydes (Bell and Henschke 2008a, Hazelwood et al. 2008). Aldehydes can then be enzymatically transformed into higher alcohols or fatty acids (Waterhouse et al. 2016). The pathway responsible for the these transformations and eventually the transition of higher alcohols into other volatile compounds is called the Ehlrich pathway (Bell and Henschke 2008a, Hazelwood et al. 2008).

Amino Acids and Volatile Aroma Compounds

A number of studies have explored the relationship between specific higher alcohols and certain amino acids (Ardö 2006, Hernández-Orte et al. 2002). For example, leucine, phenylalanine, and valine have been related to isoamyl alcohol, β-phenylethanol, and isobutanol respectively (Dickinson et al. 1998, Hernández-Orte et al. 2002, Styger et al. 2011). These are some of the major fermentation-derived higher alcohols and are associated with the following descriptors respectively; fusel, rose or honey, and solvent (Hernández-Orte et al. 2002). With the exception of β-phenylethanol, many of the higher alcohols are not necessarily desirable traits for

wine aroma (Waterhouse et al. 2016). However, these undesirable compounds are often found in levels below threshold detection or in combination with other volatile compounds that make them "detectable but indescribable" (Waterhouse et al. 2016). Not only do studies point to the importance of individual amino acids, but they also highlight the importance of total YAN concentration on volatile compound production. Studies have investigated how YAN concentrations can influence the sensory properties of higher alcohols in wine (Bell and Henschke 2008a). A few researchers have found that low YAN levels favor the formation of higher alcohols and branched chain esters (Hernandez-Orte et al. 2006, Yuan et al. 2018). However, in a study that compared different levels and types of YAN, it was found that the production of higher alcohols increased with an increase in YAN (Torrea et al. 2011). This result was true regardless of the nitrogen source used (Torrea et al. 2011). While many of the higher alcohol compounds may not be particularly desirable, nitrogen compounds are also involved in the production of some desirable esters (Styger et al. 2011).

Esters are broken up into two categories; acetate esters, and ethyl esters (Waterhouse et al. 2016). Acetate esters are formed from higher alcohols, and ethyl esters are formed from fatty acids. The formation of acetate esters is dependent on the availability of certain substrates and the expression of specific enzymes, because they are formed through the enzymatic acetylation of alcohols (Ribereau-Gayon et al. 2006). Therefore, the production of acetate esters is indirectly associated with amino acids. This indirect relationship makes predicting the transformation of specific amino acids into acetate esters incredibly complex. Ethyl esters are formed as intermediate metabolites in fatty acid metabolism from the ethanolysis of acyl-CoA (Styger et al. 2011). Fatty acid metabolism occurs with compounds from the Ehrlich pathway and is thus influenced by amino acids. Therefore, just like acetate esters, ethyl esters are also indirectly formed from amino acids metabolized by the yeast cell (Styger et al. 2011).

Despite the indirect relationship of nitrogen to acetate and ethyl esters, a number of studies have attempted to correlate the amount of YAN to the production of volatile esters. For example, Torrea et al. (2011) reported that as the total amount of YAN increased, so did the amount of acetate esters. However, as YAN increased there was not a significant increase in the amount of ethyl esters produced (Torrea et al. 2011). Torrea et al (2011) also compared two different types of nitrogen supplements, an amino acid based supplement and DAP. They found that the amino acid based supplement produced more acetate and ethyl esters than DAP at the same YAN concentration (Torrea et al. 2011). However, the differences found in acetate and ethyl ester production between the two nitrogen sources were not statistically significant at low YAN levels (Torrea et al. 2011). In contrast, Miller et al. (2007) compared similar nitrogen supplements and found that acetate esters were higher when an ammonia supplement was used rather than an amino acid supplement. It is possible that the contradicting correlations between nitrogen type and volatile compound production is due to other variables like temperature and yeast strain as these factors are known to impact volatile aroma production.

Yeast

Yeast strains have different abilities and preferences when it comes to the metabolism and production of nitrogen based compounds (Borneman et al. 2007, Hernandez-Orte et al. 2006). In *S. cerevisiae,* nitrogen is transported into the cell through different mechanisms, dependent on the type of nitrogen (Gobert et al. 2019a). The uptake of nitrogen into yeast is controlled by specific and non-specific permeases, and by the nitrogen catabolite repression system (Fleet 1993, Querol and Fleet 2006). These uptake pathways are strongly regulated by the expression of numerous genes in yeast (Fleet 1993). The expression of these genes and other regulatory components are highly variable between yeast strains. These regulatory variances cause different yeast strains to have different preferred sources of nitrogen (Crépin et al. 2012, Gobert et al. 2019a). This means that the uptake of ammonia and primary amino acids is dependent on yeast strain (Gobert et al. 2019a, Waterhouse et al. 2016). For example, in a review of *S. cerevisiae* nitrogen preferences, researchers found that

there are 17 amino acids that can theoretically be considered preferred by various yeast strains (Gobert et al. 2019a). Furthermore, not only do some yeast strains have preferred nitrogen sources, but they have different capabilities when up taking and utilizing nitrogen (Bell and Henschke 2008a). Some *S. cerevisiae* strains contain a higher expression of genes involved in the transport and metabolism of nitrogen than other strains (Barbosa et al. 2015). Yeast that have a high expression of these genes are considered high nitrogen demanding yeast, and strains that do not have a high expression of these genes are considered low nitrogen demanding yeast. As a result different *S. cerevisiae* strains can produce different volatile aromas under the same nitrogen conditions (Brice et al. 2018, Crépin et al. 2012). Therefore, when investigating nutrient effects on wine fermentation, it is important to consider the yeast strain used and the role it may play in fermentation kinetics and wine aroma.

In a study of Shiraz wine, Ugliano et al. (2010) investigated two yeast strains commonly used in red wine production with similar nutrient needs. They found that as the concentration of DAP increased the concentration of acetate esters and higher alcohols decreased (Ugliano et al. 2010). However, the changes in volatile compound production were also dependent on the yeast strain used, and not just the concentration of YAN (Ugliano et al. 2009, 2010). Ugliano et al. (2010) found that one of the yeast strains investigated produced a Shiraz wine with a greater amount of esters, and alcohols compared to the other yeast strain. Torrea et al (2003) investigated how yeast strains with different nitrogen demands influence volatile compound production. Torrea et al (2003) reported that in wine like media the strain with the higher nitrogen demand produced a higher concentration of esters, than the strain with lower nitrogen demand. In a similar manner, Gobert et al. (2019) found that high nitrogen demanding yeast strains had increased volatile aroma production. These studies highlight the importance of research that investigates both the impact of nutrients and yeast strains on volatile aroma as there are interactive effects that must be taken into account (Carrau et al. 2008, Deed et al. 2017, Rollero et al. 2015). Furthermore, they also highlight the complexity of predicating how different yeast

strains and nutrients will interact to create specific volatile aroma compounds (Hernandez-Orte et al. 2006, Vilanova et al. 2007).

Using Sensory Analysis to Determine Wine Quality

Wine quality is a challenging term to define, but the different sensory aspects of wine are useful tools for examining its quality. When tasting wine, consumers evaluate the appearance, aroma, mouthfeel, flavor, and finish (Puckette and Hammack 2015). These sensory attributes are complex and the perception of them can vary depending on the consumer, making wine tasting a subjective experience (Styger et al. 2011). In addition to consumer perception, many wine attributes like aroma are also greatly impacted by various factors including: grape variety, fermentation temperature, glass shape, and yeast selection (Dzialo et al. 2017, Styger et al. 2011). The grape variety and fermentation practices used are said to be the main sources of aroma and flavor development (Styger et al. 2011). Every grape variety contains its own unique flavor and aroma profile, but the overall volatile composition of each grape variety is actually very similar (Polášková et al. 2008). The main differences in overall wine aroma actually come from the varying ratios of these volatile compounds (Polášková et al. 2008). For example, recent studies have shown that in complex mixtures the vegetal/bell pepper aroma of Cabernet wines can be masked by the presence of fruit aroma, and that fruity aromas can be enhanced by the presence of C13-norisoprenoids and dimethyl sulfides, but suppressed by ethanol (Escudero et al. 2007, Polášková et al. 2008).

In Chardonnay and Pinot noir, wine aroma is primarily derived from fermentation, because the grape variety of both these cultivars is not very distinct (Robinson and Beazley 1992). Chardonnay and Pinot noir are both said to be vaguely fruity, and are mainly used as vehicles for showcasing vineyard and winery character (Robinson 2006). Despite this, there are still recognizable aromas associated with each variety, based off wine region. For example, in Chardonnay's famous home of Burgundy, France, the wine aroma can be muted with apple aromas, or creamy and oaky (Puckette and Hammack 2015). However, in other locations around France,

Chardonnay is described as melon, lemon, quince, or starfruit (Robinson and Beazley 1992). In the United States it is common for Chardonnay to have an exotic fruit aroma with possible pineapple or yellow apple notes (Broom and Beazley 2003, Puckette and Hammack 2015). Pinot noir is know to have raspberry or clove notes when it is from New Zealand, Chile, or Argentina, and to have cranberry or mushroom notes when it is from France, Oregon, or Italy (Puckette and Hammack 2015, Tomasino et al. 2013).

Aside from aroma and taste, a critical wine quality component is mouthfeel. Wine, mouthfeel is an important attribute for quality, particularly in red wines, but mouthfeel terminology is not well agreed upon or described (Gawel et al. 2000). To make wine mouthfeel even more challenging to understand, research has shown that there are numerous factors that can influence wine mouthfeel. For example, researchers have found that the chemical structure and perception of some wine mouthfeel compounds is dependent on factors like growing location, wine age, and consumer panel training (Gawel 2008, Gawel et al. 2000). Some production practices that influence wine mouthfeel are well understood (Gawel et al. 2000, Vidal et al. 2004a). For instance, MLF reduces acidity, therefore helping soften wine acidity, but it can change other mouthfeel properties too (Waterhouse et al. 2016). MLF is also said to impart a creaminess or increased viscosity (Jackson 2000). In red wine, like Pinot noir, many mouthfeel attributes are due to phenolic compounds extracted from grapes during primary alcoholic fermentation (Jackson 2000). Phenolic compounds can impart various astringent and drying characteristics to wine (Robinson 2006) that are important for providing wine with texture and body. Ultimately, there are many processing decisions that influence the final sensory attributes of wine, and many of these are impacted by grape composition.

Sensory Analysis and Nitrogen

Relating initial nitrogen composition to wine aroma faces a number of sensory analysis challenges. In sensory testing, it is hard to predict how consumers will perceive products. The concentration of individual volatile compounds in a wine can help researchers understand whether or not specific compounds are above or below

the detection and/or acceptability threshold (Lawless and Heymann 2010). However, it is possible to have a wine where the concentration of every volatile compound is known, but you still can't correctly predict how consumers will perceive the wine. Not only is the tasting experience subjective, but when a wine is evaluated, it is the sum of all the volatile compounds present that gives the wine its unique smell (Lawless and Heymann 2010, Styger et al. 2011).

In wine there are many interaction effects between the volatile compounds present, and these interactions are capable of altering the aroma perception of wine (Styger et al. 2011). In sensory testing acetate esters commonly have a floral or fruity aroma and play a large role in the aroma of new/young wines (Waterhouse et al. 2016). However, the perception of the acetate ester, ethyl acetate, can vary based on its concentration and the presence of other volatile compounds (Waterhouse et al. 2016). For example, ethyl acetate is described as fruity in low concentrations, but as nail polish remover at high concentrations (Waterhouse et al. 2016). Therefore, if ethyl acetate is the dominate ester, it is likely that the wine will perceived as solvent-like, but if the wine is also dominated by isoamyl acetate or ethyl butanoate it will likely have a more fruity, banana or apple aroma (Campo et al. 2005). Finally, it is also important to note that the threshold level, the intensity level that is barely perceptible, and odor activity level, the ratio between the concentration of the compound and its detection threshold, for isoamyl acetate and ethyl butanoate are much lower than ethyl acetate (Ferreira et al. 2002, Juan et al. 2012). This means that if ethyl acetate is present in the same concentration as these other esters its aroma will likely be masked.

Due to the interactive effect of esters, it can be difficult to predict wine aroma based off of volatile compound concentrations. The concentration of volatile compounds are known to be impacted by nitrogen composition, but it is challenging to predict the relationship between initial nitrogen content to volatile compound formation and sensory impact. There have been studies that have investigated the effects of different wine nutrients on wine composition, but only a few have conducted sensory

analysis (Bell and Henschke 2008a, Torrea et al. 2011, Ugliano et al. 2010). Additionally no studies have directly compared nitrogen supplements in the winery to boosting nitrogen in the vineyard. Finally, many studies investigating the impact of YAN on wine quality have used very high YAN concentrations. For example, Ugliano et al. (2010) supplemented Shiraz wines with DAP to 400 mg/L YAN while Torrea et al. (2011) boosted YAN to 480 mg/L.

The objective of this study was therefore, to investigate how the source of nitrogen, vineyard boosted vs. winery supplemented, impacts wine quality. Specifically, this study will investigate the impact of nitrogen on Pinot noir and Chardonnay wine quality. These grape varieties were chosen because of their importance to the Oregon wine industry given that Pinot noir is the most planted red varietal while Chardonnay is the second most planted white varietal. The impact of nitrogen supplementation will be assessed through chemical analysis of grapes and wines, and consumer panel sensory testing. In addition to YAN analysis, grape amino acid composition will be analyzed so that differences in nitrogen composition as well as concentration can be determined. As pointed out above, wine quality is often defined by many sensory attributes. Therefore, adding a sensory component to this study was crucial. Consumers play a large role in the wine market, so it was important they were involved in sensory testing. However describing wine mouthfeel and aroma can be challenging, so to accurately determine specific wine descriptors it was necessary to have wine experts be a part of the sensory testing as well. In this study there were two goals in sensory testing; to determine if consumers could tell a difference between wines produced from grapes supplemented with a variety of nitrogen sources, and to determine what specific wine descriptors were impacted by differences in nitrogen supplementations using expert tasters.

Chapter 2

Changes in Grape Juice Chemistry and Fermentation Kinetics Due to Nitrogen Supplementation in the Vineyard and Winery

Abstract

The impact of winery or vineyard nitrogen (N) addition on Chardonnay and Pinot noir juice/must chemistry and fermentation kinetics was examined across three years. Nitrogen was applied to Pinot noir or Chardonnay grapevines by fertigation (+SoilN) or foliar spray (+FoliarN). In the winery, diammonium phosphate (+DAP) or organic N (+Nutriferm) were used to supplement Chardonnay juice and Pinot noir grape must. The addition of nitrogen in the vineyard increased yeast assimilable nitrogen (YAN) concentration every year with both application methods being effective. Addition of DAP in the winery increased ammonia N only, while the addition of the organic N product increased the concentration of free amino nitrogen (FAN). All Pinot noir and Chardonnay treatments successfully completed fermentation each year. No significant differences in time to completion were noted between treatments even when ferments contained <150 mg/L YAN. In an additional experiment, three different yeast strains were used to ferment Chardonnay juice supplemented with N by fertigation in the vineyard, or with DAP or an organic N product in the winery. Nitrogen type had a significant affect on fermentation rate, and there was also a significant interaction effect between yeast strain and nitrogen source on fermentation rate. In addition to impacting overall YAN concentration, vineyard or winery nitrogen supplementation also affected the concentration of various free amino acids. Addition of nitrogen in the vineyard consistently boosted arginine, alanine, serine, and glutamine, while the addition of the organic N product in the winery consistently boosted threonine compared to other treatments. These results show that both nitrogen concentration and source are important for successful fermentation, and that when YAN is adequate, fermentation rate can be impacted by yeast strain and type of YAN present.

Introduction

Yeasts require nitrogen to successfully complete alcoholic fermentation. Yeast assimilable nitrogen (YAN), composed of ammonia nitrogen and primary amino acid nitrogen, is of particular importance for its role in fermentation health, and wine quality (Bell and Henschke 2008a). However, there is some dispute over how much YAN is required. A minimum of 140 mg N/L is frequently cited as the necessary amount for fermentation, but recent studies have reported that concentrations below 100 mg N/L can results in successful fermentations (Bell and Henschke 2008a, Schreiner et al. 2018). The main reason insufficient YAN is concerning, is the possibility of stuck or sluggish ferments (Bisson 1999, Gobert et al. 2019b). Additionally studies have also shown that the concentration of YAN can influence the production of hydrogen sulfide (Jiranek et al. 1995a). Hydrogen sulfide is a volatile compound that smells like rotten eggs (Fleet 1993). Therefore, many studies have sought to determine the ideal concentration of YAN for timely alcoholic fermentation, but also for reduction of hydrogen sulfide formation (Jiranek et al. 1995b, Ugliano et al. 2009). Grapes naturally contain nitrogen but YAN concentration in grapes may not always be sufficient for fermentation. Wine producers therefore often boost YAN in the vineyard and/or the winery to ensure sufficient YAN for fermentation (Henschke and Jiranek 1993, Mendes-Ferreira et al. 2011). In the vineyard, YAN can be boosted through the addition of nitrogen fertilizers (organic material or urea based fertilizers). In the winery, YAN can be boosted by the addition of a variety of commercial products that contain organic and/or inorganic nitrogen (Boulton et al. 2013, Jackson 2000).

Despite the numerous studies investigating the concentration of YAN on fermentation rate and hydrogen sulfide formation, there is still not a consensus on the “ideal” level of YAN for fermentation. This lack of consensus may be due impart to the fact that YAN is dependent on growing location, and varietal (Bell and Henschke 2008a). Furthermore, the level of YAN for successful fermentation may vary with yeast stain and varietal (Bisson 1991, Brice et al. 2014). Many studies have shown that yeast strains play a role in fermentation kinetics and that different yeasts have different

YAN demands and preferences (Brice et al. 2014, Hernandez-Orte et al. 2006). While the concentration of YAN is of clear interest, so is the yeast strain chosen. Thus, there is a demand for research investigating how different nitrogen sources and concentrations, and yeast strains impact fermentation rate.

Besides fermentation kinetics, YAN concentration and composition can play a role in other aspects of wine quality. In particular, concentrations of ammonia nitrogen vs. amino acid nitrogen are reported to impact wine volatile aromas differently. Torrea et al. (2011) and Vilanova et al. (2007) suggested that amino acid based supplements rather than ammonia based supplements can improve overall wine quality. Additionally, other studies have linked the presence of certain amino acids in grape juice/must to the production of specific volatile compounds in the final wine (Styger et al. 2011, Vilanova et al. 2007). Therefore, there is a growing interest in understanding how different sources and applications of nitrogen change the content of ammonia or primary amino acids in grape juice/must, and how these can potentially change wine quality. Furthermore, it is simpler to add nitrogen in the winery rather than the vineyard, so if the source of YAN (vineyard or winery) does not impact wine quality than it would be advisable to make adjustments at the winery. However, if the source of nitrogen does impact wine quality than wine producers will need to consider this factor when deciding how to boost YAN.

For these reasons, the goals of this study were to determine the effect of boosting YAN in the vineyard or the winery on juice/must chemistry, fermentation kinetics, and amino acid composition. In addition, interactions between yeast strain and YAN concentration and composition were explored. To achieve these goals, Chardonnay and Pinot noir wines were made from grapes produced in a commercial vineyard where nitrogen was applied in the form of urea/ammonium nitrate fertilizer. In the winery, additions of inorganic nitrogen or organic nitrogen were applied.

Materials and Methods

Grape and Wine Production

Chardonnay (CH) and Pinot Noir (PN) wines were produced in 2016, 2017, and 2018 at the Oregon State University Research Winery from grapes harvested at a commercial vineyard in Eola Amity, Oregon. The experimental plots in the vineyard consisted of five treatments in a replicated complete block design with four replicates for a total of 20 experimental units per variety. The five treatments compared for each grape variety were, Control (no N addition in vineyard, no N addition in winery), +DAP added in winery (no N in vineyard), +Nutriferm added in winery (no N in vineyard), +Soil N in vineyard (UAN, urea and ammonium nitrate in water, applied via drip system 2 or 3 times at 20 pounds of N per acre, for a total of 40-60 pounds with no N addition in winery), and +FoilarN (UAN, urea and ammonium nitrate in water, sprayed on foliage at 7.5 pounds of N per acre with no N addition at winery). The +FoilarN treatment did not begin until 2017. In 2017 the PN block was moved due to a tillage error in the original PN block being used for this trial in spring 2016. In each year, grapes were harvested upon maturity.

After harvest, CH and PN grapes were stored overnight in a cold room at 4°C before being destemmed. CH grapes were pressed at 1.5 bar for 5 minutes using a bladder press. All CH juice was stored in 5 gallon carboys for 24 hours at 4°C for cold settling following an addition of 50 mg/L sulfur dioxide (SO_2). CH juice was then racked into a clean and sanitized 5 gallon carboy and samples assessed for basic fruit maturity indices including YAN. The volume of juice in each carboy was standardized to 12 L. Twenty-eight kg of destemmed Pinot Noir grapes from each vineyard replicate were transferred into plastic fermenters and 50 mg/L SO_2 added. Fermenters were placed in a cold room set at 8°C for 24 hrs after which samples were assessed for basic fruit maturity indices including YAN.

Grape analysis

Fruit maturity indices (°Brix, pH, and titratable acidity) were determined using standard methods. YAN concentration was determined by summing free amino acid-

nitrogen obtained by the OPA (o-phthaldialdehyde) colorimetric assay (Dukes and Butzke 1998) and ammonium nitrogen by an enzymatic assay (Vintessential® Laboratories, Dromana, Vivtoia, Australia). After the initial YAN assessment, adjustments were made to the +DAP and +Nutriferm treatments by the addition of diammonium phosphate (DAP) or Nutriferm Arom Plus (Enartis, California, USA). The goal was to raise the YAN value of the +DAP and +Nutrifem treatments to the YAN value of the +Soil N vineyard treatment. The concentration of ammonium nitrogen and free amino acid nitrogen provided by DAP and Nutriferm Arom Plus was originally determined by making additions of DAP or Nutriferm Arom Plus to white grape juice (Santa Cruz Organic) and measuring ammonia nitrogen and free amino acid nitrogen before and after additions. DAP contributed 220 mg ammonia-N/g and no free amino acid-N while Nutriferm Arom Plus provided no ammonia-N and 150 mg free amino acid-N/g. DAP was weighed out, added directly to each replicate, and mixed thoroughly. Nutriferm Arom Plus was weighed out and dissolved in a small amount of juice from each fermenter before being added to the remaining juice and mixed thoroughly. After mixing samples were re-assessed for YAN. Additional samples were frozen at -80°C for later HPLC analysis of amino acid content. The complete experimental design is illustrated in Figure 2-1.

Fermentation:

After nutrient adjustments were made, CH juice was inoculated with *Saccharomyces cerevisiae* D47™ (Lallemand, Montreal, Canada) following manufacturer's instructions and fermented in a temperature controlled room at 15°C. Pinot noir must was inoculated with *Sacchromyces cerevisiae* RC212™ (Lallemand, Montreal, Canada) following the manufacturer's instructions and fermented in a temperature controlled room at 27°C. Pinot noir fermentations received twice daily punch-downs. All fermentations were monitored by daily brix measurements with an Anton Paar DMA 35N density meter (Graz, Austria). Once all CH replicates within a treatment were below 0°Brix, 50 mg/L SO_2 was added and the wines were moved to 4°C to cold settle for 48 hrs before being racked. Once all PN replicates were below 0°Brix they were pressed using a bladder press for 5 minutes at 1.0 bar. Samples were taken from each replicate after YAN adjustment and frozen at -80°C for later analysis. The

replicates for each treatment were then combined in a 100 L stainless steel tank and inoculated with *Oenococcus oeni* VP41 (Lallemand, Montreal, Canada) to induce malolactic fermentation (MLF). Tanks were held in a temperature controlled room at 21°C until the completion of MLF (< 0.10 g/L malic acid measured by enzymatic test kit (Vintessential® Laboratories)). After MLF was complete 50 mg/L SO_2 was added to the wines which were then stored cold (4°C) for 48 hrs before being racked. Both the PN and CH wines were stored at 4°C until filtration and bottling. During storage, the free SO_2 levels of both CH and PN were monitored weekly by the aeration-oxidation method (Buechsenstein and Ough, 1978) and adjusted to 30 mg/L.

An addition of 0.12 g/L bentonite was made to all of the CH replicates. After 48 hrs of cold settling the replicates of each CH treatment were racked and combined into a stainless steel tank and returned to cold storage. Preliminary sensory evaluations of the 2018 CH wine replicates determined that replicate one in each CH treatment was noticeably different from all other replicates within that treatment. Therefore, it was decided not to include replicate one and only the other three replicates were combined and used for formal sensory evaluation. Before bottling, CH wine was filtered through a 1µm nylon cartridge filter (G.W. Kent, Ypsilanti, Michigan, USA), followed by a 0.45 µm sterile PES cartridge filter (Merck-Millipore, MA, USA). PN wines were filtered through a plate and frame filter, fitted with 20 cm x 20 cm Beco K-3.0 µm filter sheets (Langenlonsheim, Germany) prior to filtration through a 1µm nylon cartridge filter (G.W. Kent), and a 0.45 µm sterile PES cartridge filter (Merck-Millipore). Wines were bottled in 750 mL screw-capped (Stelvin™, Amcor, Zurich) wine bottles and stored at 13°C until required for analysis.

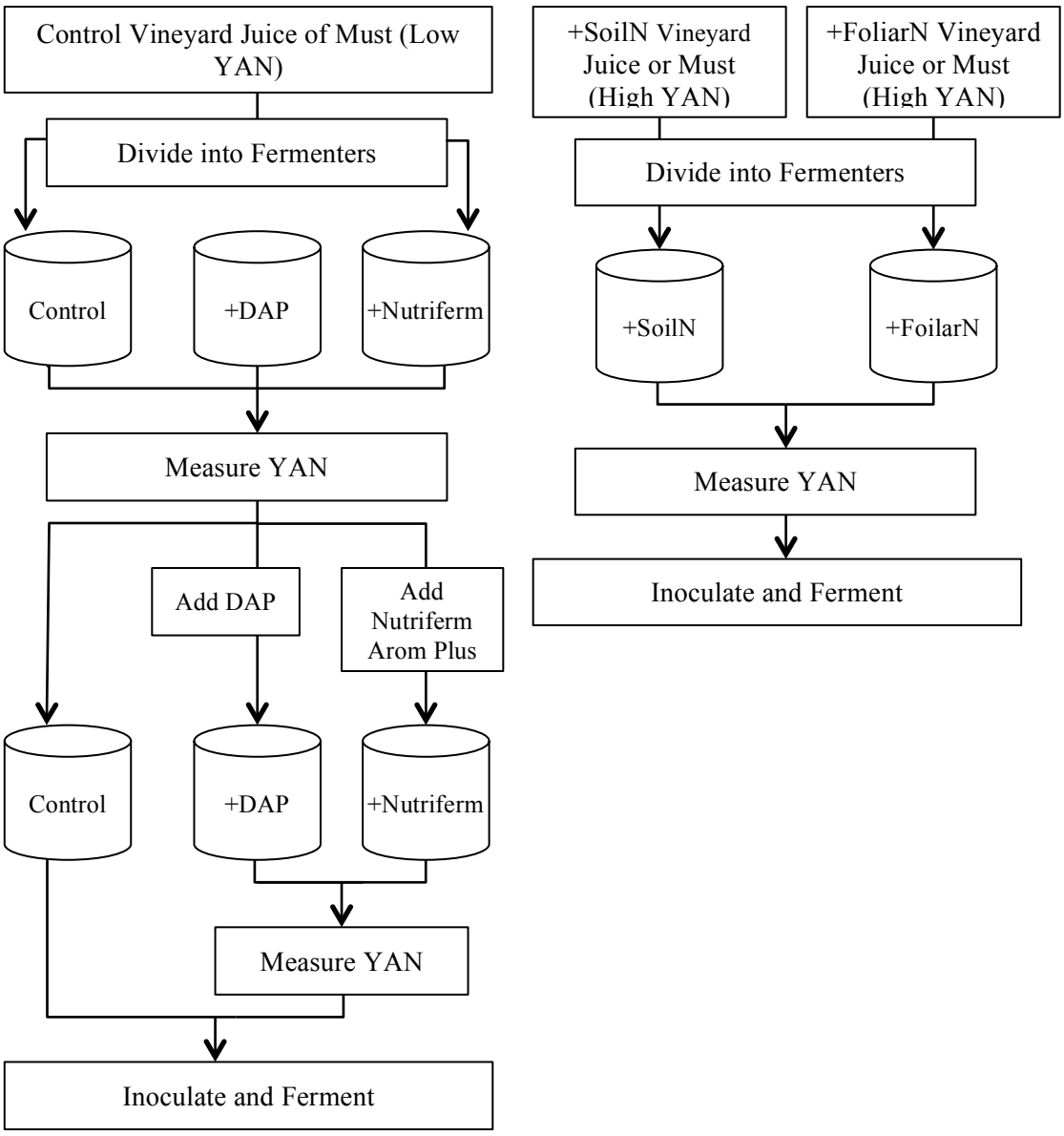

Figure 2-1. Experimental design for Pinot noir and Chardonnay fermentations with various nitrogen supplementations treatments.

Yeast Strain x Nitrogen Supplementation Experiment

In 2018, three gallons of the +SoilN and Control CH juice was set aside for an additional experiment investigating the interaction between yeast strain and nitrogen supplementation on Chardonnay wine aroma. After pressing, the juice from each treatment was stored in 3 gallon carboys for 2 days at 4°C before being racked. A sample was pulled at this time from each carboy for YAN assessment. The juice

from the +Soil N treatment was divided up into 9 sterile 500 ml PYREX glass bottles. The Control juice was divided into 27 sterile 500 ml PYREX glass bottles. All bottles were filled with 300 ml aliquots of juice. All together, there were 12 treatments, each with 3 replicates. The experimental design is outlined in Figure 2-2.

YAN concentrations in the +DAP and +Nutriferm treatments were adjusted by the addition of DAP or Nutriferm Arom Plus (Enartis) in the same manner as outlined previously. Samples for YAN measurements and HPLC amino acids analysis were taken and frozen at -80°C until needed. To eliminate naturally occurring microorganisms the juice was treated with dimethyl dicarbonate (DMDC) (Millipore, Merck KGaA, Drmstadt, Germany) at a rate of 400 ul/L as outlined by Deed et al. (2017) and stored at 15°C for 24 hours. Juice samples were plated on YPD media (10 g/L yeast extract, 20 g and /L peptone, 20 g/L D-glucose, 20 g.L agar, pH 6.5) and incubated at 25 °C for 24hrs to confirm that any naturally occurring yeast were eliminated. Juice was then inoculated with either *S. cerevisiae* strain DV10, *S. cerevisiae* strain ICV D47, or *S. cerevisiae bayanus* CVW5. All *S. cerevisiae* strains were sourced from Lallemand (Montreal, Canada) and prepared by streaking from frozen glycerol cultures onto YPD agar plates and incubating at 25 °C for 48 hours. A single colony was then inoculated in YPD broth and incubated at 25°C for 48 hrs. Broth was mixed to re-suspend the yeast and used to inoculate each treatment at approx. 10^6 cfu/mL. All treatments were placed in a temperature controlled room at 15°C and monitored by twice daily weight measurements. Ferments were considered complete when the weight loss stabilized (Miller et al. 2007). When all replicates within each treatment were complete, 50 mg/L SO_2 was added. Wines were centrifuged to remove solids and decanted samples were taken and stored at -80°C for later analysis by GC-MS.

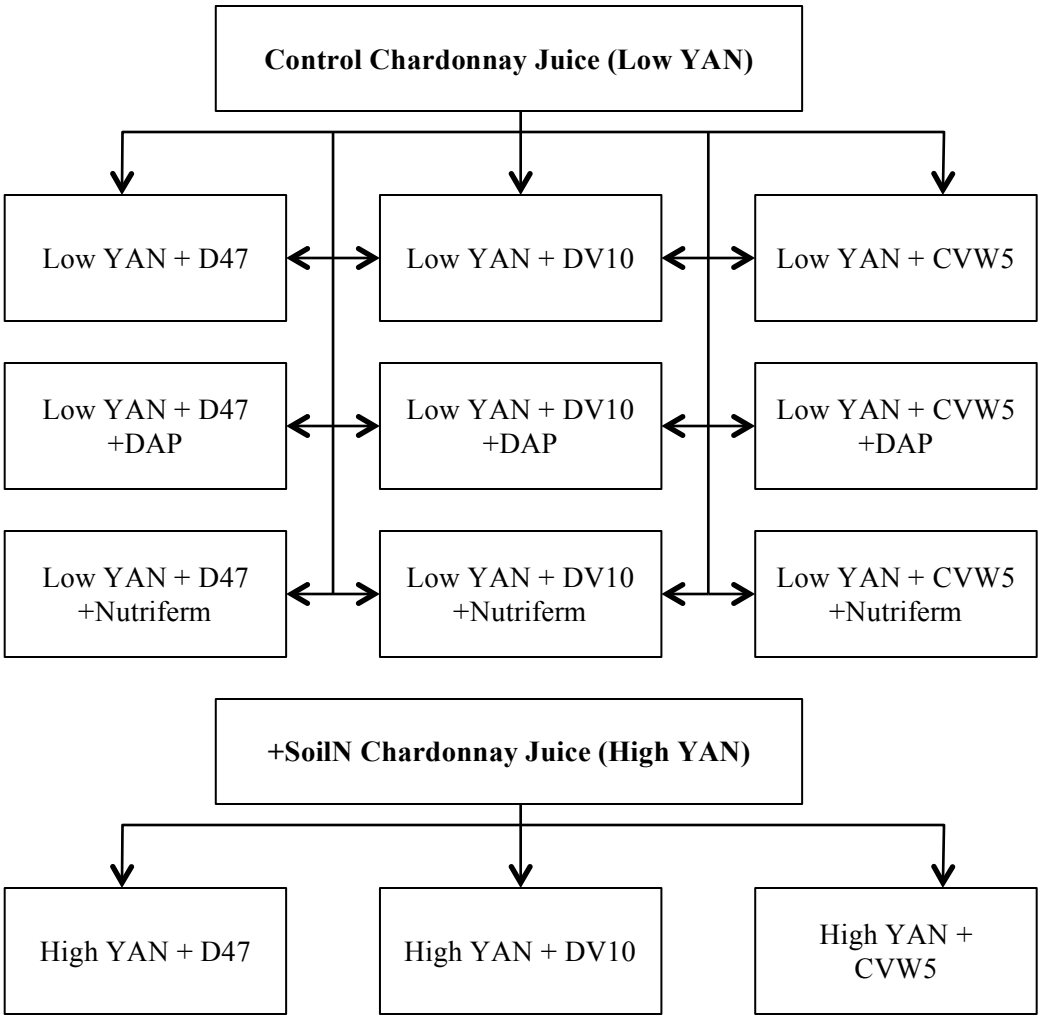

Figure 2-2 Experimental design for determining the interaction effect of yeast strain and nitrogen source on Chardonnay wine quality

Amino Acid Analysis

Free amino acids were analyzed by high performance liquid chromatography (HPLC) using a Hewlett-Packard/Agilent Series 1100 (Palo Alto, CA, USA) equipped with HP ChemStation Software and photodiode array detector (DAD). Free amino acid analysis was performed according to Henderson et al. (2006) with some modifications. In brief, samples of grape juice were taken after YAN adjustment and centrifuged in 1.5ml microcentrfigure tubes (VWR, Radnor, PA, USA) for 5 minutes at 8000 rpm with an Allegrea X-22 centrifuge (Beckman Coulter, Brea, Ca, USA). Juice samples were then filtered through 0.45 um syringe filters (Pall Corporation, Port Washington, NY, USA). Before injection, inline derivitization with o-

phthaldehyde (OPA) (TCI America, Portland, OR, USA) and 9-fluorenylmethyl chloroformate (FMOC) (Sigma Aldrich, Saint Louis, MO, USA) was performed to react primary and secondary amino acids into fluorescent products, respectively.

The instrument was fitted with a Zorbax Eclipse AAA analytical column (150 mm x 4.6 m 5 um, Agilent Technologies, Santa Lara, CA, USA) and guard column (12.5 mm x 4.6 mm, 5 um, Agilent). Mobile phase A was a 40 mM sodium phosphate solution, adjusted to pH 7.8 with a 20% phosphoric acid solution. The sodium phosphate solution was prepared with Milli-Q water (Millipore, Bedford, MA, USA) and sodium phosphate dibasic, anhydrous (Fisher Chemical, Fair Lawn, NJ, USA). Mobile phase B was acetonitrile, methanol, and water (45:45:10 v/v/v). Mobile phase B solvents were from EMD Millipore (Billerica, MA, USA). Gradients of mobile phase A and mobile phase B were applied as followed: 0% B (1.5 ml/min) from 0 to 22.6 min, 57% B linear (1.5 ml/min) from 22.6 to 23.2 min, 100% B linear (1.5 ml/min) from 23.2 to 27.9 min, 0% B linear (1.5 ml/min) from 27.9 to 32.5 min. Quantification of amino acids was determined from calibration curves using amino acid standards (Sigma-Aldrich). Additional single amino acid solutions for extension of STD curves and peak identification were made using pure amino acids (Sigma-Aldrich) dissolved in a solution of 50% 1 N HCl and 50% Milli-Q water. OPA and FMOC were prepared as follows: 2.5 mg OPA was dissolved 500 ul of methanol, 4.5 ml of Borate Buffer (pH of 10.8), and 21 ul of 3-MPA, and 0.25mg of FMOC was dissolved in 1 ml of acetonitrile.

Data Analysis

All statistical analysis tests were completed using Minitab 16 Statistical Software. Statistical analysis of juice and must parameters in Chardonnay and Pinot noir was performed using a one-way ANOVA test to determine if a significant difference (p-value<0.05) existed between the treatment means. Two-way ANOVA test was performed to determine if any significant effects (p-value<0.05) were observed between the different yeast strain and nutrient supplement data. Following significant findings in ANOVA, Tukey's HSD multiple comparison was performed to test least squares means of treatment effects at the $p<0.05$ significance level.

Results

Chardonnay Juice Composition and Fermentation

After processing and nutrient amendments, basic juice parameters for all treatments were measured. No significant differences between the treatments in Brix, pH, or TA were noted in 2016 or 2018 (Table 2-1, 2-3). In 2017, juice from the +FoliarN treatment contained significantly higher Brix than all other treatments, but the difference was small (Table 2-2). In 2017 the control juice and the Nutriferm Arom Plus treatment juice had significantly lower pHs than the +SoilN treatment (Table 2-2). However, as with Brix, these differences were minor.

In each year, the application of nitrogen in the vineyard increased juice YAN concentration compared to the control (Table 2-1, 2-2, 2-3). In addition, the +SoilN treatment contained the highest YAN concentration in each year (Table 2-1, 2-2, 2-3). In 2016 and 2017 the application of YAN in the vineyard significantly increased both free amino acid nitrogen and ammonia nitrogen (Table 2-1, 2-2). In 2018, free amino nitrogen was higher than the control in both the +SoilN and +FoliarN treatments but ammonia N was significantly higher only in the +SoilN treatment (Table 2-3). DAP or Nutriferm Arom Plus additions to the juice resulted in increased YAN concentrations compared to the control juice (Table 2-1, 2-2, 2-3). In 2016 and 2017 the YAN levels of DAP adjusted juice to the same YAN levels as the vineyard nitrogen amended treatments (Soil and/or Foliar N). When DAP was added an increase in ammonia N was observed, while when Nutriferm Arom Plus was added an increase in free amino acid N was observed. While the addition of Nutriferm Arom Plus raised YAN concentration above that of the control, the juice contained lower YAN than the +DAP and +SoilN and treatments (Table 2-1, 2-2). For example, in 2016 the addition of DAP raised the concentration of YAN to 191.1 mgN/L which was not statistically different from the +SoilN YAN of 189.6 mgN/L (Table 2-1). In contrast, the concentration of YAN in the +Nutriferm juice was only raised to 161.7 mgN/L, which was significantly different from the YAN in the +SoilN juice in 2016 (Table 2-1). In 2018, the YAN concentration in juices amended with either DAP or Nutriferm Arom Plus was statistically the same as the Soil N treatment (Table 2-3).

In each year all Chardonnay treatments completed alcoholic fermentation within 770 hours (Table 2-1, 2-2, 2-3). In 2016 and 2018 there were no significant differences between treatments in the time taken to complete fermentation. However, in 2017, the +SoilN treatment finished fermentation significantly faster than the control. While the control treatment, where no nitrogen was added, took the longest to complete fermentation in each year (Figure 2-1, 2-2, 2-3) the time differences were not significant in 2016 and 2018.

In 2018, an additional experiment was conducted to investigate interactions between yeast strain and nitrogen composition. Chardonnay juice from the 2018 control and +SoilN treatments were used. There were no significant Brix, pH, or TA differences between any of the juices (Table 2-4). As previously noted, the addition of nitrogen in the vineyard boosted YAN. For example, the control juice contained 96.4 mg N/L while the Soil N treatment juice contained 147.0 mg N/L (Table 2-4). The addition of DAP boosted ammonia N while Nutriferm Arom Plus increased free amino acid N. The addition of DAP raised the amount of YAN to 137.9 mg N/L which was not statistically different from the amount of YAN in the +SoilN juice (Table 2-4). However, the addition of Nutriferm Arom Plus raised the amount of YAN to 181.8 mgN/L which was significantly higher than the amount of YAN in the +SoilN juice (Table 2-4).

All Chardonnay treatments completed alcoholic fermentation within 610 hours (Table 2-5). There was no significant effect of yeast strain on fermentation time (Table 2-6). However, there was a significant effect due to nutrient and a significant interaction effect between yeast strain and nutrient on fermentation time (Table 2-6). *S. cerevisiae* strain CVW5 and DV10 completed alcoholic fermentation significantly faster in Chardonnay juice with an addition of DAP compared to the control, where no nutrients were added (Table 2-5). In contrast, *S. cerevisiae* strain D47 finished fermentation significantly faster with the addition of Nutriferm Arom Plus compared to the control (Table 2-5).

Pinot Noir Must Composition and Fermentation

As was seen with Chardonnay, there were minor differences in Brix, pH, and TA of the Pinot noir must from each treatment, but very few significant differences. In 2016 there were no differences between Brix, pH, and TA of any of the treatments (Table 2-7). In 2017, only small differences between the Brix content of various treatments were noted while in 2016 and 2018, no differences in Brix, pH and TA were measured (Table 2-7, 2-8, 2-9). In 2017, there were minor differences between some treatments in pH and TA (Table 2-8). The control treatment had the lowest pH while the addition of DAP resulted in the highest pH. Control also had lower TA than the +Nutriferm and +DAP treatments.

In every year, YAN levels were raised by the application of nitrogen in the vineyard (Table 2-7, 2-8, 2-9). How nitrogen was applied in the vineyard did not impact the concentration of YAN in the grapes, as there was no significant difference between the YAN of grapes from +SoilN or +FoliarN treatments in any year (Table 2-8, 2-9). As noted for Chardonnay, the addition of DAP raised the level of ammonia N while the addition of Nutriferm Arom Plus raised the level of free amino acid N. In each year, the addition of either DAP or Nutriferm Arom Plus was able to increase the level of YAN to similar a similar YAN concentration to the +SoilN treatment. For example, in 2018 the addition of DAP and Nutriferm Arom Plus raised the concentration of YAN to 244.5 mgN/L and 209.7 mgN/L respectively, which were statistically the same as the +SoilN YAN of 211.4 mgN/L (Table 2-9).

All Pinot noir treatments in each year completed alcoholic fermentation within 221 hours (Table 2-7, 2-8, 2-9). Minor differences between fermentation times occurred in some years. For example, in 2016 the +SoilN treatment finished significantly quicker than all other treatments and significantly quicker than the +Nutriferm treatment in 2017 and 2018. In each case, the difference in time to complete fermentations was less than 48 hours.

Chardonnay juice amino acid content

Concentrations of primary amino acids in the Chardonnay juices were quantified using HPLC-DAD. Sixteen amino acids were quantified in each year although not all amino acids were detectable. In all three years (2016, 2017, 2018) the control juice contained the lowest concentration of the majority of the primary amino acids while the +SoilN, had the highest concentrations (Table 2-10, 2-11 , 2-12). For example, in 2016 the control juice contained 18.36 mg/L of glutamine and 44.67 mg/L of alanine, while the +SoilN juice contained 58.45 mg/L of glutamine and 126.50 mg/L of alanine (Table 2-10). In 2016, glutamate, serine, glutamine, arginine, and alanine were significantly higher in the +SoilN juice than in any other treatment (Table 2-10). Except for glutamate, these amino acids were also highest in the 2017 +Soil N treatment while in 2018, only alanine, and arginine were significantly higher (Table 2-11, 2-12). A +FoliarN treatment was included in the 2017 and 2018 experiments and affected amino acid concentrations in the same manner as the +SoilN treatment (Table 2-11, 2-12). Significantly higher concentrations of serine, glutamine, arginine, and alanine were present in the +FoliarN treatment compared to the control in 2017, and higher concentrations of serine, arginine, and alanine were present in 2018.

Chardonnay juices where DAP had been added contained the same concentration of primary amino acids as the control juice in most cases (Table 2-10, 2-11, 2-12). However, if juices were supplemented with Nutriferm Arom Plus then concentrations of certain primary amino acids were significantly higher than the control. In 2016 and 2018, juice that received an addition of Nutriferm Arom Plus contained significantly higher concentrations of threonine compared to any of the other treatments (Table 2-10, 2-12). In addition, the concentrations of phenylalanine and isoleucine were significantly higher in the +Nutriferm treatment in 2016 compared to all other treatments (Table 2-10), while in 2018, valine was significantly higher in the +Nutriferm treatment compared to all other treatments (Table 2-12).

Table 2-1 2016 Chardonnay grape juice parameters before and after YAN was adjusted with DAP (+DAP), Nutriferm Arom Plus (+Nutriferm) or where adjustments were made in the vineyard (+SoilN) or no adjustments were made (Control).

	Treatment			
	Control	**+DAP**	**+Nutriferm**	**+SoilN**
Must Sol. Solids (Degree Brix)	22.4 a	22.3 a	22.9 a	23.0 a
Must pH	3.13 a	3.14 a	3.14 a	3.20 a
Must TA (g/L)	5.61 a	5.70 a	5.48 a	5.96 a
Initial Must NH4 - N (mg N/L)	23.2 b*	25.3 b	26.4 b	40.0 a
Initial Must FAA - N (mg N/L)	76.0 b	87.0 b	86.4 b	148.6 a
Initial Must YAN(mg N/L)	99.0 b	112.3 b	112.8 b	188.6 a
Post-Add Must NH4 - N (mg N/L)	23.2 c	104.0 a	26.4 c	40.0 b
Post-Add Must FAA - N (mg N/L)	76.0 b	87.0 b	135.4 a	149.6 a
Post-Add Must YAN (mg N/L)	99.0 c	191.1 a	161.7 b	189.6 a
Hours to reach 0° Brix	441.6 a	381.6 a	381.6 a	379.2 a

*Means followed by a different letter within a row are significantly different based on Tukeys HSD at 95% confidence.

Table 2-2 2017 Chardonnay grape juice parameters before and after YAN was adjusted with DAP (+DAP), Nutriferm Arom Plus (+Nutriferm) or where adjustments were made in the vineyard (+SoilN, +FoliarN) or no adjustments were made (Control).

	Treatment				
	Control	**+DAP**	**+Nutriferm**	**+SoilN**	**+FoliarN**
Must Sol. Solids (Degree Brix)	21.4 b	21.8 b	21.8 b	21.6 b	22.7 a
Must pH	3.31 c	3.41 ab	3.29 c	3.50 a	3.37 bc
Must TA (g/L)	5.54 b	6.14 ab	6.28 a	5.77 ab	5.68 ab
Initial Must NH4 - N (mg N/L)	15.4 c	13.5 c	10.6 c	31.3 a	22.9 b
Initial Must FAA - N (mg N/L)	43.3 c	44.5 c	43.5 c	148.1 a	98.2 b
Initial Must YAN(mg N/L)	58.7 c	58.0 c	52.9 c	179.2 a	121.0 b
Post-Add Must NH4 - N (mg N/L)	15.4 cd	121.5 a	10.6 d	31.3 b	22.9 bc
Post-Add Must FAA - N (mg N/L)	43.3 c	44.5 c	113.5 b	148.1 a	98.2 b
Post-Add Must YAN (mg N/L)	58.7 c	165.9 a	123.0 b	179.2 a	121.0 b
Hours to reach 0° Brix	768.0 a	552.0 ab	568.8 ab	417.6 b	568.8 ab

*Means followed by a different letter within a row are significantly different based on Tukeys HSD at 95% confidence.

Table 2-3 2018 Chardonnay grape juice parameters before and after YAN was adjusted with DAP (+DAP), Nutriferm Arom Plus (+Nutriferm) or where adjustments were made in the vineyard (+SoilN, +FoliarN) or no adjustments were made (Control).

	Treatment				
	Control	**+DAP**	**+Nutriferm**	**+SoilN**	**+FoliarN**
Must Sol. Solids (Degree Brix)	21.9 a	21.7 a	21.6 a	22.2 a	22.6 a
Must pH	3.25 a	3.32 a	3.28 a	3.26 a	3.32 a
Must TA (g/L)	7.41 a	7.84 a	7.29 a	7.78 a	6.62 a
Initial Must NH4 - N (mg N/L)	10.8 b	11.8 b	13.9 b	40.4 a	20.5 b
Initial Must FAA - N (mg N/L)	53.0 c	56.4 ac	60.0 ac	106.2 b	82.7 ab
Initial Must YAN(mg N/L)	63.8 c	68.2 ac	73.9 ac	146.5 b	103.2 a
Post-Add Must NH4 - N (mg N/L)	10.8 c	93.5 b	13.9 c	40.4 a	20.5 ac
Post-Add Must FAA - N (mg N/L)	53.0 b	56.4 b	96.6 a	106.2 a	82.7 ab
Post-Add Must YAN (mg N/L)	63.8 c	149.8 b	110.6 bc	146.5 ab	103.2 ac
Hours to reach 0° Brix	700.8 a	518.4 a	475.2 a	480.0 a	621.6 a

*Means followed by a different letter within a row are significantly different based on Tukeys HSD at 95% confidence.

Table 2-4. 2018 Chardonnay grape juice parameters for mini-fermentations before and after YAN was adjusted with DAP (+DAP), Nutriferm Arom Plus (+Nutriferm) or where adjustments were made in the vineyard (+SoilN) or no adjustments were made (Control).

	Treatment			
	Control	**+ DAP**	**+ NutriFerm**	**+SoilN**
Soluble solids (Degree Brix)	20.5 a	20.5 a	20.5 a	21.7 a
pH	3.25 a	3.25 a	3.25 a	3.27 a
TA (g/L)	7.87 a	7.87 a	7.87 a	7.67 a
Initial NH4 - N (mg N/L)	18.0 b*	18.0 b	18.0 b	23.5 a
Initial FAA - N (mg N/L)	78.3 b	78.3 b	78.3 b	123.5 a
Initial YAN (mg N/L)	96.4 b	96.4 b	96.4 b	147.0 a
Post-Add NH4 - N (mg N/L)	18.0 c	60.0 a	18.0 c	23.5 b
Post-Add FAA - N (mg N/L)	78.3 c	78.3 c	163.7 a	123.5 b
Post-Add YAN (mg N/L)	96.4 c	137.9 b	181.8 a	147.0 b

*Means followed by a different letter within a row are significantly different based on Tukeys HSD at 95% confidence

Table 2-5 Time to complete fermentation of 2018 Chardonnay grape juice supplemented with DAP (+DAP), Nutriferm Arom Plus (+Nutriferm), or where no adjustments were made (Control, +SoilN). Juice was inoculated with either *S cerevisiae* strain D47, DV10, or CVW5.

Yeast Strain	**Fermentation Time (hours)**			
	Control	**+DAP**	**+Nutriferm**	**+SoilN**
D47	609.6 a*	588.0 ab	506.4 bc	537.6 abc
DV10	595.2 ab	487.2 c	537.6 abc	513.6 bc
CVW5	585.6 ab	487.2 c	561.6 abc	547.2 abc

*Means followed by a different letter are significantly different based interaction groupings using Tukeys HSD at 95% confidence.

Table 2-6 Effects of yeast strain, nutrient, and the interaction effect of yeast strain x nutrient on the fermentation rate of the 2018 Chardonnay grape juice supplemented with DAP (+DAP), Nutriferm Arom Plus (+Nutriferm), or where no adjustments were made (Control, +SoilN). Juice was inoculated with either *S cerevisiae* strain D47, DV10, or CVW5.

ANOVA: Interactions of Nitrogen Nutrients and Yeast Strains	
Variable	**P-Value**
Yeast strain	0.146
Nutrient	0.000*
Yeast x Nutrient	0.010*

*P-value is significant (below an alpha value of 0.05), indicates a variable effect on fermentation time based on a two-way ANOVA test.

Table 2-7. 2016 Pinot noir grape must parameters before and after YAN was adjusted with DAP (+DAP), Nutriferm Arom Plus (+Nutriferm) or where adjustments were made in the vineyard (+SoilN) or no adjustments were made (Control).

	Treatment			
	Control	**+DAP**	**+Nutriferm**	**+SoilN**
Must Sol. Solids (Degree Brix)	24.9 a	25.1 a	25.0 a	25.2 a
Must pH	3.23 a	3.24 a	3.24 a	3.34 a
Must TA (g/L)	5.75 a	5.55 a	5.77 a	5.75 a
Initial Must NH4 - N (mg N/L)	36.6	37.2	36.7	37.4
Initial Must FAA - N (mg N/L)	138.6 b*	143.0 b	147.7 b	205.7 a
Initial Must YAN(mg N/L)	175.2 b	180.1 b	184.4 b	243.0 a
Post-Add Must NH4 - N (mg N/L)	36.6 b	114.0 a	36.7 b	37.4 b
Post-Add Must FAA - N (mg N/L)	138.6 b	143.0 b	215.7 a	205.7 a
Post-Add Must YAN (mg N/L)	175.2 b	256.9 a	252.4 a	243.0 a
Hours to reach 0° Brix	141.6 a	141.6 a	141.6 a	117.6 b

*Means followed by a different letter within a row are significantly different based on Tukeys HSD at 95% confidence.

Table 2-8. 2017 Pinot noir grape must parameters before and after YAN was adjusted with DAP (+DAP), Nutriferm Arom Plus (+Nutriferm) or where adjustments were made in the vineyard (+SoilN, +FoliarN) or no adjustments were made (Control).

	Treatment				
	Control	**+DAP**	**+Nutriferm**	**+SoilN**	**+FoliarN**
Must Sol. Solids (Degree Brix)	24.4 ab*	24.8 a	24.7 a	24.3 ab	24.0 b
Must pH	3.4 d	3.75 a	3.59 b	3.50 c	3.49 c
Must TA (g/L)	5.45 c	6.95 ab	7.18 a	6.48 abc	6.37 bc
Initial Must NH4 - N (mg N/L)	23.3 b	19.7 b	18.6 b	40.0 a	40.7 a
Initial Must FAA - N (mg N/L)	110.9 b	119.1 b	112.9 b	178.4 a	195.2 a
Initial Must YAN(mg N/L)	134.2 b	138.8 b	131.6 b	218.2 a	235.9 a
Post-Add Must NH4 - N (mg N/L)	23.3 c	125.5 a	18.6 c	40.0 b	40.7 b
Post-Add Must FAA - N (mg N/L)	110.9 b	119.1 b	187.4 a	178.4 a	195.2 a
Post-Add Must YAN (mg N/L)	134.2 c	244.6 a	206.0 b	218.2 ab	235.9 ab
Hours to reach 0° Brix	155.8 a	147.5 ab	155.5 a	135.5 ab	127.5 b

*Means followed by a different letter within a row are significantly different based on Tukeys HSD at 95% confidence.

Table 2-9. 2018 Pinot noir grape must parameters before and after YAN was adjusted with DAP (+DAP), Nutriferm Arom Plus (+Nutriferm) or where adjustments were made in the vineyard (+SoilN, +FoliarN) or no adjustments were made (Control).

	Treatment				
	Control	**+DAP**	**+Nutriferm**	**+SoilN**	**+FoliarN**
Must Sol. Solids (Degree Brix)	25.3 b	25.9 a	25.9 a	25.1 b	25.6 ab
Must pH	3.24 a	3.38 a	3.31 a	3.15 a	3.17 a
Must TA (g/L)	5.45 a	5.29 a	5.33 a	6.40 a	6.59 a
Initial Must NH4 - N (mg N/L)	24.4 b	25.6 b	19.4 b	48.2 a	49.6 a
Initial Must FAA - N (mg N/L)	115.3 b	119.7 b	119.7 b	163.2 a	156.8 a
Initial Must YAN(mg N/L)	139.7 b	145.4 b	139.0 b	211.4 a	206.5 a
Post-Add Must NH4 - N (mg N/L)	24.4 b	124.8 a	19.4 b	48.2 b	49.6 b
Post-Add Must FAA - N (mg N/L)	115.3 c	119.7 c	190.3 b	163.2 b	156.8 ab
Post-Add Must YAN (mg N/L)	139.7 b	244.5 a	209.7 a	211.4 a	206.5 a
Hours to reach 0° Brix	216.0 ac	208.8 ac	220.8 a	177.6 bc	189.6 c

*Means followed by a different letter within a row are significantly different based on Tukeys HSD at 95% confidence.

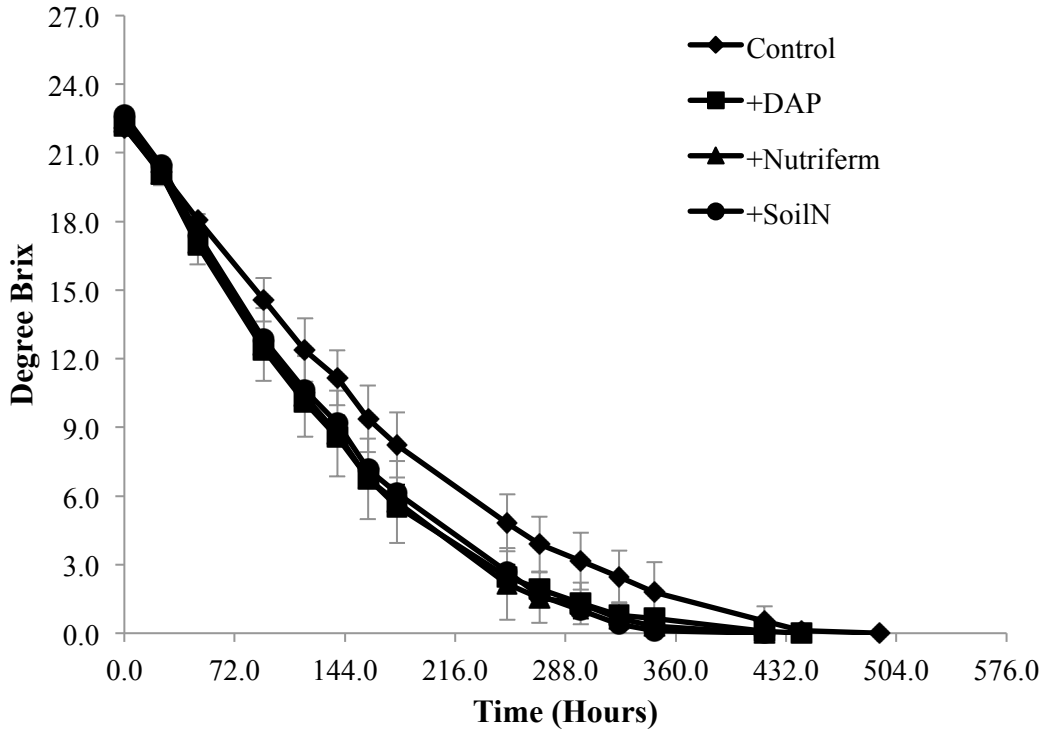

Figure 2-3 Change in °Brix during fermentation of 2016 Chardonnay juice where YAN was either adjusted in the vineyard (+SoilN), at the winery (+DAP, +Nutriferm) or where no adjustments were made (Control).

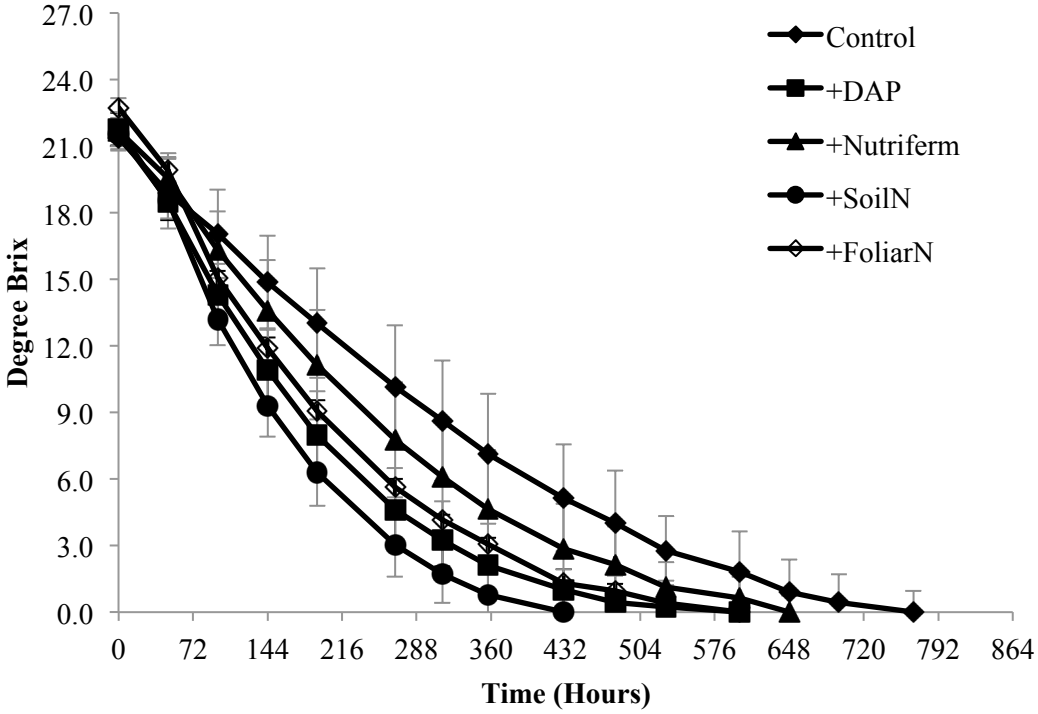

Figure 2-4 Change in °Brix during fermentation of 2017 Chardonnay juice where YAN was either adjusted in the vineyard (+SoilN, +FoliarN), at the winery (+DAP, +Nutriferm) or where no adjustments were made (Control).

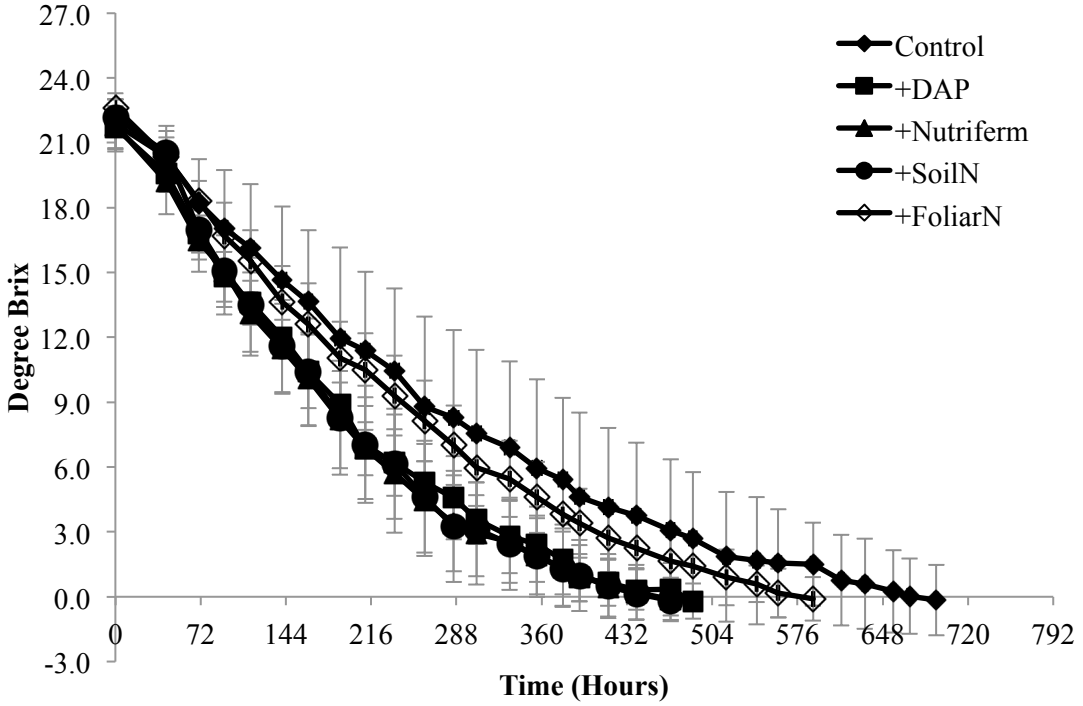

Figure 2-5 Change in °Brix during fermentation of 2018 Chardonnay juice where YAN was either adjusted in the vineyard (+SoilN, +FoliarN), at the winery (+DAP, +Nutriferm) or where no adjustments were made (Control).

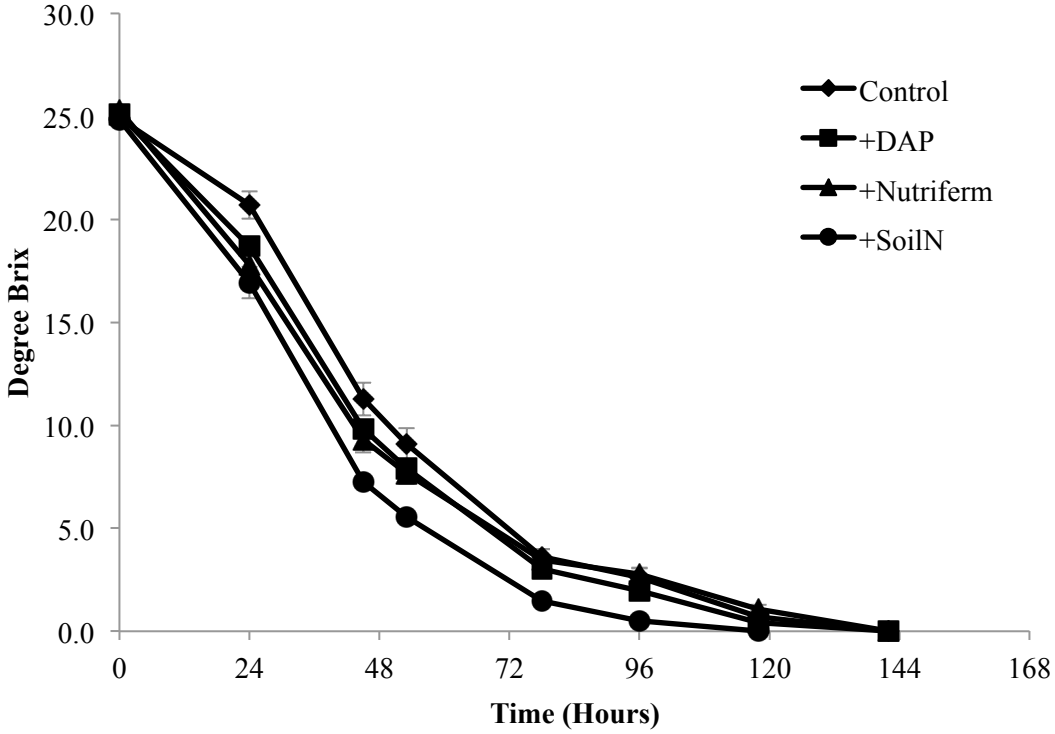

Figure 2-6 Change in °Brix during fermentation of 2016 Pinot noir grape must where YAN was either adjusted in the vineyard (+SoilN), at the winery (+DAP, +Nutriferm) or where no adjustments were made (Control).

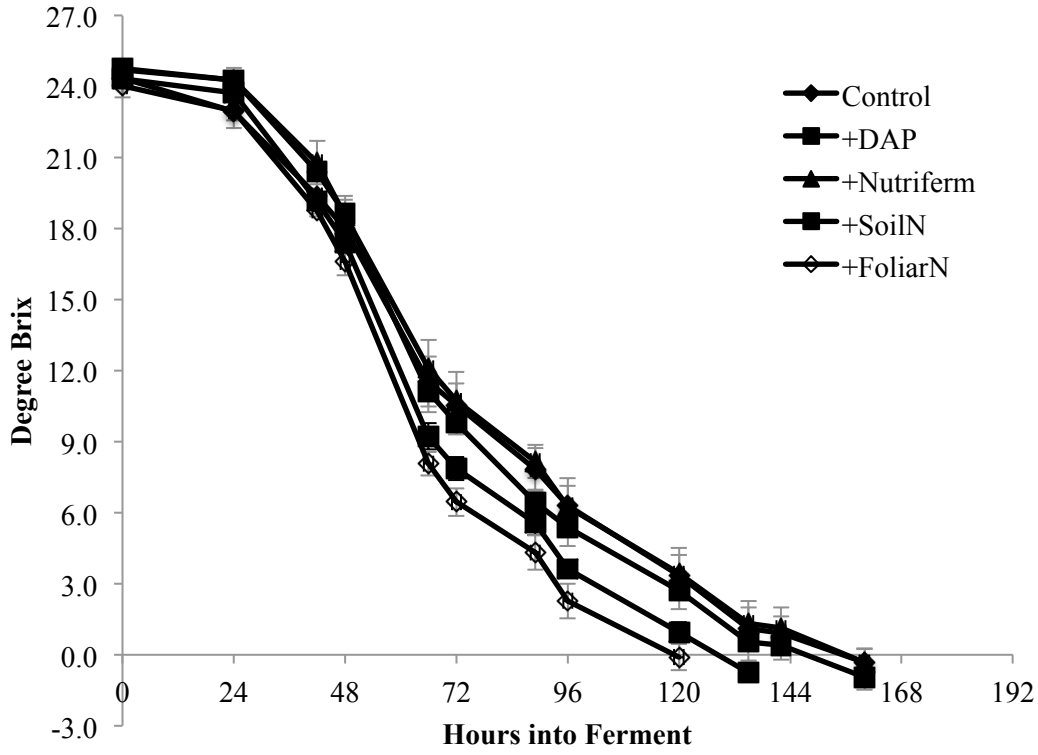

Figure 2-7 Change in °Brix during fermentation of 2017 Pinot noir grape must where YAN was either adjusted in the vineyard (+SoilN, +FoliarN), at the winery (+DAP, +Nutriferm) or where no adjustments were made (Control).

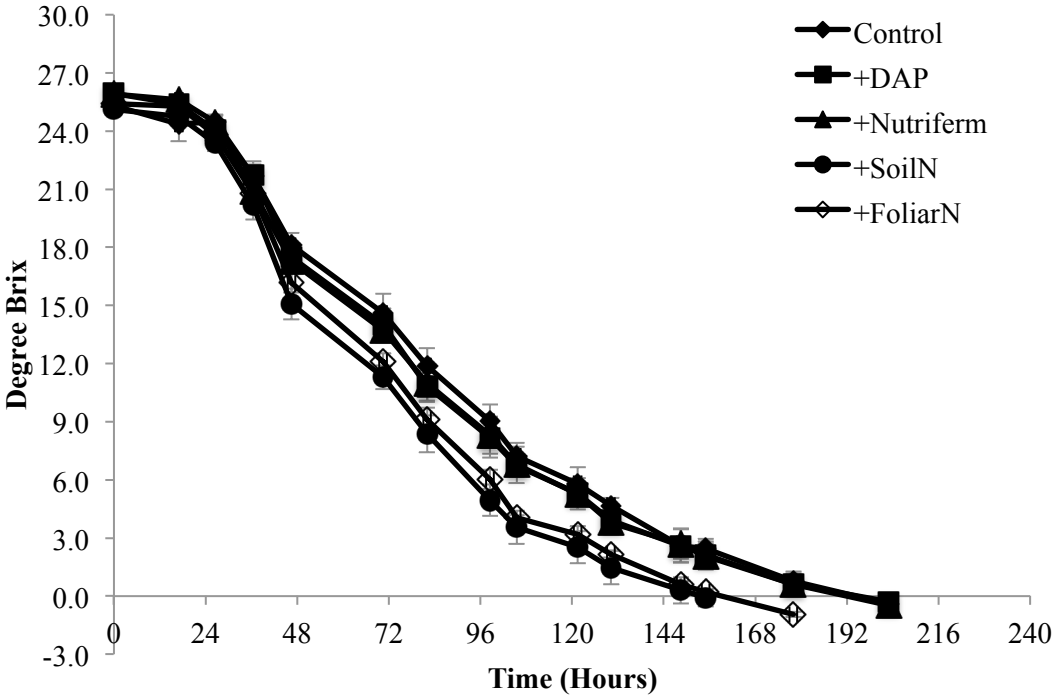

Figure 2-8 Change in °Brix during fermentation of 2018 Pinot noir grape must where YAN was either adjusted in the vineyard (+SoilN, +FoliarN), at the winery (+DAP, +Nutriferm) or where no adjustments were made (Control).

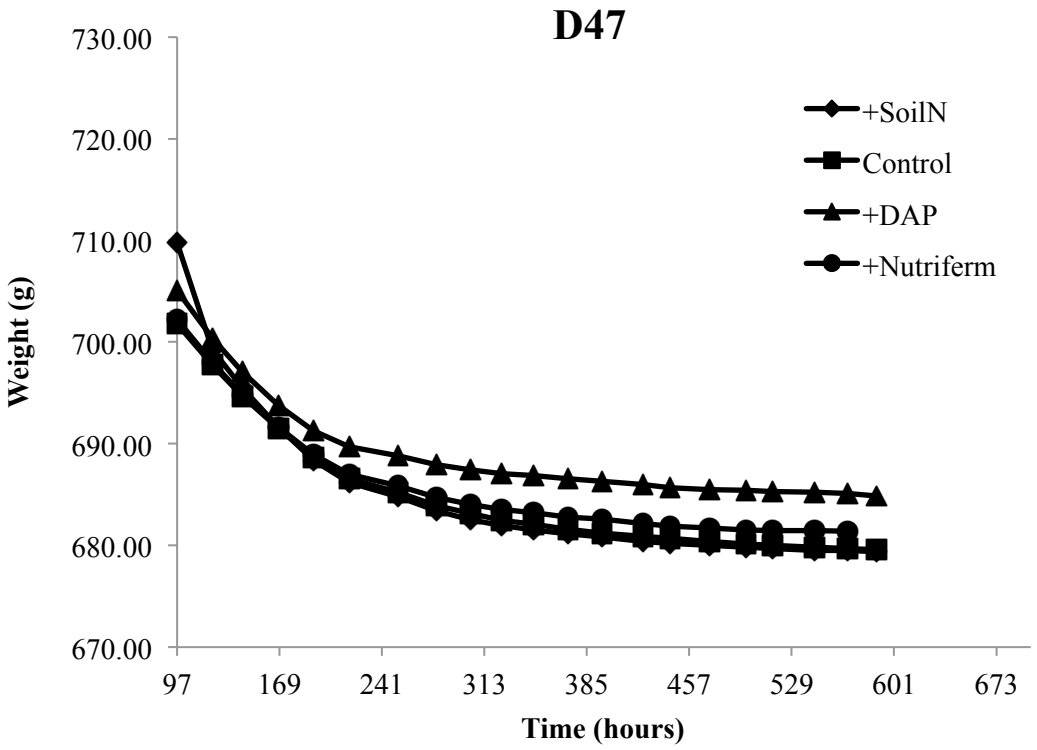

Figure 2-9 Change in weight of 2018 Chardonnay grape juice inoculated with *S. cerevisiae* strain D47 and supplemented with DAP (+DAP), Nutriferm Arom Plus (+Nutriferm), or where no adjustments were made (Control, +SoilN).

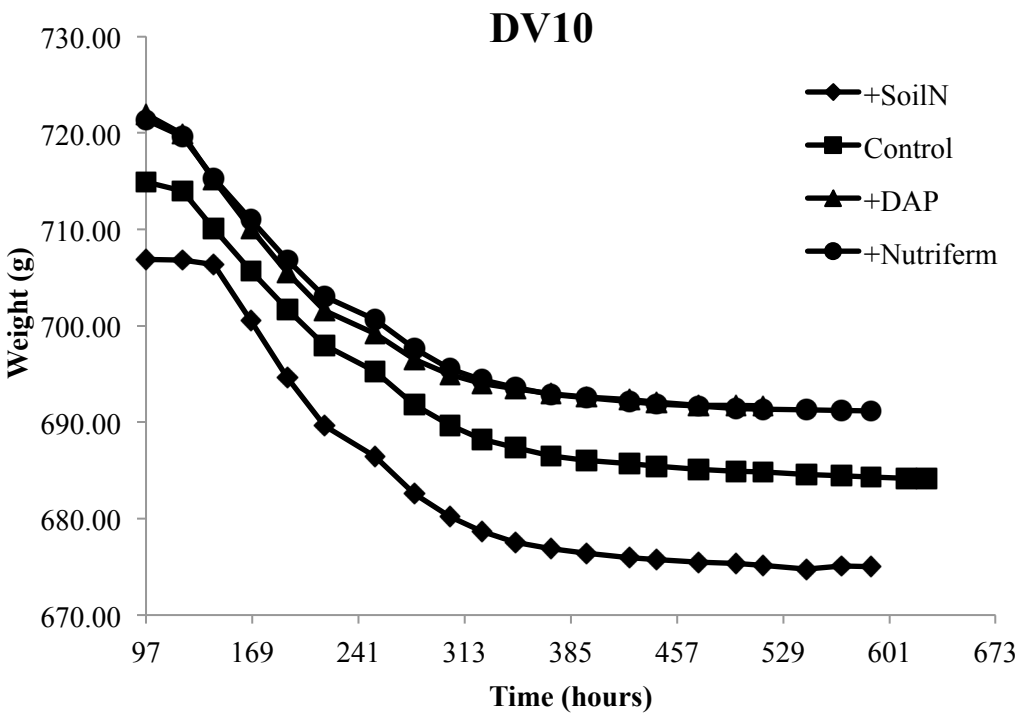

Figure 2-10 Change in weight of 2018 Chardonnay grape juice inoculated with *S. cerevisiae* strain DV10 and supplemented with DAP (+DAP), Nutriferm Arom Plus (+Nutriferm), or where no adjustments were made (Control, +SoilN).

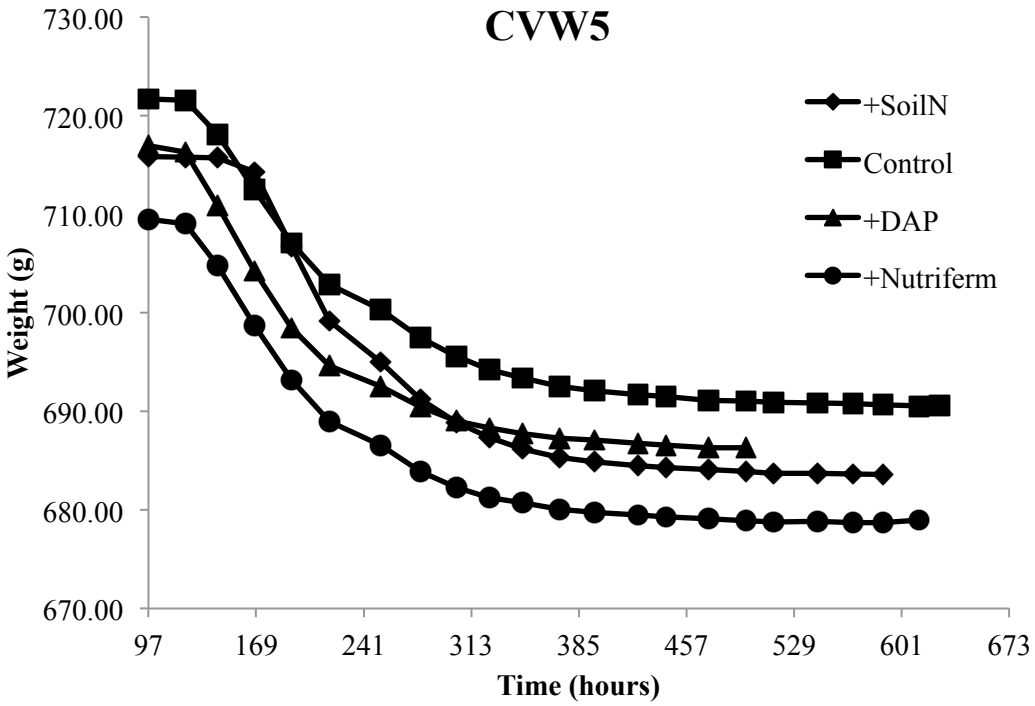

Figure 2-11 Change in weight of 2018 Chardonnay grape juice inoculated with *S. cerevisiae* strain CVW5 and supplemented with DAP (+DAP), Nutriferm Arom Plus (+Nutriferm), or where no adjustments were made (Control, +SoilN).

Table 2-10 Concentration of amino acids (mg/L) in 2016 Chardonnay grape juice after nutrient adjustments with either DAP (+DAP) or Nutriferm Arom Plus (+Nutriferm) or where no adjustments were made (Control and +SoilN) Means followed by a different letter in each row differ significantly based on Tukey's HSD at 95% confidence.

Amino Acid	**Control**	**+DAP**	**+Nutriferm**	**+SoilN**
Aspartate	5.28 b (.38)	5.05 b (.58)	5.41 ab (.61)	5.93 a (.47)
Glutamate	7.91 c (.61)	7.60 c (.41)	9.61 b (.49)	10.15 a (.35)
Asparagine	1.22 a (0.66)	1.01 a (0.91)	1.18 a (0.74)	ND*
Serine	23.55 b (2.68)	24.27 b (6.06)	28.04 b (4.96)	40.96 a (2.89)
Glutamine	18.36 b (3.30)	24.87 b (12.80)	24.77 b (8.96)	58.45 a (9.59)
Threonine	ND	ND	17.87 (1.62)	0.93
Arginine	27.17 b (.71)	28.61 b (3.07)	29.34 b (1.86)	40.23 a (2.43)
Alanine	44.67 b (7.78)	51.85 b (21.72)	56.81 b (12.45)	126.50 a (9.49)
Tyrosine	ND	ND	0.69 a (1.61)	0.77 a (1.80)
Valine	4.63 ab (0.25)	4.97 a (0.30)	4.74 ab (0.34)	4.04 b (1.31)
Methionine	13.38 c (2.00)	18.68 b (3.62)	28.41 a (3.92)	25.11 a (4.12)
Norvaline	ND	ND	0.79 a (0.07)	0.57 a (0.73)
Tryptophan	5.86 a (1.69)	6.84 a (4.99)	6.22 a (2.95)	9.43 a (4.88)
Phenylalanine	6.13 b (0.62)	6.31 b (0.76)	7.98 a (0.99)	5.90 b (0.60)
Isolecuine	6.63 bc (0.78)	6.17 c (3.50)	12.17 a (1.62)	9.05b (2.57)
Leucine	5.16 b (1.70)	5.34 b (2.89)	8.25 a (1.66)	8.67 a (1.37)

*(ND) Not detectable

Table 2-11 Concentration of amino acids (mg/L) in 2017 Chardonnay grape juice after nutrient adjustments with either DAP (+DAP) or Nutriferm Arom Plus (+Nutriferm) or where no adjustments were made (Control, +SoilN, and +FoliarN) Means followed by a different letter in each row differ significantly based on Tukey's HSD at 95% confidence.

Amino Acid	**Control**	**+DAP**	**+Nutriferm**	**+SoilN**	**+FoliarN**
Aspartate	5.29 a (.37)	5.07 a (.56)	4.32 b (0.60)	5.24 a (.42)	5.22 a (.45)
Glutamate	8.46 ab (.61)	8.00 b (.92)	8.64 ab (.94)	9.42 a (.93)	9.36 a (.79)
Asparagine	ND*	ND	ND	ND	0.77 (1.05)
Serine	17.68 c (2.24)	16.33 c (3.87)	13.84 c (2.92)	36.65 a (4.73)	29.20 b (2.58)
Glutamine	11.62 c (1.81)	9.98 c (1.28)	3.91 c (4.13)	42.90 a (17.7)	27.5 b (3.73)
Threonine	3.12 b (2.37)	3.00 b (1.83)	12.70 a (1.95)	10.09 a (3.79)	3.40 b (4.40)
Arginine	26.33 b (1.56)	26.19 b (1.80)	25.63 b (1.33)	42.92 a (5.49)	33.92 b (1.00)
Alanine	28.94 c (7.27)	26.68 c (10.93)	21.22 c (6.49)	109.29 a (12.39)	62.55 b (6.82)
Tyrosine	ND	ND	ND	ND	ND
Valine	4.14 c (0.64)	6.25 b (0.83)	6.30 b (0.28)	7.47 a (0.76)	7.85 a (0.80)
Methionine	8.31 a (4.04)	1.01 b (3.49)	6.78 a (1.91)	0.73 b (2.43)	1.01 b (3.48)
Norvaline	ND	ND	0.63 (0.30)	ND	ND
Tryptophan	1.91 a (1.32)	4.07 a (3.15)	ND	3.32 a (2.35)	1.98 a (1.92)
Phenylalanine	3.67 ab (2.72)	1.42 b (2.60)	6.32 a (0.74)	6.01 a (3.07)	5.62 a (3.43)
Isolecuine	1.16 a (2.73)	1.73 a (4.35)	0.99 a (2.32)	ND	ND
Leucine	0.50 a (1.72)	ND	ND	1.09 a (2.17)	1.37 a (2.50)

*(ND) Not detectable

Table 2-12 Concentration of amino acids (mg/L) in 2018 Chardonnay grape juice after nutrient adjustments with either DAP (+DAP) or Nutriferm Arom Plus (+Nutriferm) or where no adjustments were made (Control, +SoilN, and +FoliarN) Means followed by a different letter in each row differ significantly based on Tukey's HSD at 95% confidence.

Amino Acid	**Control**	**+DAP**	**+Nutriferm**	**+SoilN**	**+FoliarN**
Aspartate	4.17 bc (0.48)	4.63 abc (0.45)	4.70 a (0.39)	4.05 c (0.20)	4.59 ab (0.47)
Glutamate	7.34 b (1.36)	8.21 ab (0.25)	9.31 a (0.44)	8.34 ab (0.78)	8.22 ab (0.96)
Asparagine	0.63 ab (0.98)	ND*	0.52 ab (0.80)	ND	1.37 a (0.45)
Serine	19.06 b (3.20)	19.46 b (1.63)	20.46 b (3.43)	25.85 a (2.33)	24.98 a (3.64)
Glutamine	10.89 a (4.73)	14.44 a (1.14)	13.91 a (2.18)	13.36 a (6.32)	10.63 a (3.20)
Threonine	1.70 c (1.59)	ND	11.27 a (1.98)	6.31 b (1.35)	ND
Arginine	25.45 c (0.776)	27.46 bc (1.35)	27.62 b (1.65)	31.90 a (1.56)	29.03 b (2.40)
Alanine	22.96 c (3.60)	30.78 bc (3.37)	30.65 bc (7.01)	45.14 a (8.76)	35.65 b (9.96)
Tyrosine	ND	ND	ND	ND	ND
Valine	5.91 c (0.59)	6.01 c (0.27)	8.10 a (0.34)	6.99 b (0.33)	6.85 b (0.57)
Methionine	ND	ND	ND	ND	ND
Norvaline	ND	ND	ND	ND	ND
Tryptophan	1.50 b (1.65)	4.10 a (2.01)	0.58 b (0.93)	3.88 a (1.37)	1.17 b (1.24)
Phenylalanine	ND	5.41 a (0.34)	ND	5.75 a (0.75)	5.20 a (1.77)
Isolecuine	ND	ND	ND	ND	ND
Leucine	ND	ND	ND	ND	ND

*(ND) Not detectable

Table 2-13 Concentration of amino acids (mg/L) in 2018 mini Chardonnay grape juice ferments after nutrient adjustments with either DAP (+DAP) or Nutriferm Arom Plus (+Nutriferm) or where no adjustments were made (Control and +SoilN) Means followed by a different letter in each row differ significantly based on Tukey's HSD at 95% confidence.

Amino Acid	**Control**	**+DAP**	**+Nutriferm**	**+SoilN**
Aspartate	4.74 a (0.33)	4.70 a (0.46)	4.70 a (0.39)	4.71 a (0.36)
Glutamate	7.31 c (0.13)	7.92 c (0.68)	8.80 b (0.86)	9.19 a (0.83)
Asparagine	1.11 a (0.49)	2.02 a (1.12)	1.43 a (3.40)	1.54 a (2.07)
Serine	20.60 b (1.50)	19.94 b (3.67)	19.28 b (2.90)	29.24 a (7.40)
Glutamine	19.68 b (9.70)	19.26 b (3.21)	15.69 b (2.31)	28.62 a (4.93)
Threonine	3.97 c (0.64)	5.51 bc (2.16)	6.77 b (6.21)	9.61 a (2.37)
Arginine	27.75 b (0.63)	28.48 b (2.05)	28.73 b (3.03)	35.38 a (3.92)
Alanine	34.77 bc (1.94)	33.19 c (4.45)	38.86 b (6.86)	56.43 a (11.10)
Tyrosine	ND*	ND	ND	ND
Valine	6.42 c (0.60)	7.57 b (1.42)	9.16 a (0.87)	8.12 b (1.52)
Methionine	ND	ND	ND	ND
Norvaline	1.64 a (0.40)	1.47 a (0.54)	0.91 b (0.17)	0.86 b (0.50)
Tryptophan	ND	ND	ND	ND
Phenylalanine	6.05 ab (0.39)	6.72 a (1.17)	3.68 c (0.69)	5.52 b (1.42)
Isolecuine	4.29 bc (0.89)	5.98 a (0.73)	3.50 c (0.70)	5.37 ab (2.51)
Leucine	ND	ND	ND	ND

*(ND) Not detectable

Discussion

Nitrogen is a vital nutrient required for yeast to complete alcoholic fermentation and a number of studies have sought to understand the relationship between YAN and fermentation kinetics (Bisson 1991, 1999, Henschke and Jiranek 1993, Ingledew and Kunkee 1985). The present study adds to our understanding of this relationship, but also determines how the application of nitrogen in the vineyard or in the winery differentially affects YAN composition. In agreement with others (Bell et al. 1979, Bell and Henschke 2008a, Spayd et al. 1994), the application of UAN (urea and ammonium nitrate in water) in the vineyard raised YAN concentrations in both the Chardonnay and Pinot Noir grapes. Both methods of application (fertigation and foliar application) were effective at raising YAN levels. For Chardonnay, the addition of UAN through the irrigation system was more effective at boosting YAN than the addition of urea through the foliar spray. This difference was not observed in Pinot noir. While interesting, the reasoning for this variation in effectiveness between the two applications is not currently understood, but could be due to difference in common practices like cropping thinning between the two varieties. However, further research would be needed to confirm this theory or to determine if for some grape varieties it is more effective to apply nitrogen through foliar application or through the drip system.

As expected, the addition of DAP or an organic nitrogen product to either Chardonnay juice or Pinot noir grape must boosted YAN concentration. Others have reported the effectiveness of a variety of winery supplements at raising the level of YAN (Torrea et al. 2011, Ugliano et al. 2008). DAP was effective at raising the level of YAN, by boosting ammonia in a similar manner as reported by others (Henschke and Jiranek 1993). The addition of Nutriferm Arom Plus, was also effective at raising the level of YAN, by increasing the amount of primary amino acids. While the addition of DAP allowed us to closely match the YAN of vineyard supplemented fruit, the treatments with Nutriferm Arom Plus often contained lower YAN than vineyard supplemented treatments due to a maximum allowable addition rate. Additionally, research is inconclusive about the impact of nitrogen additions in the

vineyard or winery on pH, TA, and sugar, so the lack of trends related to must composition in this study are not surprising (Bell and Henschke 2008a).

The major reason for boosting YAN in the vineyard or winery is to ensure there is sufficient YAN for yeasts to complete alcoholic fermentation. How much YAN is needed is still a point of debate but a number of studies suggest that a "moderate" level of 150 mg N/L is sufficient (Bell and Henschke 2008a). However, others suggest a minimum of 200 mg N/L YAN is required to complete fermentation of a 21°Brix must, with an additional 25 mg N/L for every one degree increase in Brix (Bisson and Butzke 2000). Often fermentation kinetic studies that explore fermentation rate, boost YAN levels up to 400 mg N/L, and while these concentrations are useful for preventing stuck and sluggish ferments, they may be excessive (Bisson 1999, Ugliano et al. 2009, Vilanova et al. 2007). Others have noted that YAN requirements are dependent on the grape variety and yeast strain (Bisson 1999, Blateyron et al. 2003, Ribereau-Gayon et al. 2006). Recent work on Oregon Pinot noir reported that YAN levels even lower than 100 mg N/L were adequate for successful completion of fermentation (Schreiner et al. 2018). The results from this study support these findings as Pinot Noir musts with YAN levels less than 150 mg N/L successfully complete alcoholic fermentation. Specifically, the control treatment in Pinot noir always finished fermentation within 48 hours of the treatments that had boosted YAN levels. Chardonnay control treatments reached YAN levels below 100 mg N/L in each vintage and took longer to complete fermentation. However, Chardonnay fermentations with YANs as low as 60 mg N/L still completed fermentation successfully.

While fermentation rate is heavily dependent on YAN, it is also influenced by other factors like oxygen, temperature, and yeast strain (Bell and Henschke 2008a). Different yeast strains may preferentially utilize certain nitrogen sources, but there is not yet a consensus on which sources are preferred across all yeast strains. Some researchers have classified ammonium, asparagine, and glutamine as being preferred nitrogen sources, but in total, 17 sources of YAN have been identified as preferred

(Gobert et al. 2019a). In this study, we used three different yeast strains to determine what, if any, impact yeast strain would have on the fermentation rate of Chardonnay juices containing different YAN concentrations and compositions. If no nutrient treatment was applied the three yeast strains did not influence fermentation time. However, there were differences between yeast strains if different nutrient treatments were used. For example, the addition of DAP significantly decreased the time to complete fermentation for strains DV10 and CVW5 compared to the control, but this impact was not seen for strain D47. This finding supports the findings of others that ammonium is a preferred YAN source for yeast, but this maybe strain dependent (Boer et al. 2007, Grenson 1992, Jiranek et al. 1995b).

The addition of Nutriferm Arom Plus or vineyard supplementation (+Soil N) increased the concentrations of primary amino acids, but did not result in quicker fermentation times for strains DV10 or CVW5. In contrast, the addition of Nutriferm Arom Plus decreased the time to complete fermentation for strain D47 compared to the control. This may be due to strain differences in nitrogen utilization as noted by others (Gutiérrez et al. 2013, Ough et al. 1991). Our results support the findings of Brice et al (2018), in that yeast strains differ in their ability to adapt to the nitrogen composition of the environment, influencing their fermentation performance. However, in a study of kinetic profiles of nitrogen consumption in 14 different strains of *S. cerevisiae,* Crepin et al. (2012) reported that while the kinetic profiles were diverse, the order of nitrogen source consumption was similar for all strains.

YAN concentration and yeast strain are important for successful completion of fermentation, but they can also impact other aspects of wine quality such as wine aroma (Styger et al. 2011). A number of studies have investigated how different concentrations of YAN impact wine volatile aroma compounds and sensory attributes (Carrau et al. 2008, González-Marco et al. 2010, Vilanova et al. 2007). For example, Ugliano et al. (2008) reported that increasing YAN in Syrah grape must, by the addition of DAP, increased the concentrations of acetates, straight chain fatty acids, and straight chain fatty acid ethyl esters, but lowered concentrations of branched-

chain fatty acids and their ethyl esters in the wines. These differences are primarily due to varied production of volatile aroma compounds from nitrogen by *S. cerevisiae* during fermentation (Garde-Cerdán et al. 2014, Hernandez-Orte et al. 2006, Jiménez-Martí et al. 2007, Mendes-Ferreira et al. 2009). Similarly, in Pinot noir, Yuan et al (2018) showed that increase in nitrogen supplementation through fertilizers increased straight chain esters and alcohols, and decrease branched-chain esters and alcohols in wine. Our study noted some differences in YAN concentration due to vineyard or winery nitrogen supplementation that could be expected to impact the production of wine aroma compounds by yeast.

The amino acid compositions of the Chardonnay juices in our study were similar to those reported by others. Others have reported that arginine, alanine, serine, and glutamine are usually present in the highest concentrations (Guitart et al. 1999, Huang and Ough 1991, Treeby et al. 2000), and these findings match our amino acid profiles (Table 2-10, 2-11, 2-12). One point of difference however, was that while many other studies report arginine as the most abundant free amino acid in Chardonnay grape juice (Guitart et al. 1999, Hernández-Orte et al. 2002, Treeby et al. 2000), in our study, alanine was the most abundant free amino acid present. In addition, the concentrations of primary amino acids measured in the present study were generally in lower concentrations compared to others. However, it is challenging to directly compare the concentrations of amino acids in our study to others without knowing total YAN concentrations, which were often not reported. When relative amino acid ratios from our study were compared to previous studies they were similar. The only exception to this was the concentrations arginine and alanine where the ratio of arginine to total primary amino acids was lower than other studies, while the ratio of alanine was higher. It should also be remembered that other factors in the current study such as location, climate, rootstock, clone could impact amino acid composition and may explain some of the differences relative to other studies (Gutiérrez-Gamboa et al. 2018, Huang and Ough 1991).

The application of nitrogen in the vineyard boosted total YAN as well as a number of individual amino acids. Application of nitrogen through the soil or foliage resulted in increased concentrations of arginine, alanine, serine, and glutamine in every year compared to the control (Table 2-10, 2-11, 2-12). Application of nitrogen through the soil increased YAN to a greater extent than foliar application and this was reflected in the concentration of some amino acids. Interestingly, the ratio of free amino acids does not stay consistent as YAN is raised. Some amino acids increased significantly compared to the control while others, such as tryptophan, isolecuine, and leucine, were not significantly boosted by vineyard fertilization (Table 2-10, 2-11). In support, Schreiner et al (2009) noted that as nitrogen supply was lowered in the vineyard amino acids were also lowered, but some free amino acids were reduced more than others. This suggests that the boosting of YAN in the vineyard does not necessarily boost individual amino acids in a consistent manner.

The addition of DAP in the winery reached YAN concentrations similar to the vineyard nitrogen supplemented treatments, but there were significant differences in the concentration of a number of amino acids. This was expected given that DAP only contains ammonia nitrogen and should not alter amino acid concentrations. However, it was expected that the addition of Nutriferm Arom Plus would increase amino acid concentrations given that based on YAN analysis it boosted free amino nitrogen content. As such, it was not surprising that the addition of Nutriferm Arom Plus significantly increased the concentration of some amino acids. Similar to the vineyard supplementation treatments, the addition of the Nutriferm Aroma Plus did not raise all of the free amino acids. Threonine and methionine were significantly increased compared to the control, but aspartate, glutamine, and serine were not statistically significant from the control (Table 2-10, 2-11, 2-12). Nutriferm Arom Plus is a commercial product, so we did not know what amino acids it contains. Compared to the other treatments, the one amino acid that increased every year with the addition of Nutriferm Arom Plus was threonine. Based on this, Nutriferm Arom Plus contains threonine and it is one of the amino acids contained in the product that boosts YAN.

Supplementation of nitrogen in the vineyard or in the winery resulted in different concentrations of YAN as well as individual amino acids. This has consequences for wine aroma due to how both total YAN and amino acids impact yeast production of esters and higher alcohols. Styger et al (2011), describes many of the amino acid utilizing pathways known to influence wine aroma. Based on these pathways, predictions can be made regarding how increases in certain amino acids would result in increased production of certain higher alcohols and/or esters. For example, in the +Nutriferm treatment there were higher concentrations of threonine which has been linked to acetaldehyde formation (Ardö 2006). The +Soil N treatment contained elevated concentrations of glutamine which is positively linked to the production of fruity thiols (Pinu et al. 2014). While the amino acid profiles can allow some predictions to be made, many other factors such as total YAN, yeast strain, and fermentation temperature, will have a significant effect on the production of volatile aroma compounds by yeast (Miller et al. 2007, Rollero et al. 2015, Ugliano et al. 2010, Yuan et al. 2018). Volatile aroma analysis of wines produced from this study should provide clearer links between amino acid composition and wine aroma. Coupled with sensory analysis detailed in Chapter 3, this will provide key information on how the concentration and composition of YAN is important for wine quality and what affect supplementation in the vineyard versus the winery makes.

Conclusions

YAN in Chardonnay and Pinot noir can be successfully increased through the use of vineyard fertilizers and winery supplements. YAN concentration, but not YAN source impacted fermentation time in both Chardonnay and Pinot noir. The successful fermentation of all Pinot noir treatments demonstrates that YAN levels do not need to be as high as previously thought, as levels lower than 150 mg N/L were successful. A significant interaction effect was found between yeast strain and nitrogen source on fermentation rate. The addition of nitrogen in the vineyard or in the winery resulted in Chardonnay juices that contained different concentrations of certain amino acids. In particular, nitrogen applied in the vineyard consistently elevated the concentration of arginine, alanine, serine, and glutamine, while the addition of Nutriferm Arom Plus in the winery consistently boosted threonine.

Amino acid profiles can be used to predict production of some volatile aroma compounds, but volatile aroma analysis is needed in order to better understand what volatile aromas are produced given the different amino acids present and yeast strains used. This study has shown how both YAN concentration and composition is affected by whether you add nitrogen in the vineyard or in the winery. Additional aroma analysis and wine sensory evaluation will help determine what impact this has on wine quality. This will provide information for winemakers regarding whether, from a wine quality point of view, it is better to try and boost YAN in the vineyard or in the winery.

Chapter 3

Changes in Wine Sensory Attributes Due to the Nitrogen Supplementation in the Vineyard and Winery

Abstract

Yeast assimilable nitrogen (YAN) is an important nutrient for yeast in the production of wine. Its effects on fermentation kinetics are well understood, but it may also influence wine sensory attributes. Some studies have linked ammonium and free amino acids to the production of certain volatile compounds that cause different aromas, but limited sensory testing has been done to confirm these compositional differences. The main objective of this study was to investigate how the source (vineyard or winery supplementation) and concentration of YAN impacted Pinot Noir (PN) and Chardonnay (CH) wine sensory attributes. Five treatments were used; a control (no N addition), addition of either diammonium phosphate (+DAP), or organic N (+Nutriferm) in the winery, and addition of either N to the soil (+Soil N), or the foliage (+Foliar N) in the vineyard. Treatments were established with four replicates for each variety. The +Foliar N treatment did not begin until 2017 for both varieties while all other treatments were conducted in 2016-2018. Wines were produced under standard conditions. Sensory analysis of the 2016, 2017, and 2018 CH, and 2016 and 2017 PN wines were conducted using triangle tests and Napping® with Ultra Flash Profiling (UFP) for aroma and mouthfeel. In sensory testing, PN and CH control wines were significantly different from +SoilN, except for the 2017 PN and 2018 CH. In both CH and PN the vineyard treatments (+SoilN and +FoliarN) were not significantly different from each other. In the 2016 and 2017 PN no significant differences were found between the winery treatments (+DAP and +Nutriferm). Chardonnay and Pinot noir, Napping® showed that the nitrogen treatments were well grouped and differences could be described based on aroma, but not mouthfeel. Panelists were successful at describing the CH wines, such that the +SoilN CH wines were tropical in each year. However, the panelists struggled to provide unique terms for Pinot noir treatments, as they used red fruit descriptors for

every treatment. These findings demonstrate the impact that YAN source can have on wine sensory, and show how the influence of nitrogen can vary with varietal.

Introduction

As previously mentioned, the aroma of Chardonnay and Pinot noir wine is primarily derived during fermentation (Robinson 2006). Thus, the sensory attributes of Chardonnay and Pinot noir are heavily dependent on growing location and different winemaking processes. In some regions Chardonnay is known for strong apple and oak notes, and in others it is known for lemon and pineapple notes (Broom and Beazley 2003, Robinson and Beazley 1992). Similarly in some regions Pinot Noir can have raspberry and clove notes, and in others it can have cranberry and mushroom notes (Puckette and Hammack 2015, Tomasino et al. 2013). To enhance or preserve the varietal characteristics of Chardonnay and Pinot noir, producers carefully select specific fermentation vessels, fermentation temperatures, extraction techniques, nutrient supplements, and yeast strains (Boulton et al. 2013, Fleet 1993). However, the impact of nutrient supplements, namely nitrogen, on wine sensory attributes has not been well studied. Many producers understand the importance of nitrogen supplements as related to fermentation kinetics, but do not yet understand how different sources and concentrations of nitrogen can affect wine aroma and mouthfeel (Bell and Henschke 2008a).

Previous research has highlighted the role of nitrogen in the formation of both negative sulfur containing compounds and positive ester compounds (Bell and Henschke 2008a). These negative and positive aromas are formed from nitrogenous compounds that proceed down the Sulfate Reduction Sequence or Ehlrich pathway respectively (Ugliano et al. 2009, Waterhouse et al. 2016). It is well understood that primary amino acids are involved in each of the above pathways. Therefore, it is not surprising that wines made from different amino acid containing juice/must will contain different aroma compounds (Bell and Henschke 2008a, Carrau et al. 2008). Many studies that have explored the production of aroma compounds from different nitrogen supplemented juice/must fail to include sensory testing, and it is unclear if the known aroma compound changes actually change wine sensory.

In addition to aroma, the impact of different nitrogen supplements and concentrations on wine mouthfeel may also be important. Similar to aroma, the impact of nitrogen on mouthfeel has not been explored through sensory testing. Phenolic compounds are thought to largely influence mouthfeel. In white wine hydroxycinnamic acids are the major phenolic compounds present (Waterhouse 2002). Hydroxycinnamates are carbon based compounds found in the skin of grapes and are known only for their role in white wine browning (Fernández-Zurbano et al. 1998, Waterhouse 2002). Since hydroxycinnametes are not known to influence astringency or bitterness in wine, it is unlikely that changes to nitrogen will impact white wine mouthfeel. Even though, hydroxycinnametes are the major phenolics present, white wine grapes do contain small amounts of other phenolics that are known to contribute to mouthfeel attributes in red wine (Garrido and Borges 2013). Therefore, it is important to investigate the influence of nitrogen on white wine mouthfeel as the concentration of these minor phenolics compounds could be altered.

In contrast to white wine, many phenolic compounds play a large role in red wine mouthfeel (Waterhouse 2002). The phenolics compounds studied in red wine are mainly derived from the skin and seed of grapes and directly impact astringency and bitterness (Gawel 2008, Kennedy et al. 2006). Due to this direct relationship some studies have investigated the effects of different nitrogen fertilizers on the development of phenolics (Bell and Henschke 2008a, Schreiner et al. 2014, Yuan et al. 2018). However, many of these studies have yielded conflicting results and did not include sensory testing (Bell and Henschke 2008a, Delgado et al. 2004, Gutiérrez-Gamboa et al. 2017). Thus, because mouthfeel plays a large role in the perceived quality of red wine it is important that this relationship is examined. In conclusion, it is clear that nitrogen plays a key role in the development of wine aroma and potentially wine mouthfeel. Therefore, it was important that this study explored how different sources and concentrations of nitrogen impacted the sensory attributes of Chardonnay and Pinot noir wine.

Materials and Methods

Pinot Noir

After approximately 1 year of bottle aging sensory analysis was conducted on the Pinot Noir Wine. For the 2016 Pinot Noir, sensory testing was conducted on the 1st, 8th, 15th, and 29th of May 2018, and on the 1st of June 2018. In 2019 sensory testing was conducted on 22nd and 26th of March 2019, and the 5th, 12th, and 13th of April 2019 for the 2017 Pinot Noir. The first three panel dates in 2018 and the first two panel dates in 2019 were conducted with expert wine tasters from Willamette Valley, Oregon. In 2018 there were 19 panelists, of which 12 were male and 7 were female. In 2019 there were 20 panelists, of which 16 were male and 4 were female. Inclusion criteria require participants to be an expert wine taster were; a minimum of five years' experience with Pinot Noir, free of any taste deficits or oral disorders, and free of oral lesions, cankers sores, and piercings of the lip, tongue or cheek. These expert wine taster sessions were held in McMinnville, Oregon at Oregon State University's Yamhill County Extension Office. Panelists participated in triangle tests and Napping®.

The remaining sessions in both 2018 and 2019 were held at Oregon State University in the Arbuthnot dairy lab (Corvallis, Oregon). In 2018 there were 19 panelists consisting of 3 males and 16 females. In 2019 there were 40 panelists consisting of 11 males and 29 females. Panelists chosen for this study were Pinot Noir consumers. To be included in the study; panelists had to be over 21 years of age, a non-smoker, not currently pregnant, free of any taste deficits or oral disorders, free of oral lesions, cankers sores, and piercings of the lip, tongue or cheek, and have no allergies to wine. Panelist also had to consume at least one glass of red wine a week on average.

At each location the testing rooms had any background odors eliminated with air purifiers and the temperature of each room was kept at 20+/-2 degrees Celsius. In each room tables were fitted with white trifold boards to provide each panelist with a private testing booth free of distractions.

Chardonnay

After approximately 6 months of bottle aging sensory analysis was conducted on the Chardonnay Wine. For the 2016 Chardonnay sensory testing was conducted on the 17th, 23rd, and 29th of August 2017. In 2018 sensory testing was conducted on 7th, 10th, 29th, and 30th of August 2019, and the 11th of September 2018 for the 2017 Chardonnay. In 2019 sensory testing was conducted on 12th, 13th, 14th, 27th, 28th, and 29th of August 2019 for the 2018 Chardonnay. In 2017 the panels on the 17th and 23rd of August were conducted with expert wine tasters. In 2018 the panels on August 7th and 19th, and Sept 11th were conducted with expert wine tasters. In 2019 the panels on August 12th, 13th, 14th, and 29th were conducted with expert wine tasters. The remaining panel dates in each year were conducted with Chardonnay wine consumers. In 2017 there were 7 panelists, of which 12 were male and 7 were female. In 2018 there were 20 panelists, of which 10 were male and 10 were female. In 2019 there were 22 panelists, of which 11 were male and 11 were female. Inclusion criteria require participants to be an expert wine taster were a minimum of five years' experience with Chardonnay, free of any taste deficits or oral disorders, and were free of oral lesions, cankers sores, and piercings of the lip, tongue or cheek. These expert wine taster sessions were held in McMinnville, Oregon at Oregon State University's Yamhill County Extension Office and at Oregon State University in the Arbuthnot dairy lab (Corvallis, Oregon). Panelists participated in triangle tests and Napping®.

The remaining sessions in all 3 years were held at Oregon State University in the Arbuthnot dairy lab (Corvallis, Oregon). In 2017 there were 33 panelists consisting of 15 males and 18 females. In 2018 there were 36 panelists consisting of 26 males and 10 females. In 2019 there were 45 panelists consisting of 19 males and 26 females. Panelists chosen for this study were Chardonnay consumers. Panelists chosen for this study were Chardonnay consumers. To be included in the study; panelists had to be over 21 years of age, a non-smoker, not currently pregnant, free of any taste deficits or oral disorders, free of oral lesions, cankers sores, and piercings of the lip, tongue or cheek, and have no allergies to wine. Panelists also had to consume

at least one glass of white wine a week on average. In these sessions each panelist completed six triangle tests. The testing conditions for the rooms were the same in as described earlier in the PN sensory methods.

Triangle Tests

All panelists were presented with 18 wines samples for six complete triangle tests. Wines were served in INAO black glasses to eliminate color bias and were labeled with randomly generated three digit identifiers. PN wines were served at room temperature and CH wines were served chilled. The wines were presented in a random order using a complete block design**.** They were asked to smell and taste each of the three wines in each test from left to write and then write down the 3 digit code that corresponds to the wine they thought was different. If desired the panelists could go back through and re-taste each wine. The directions instructed each panelist to describe how that wine was different. They were also instructed to take a one minute break, a bite of cracker (unsalted saltine) and a sip of water between each tests.

Napping® with Ultra-flash profiling

Expert wine consumer panelists also completed Napping® tests and ultra-flash-profiling (Pagès 2005, Reinbach et al. 2014). The panelists tasted all 4 or 5 treatments, depending on the vintage, in duplicate for a total of 8 or 10 wines in each test. Wines were presented in random order using a complete block design. Wines were served in INAO black glasses to eliminate color bias and were labeled with randomly generated three digit identifiers. PN wines were served at room temperature and CH wines were served chilled.

Each panelist was to complete two Napping® tests, one for aroma and one for mouthfeel. Napping® test order was randomly assigned to panelists. Sketch paper (18 x 14 inches, Strathmore Drawing Paper Pad) and pens were placed in front of panelists. For the aroma napping, panelists were asked to refrain from tasting the wine, as aroma analysis was the main objective of this test. For the mouthfeel Napping® test the same setup was put into place as stated above for the aroma

Napping®. This time the panelists were asked to refrain from smelling the wines as the aim of the sensory study was mouthfeel analysis. They were instructed to smell/taste the eight wines from left to right and place wines close together on the paper that were similar and place wines that are very different farther apart. Once the wines were placed on the paper they were instructed to describe each wine/group with aroma descriptors (UFP). When the panelists finished with the test the location of the wine glasses was marked by the instructors of the sensory tests. In 2019 for the Mouthfeel Napping® test the same setup from 2018 was used except panelists were required to where nose clips during this portion of the test (Sereni et al. 2016).

Data Analysis

Significant differences were calculated by a one-tailed binomial test. For this method z-scores were calculated following the method outlined in the Sensory Evaluation of Foods: Principles and Practices 2 textbook (Lawless and Heymann 2010). Once z-scores were calculated, p-values were found using a one tailed Z-score table. Z-scores of 1.65 or greater were considered significant at an alpha value of 0.05 or less.

Sensory data was analyzed using XLSTAT ver 2013.2.01 (Addinsoft, NY, NY). Napping Data was obtained using a ruler (inches) and measuring from left (X) and bottom edges (Y) relative to the original paper orientation to the panelist. Multiple factor analysis (MFA) was run on the X and Y co-ordinates for each wine to analyze the effects of the four treatments. For ultra-flash profiling the frequency of the terms were placed into a matrix (treatment by term) UFP terms were condensed by removing any terms that were used less than 15%. Correspondence analysis was used to evaluate the UFP terms.

Results

2016 PN Triangle Test

Results show that the control wine treatment was different from all other nitrogen treatments expect for +DAP (Table 3-1). The +Soil N treatment was found to be significantly different from all other treatments except +Nutriferm. The two winery treatments +DAP and +Nutriferm were not significantly different from each other.

2017 PN Triangle Test

Results show that the control wine treatment was not different from any other treatment (Table 3-1). The two winery treatments +DAP and +Nutriferm were not significantly different from each other. The two vineyard treatments +SoilN and +FoliarN were not significantly different from each other. The only significant different in the 2017 PN treatments was between the +DAP and +Foliar N treatments.

2016 PN Mouthfeel Napping®

Multifactor analysis (MFA) incorporated the spatial Napping® data (Figure 3-1). In total F1 and F2 account for 46.55 of the total variance (F1-28.31%, F2-18.24%). Some of the replicated treatments paired well with each other while other did not. Replicates of treatment A and treatment D were grouped well within the lower left quadrant and upper right quadrant respectively. Replicates of treatment B and treatment C were spatially separated by variance on the F1 axis.

Correspondence analysis utilized the UFP data (Figure 3-2). The first 2 factors incorporated 59.9% of the total variance. While panelists had been able to spatially group some of the replicates based off similarities in Napping® they were not in agreement over the mouthfeel terms used to describe the wines, as shown in UFP. The replicates of both treatments A and C are spatially separated due to variance in F2. Treatment B replicates were grouped closer together in the correspondence analysis compared to MFA. Treatment D replicates were grouped near each other and away from all other treatments. Each treatment was characterized with mouthfeel descriptors; treatment A was tannic, balanced, acidic, and astringent; treatment B was thin, round, and had a long finish; treatment C was light, astringent, and had full and light tannins; treatment D was flat, plush, soft, and smooth.

2017 PN Mouthfeel Napping®

MFA incorporated the spatial Napping® data (Figure 3-3). In total F1 and F2 account for 37.2% of the total variance (F1-20.81%, F2-16.39%). Some of the replicated treatments paired well with each other while other did not. Replicates of treatment A and treatment B were separated by variance in F1. Replicates of

treatment C and E were grouped well in the upper right and left quadrants respectively. Replicates of treatment were spatially separated between the two left quadrants.

Correspondence analysis utilized the UFP data (Figure 3-4). The first 2 factors incorporated 43.98% of the total variance. While panelists had been able to spatially group some of the replicates based off similarities in Napping® they were not in agreement over the mouthfeel terms used to describe the wines, as shown in UFP. The replicates of both treatments A and B were separated by variance in F1. Replicates of treatment C and E were closely grouped but separated by variance in F2. Treatment D replicates close to each other, but slightly separated due to variance in F2. Each treatment was characterized with mouthfeel descriptors; treatment A was juicy, big/volume, and acid; treatment B was firm tannins and tight; treatment C was drying, mid pallet, and sticky tannins; treatment D was unripe, balanced, sweet, and raw; and treatment E was fresh, high acid and supple.

2016 PN Aroma Napping®

MFA incorporated the spatial Napping® data (Figure 3-5). In total F1, and F2 explain 46.55% of the total variance (F1-26.68%, F2-19.87 %). All of the replicates but D were grouped fairly well. Treatment A and B replicates were grouped well within the upper left quadrant and lower left quadrant respectively. Treatment C replicates were grouped close together, but somewhat separated due to variance in F1. Treatment D replicates were not spatially close due to variance in F2.

Correspondence analysis utilized the UFP data (Figure 3-6). While panelists had been able to spatially group the replicates based off similarities using Napping®, they were not in agreement over the aromatic terms shown in UFP. The replicates of treatment A were still grouped well, with some separation due to variance in F1. Treatment B and D replicates were grouped well fairly well with some separation due to variance in F2 and F1 respectively. Unlike in Napping® replicates of treatment C were described very different in the correspondence analysis, with variance due to F2. Each treatment was characterized with aroma descriptors as follows: treatment A with

spice, raspberry, cherry, and wood/barrel; treatment B with cooked dry fruit, and savory; treatment C with dark fruit, chemical, herbal, and fruit; treatment D with berry, bramble berry, vegetal, and earthy.

2017 PN Aroma Napping®

MFA incorporated the spatial Napping® data (Figure 3-7). In total F1, and F2 explain 37.06% of the total variance (F1-19.76%, F2-17.30 %). Treatment replicates were not placed well together as most replicates were separated by an axis. Replicates of treatments A, C, and E were separated by variance in F1. Replicates of treatment B and D were grouped near each other but each was separated by variance in F2.

Correspondence analysis utilized the UFP data (Figure 3-8). Similarly to the struggle panelists faced during Napping®, they also struggled when describing the treatments with aroma terms. Replicates of treatment A and E were separated due to variance in F1. Treatment C replicates were separated due to variance in F1. The replicates of B and D were each tightly grouped. Each treatment was characterized with aroma descriptors as follows: treatment A with red fruit, blackberry, herbal, and strawberry; treatment B with green, dark fruit, floral, and cedar plank; treatment C with reduction, spicy, jammy, and metallic; treatment D with berry, cherry, and dusty; treatment E with clean, cherry, metallic, and cola.

2016 CH Triangle Tests

In 2016 the control was found to be significantly different from all other treatments except +Nutriferm (Table 3-2). The +SoilN treatment was found to be significantly different from all other treatments. There were no significant differences between the two winery treatments, +DAP and +Nutriferm.

2017 CH Triangle Tests

In 2017 the control was found to be significantly different from all other treatments (Table 3-2). The two winery treatments, +DAP and +Nutriferm, were found to be significantly different form each other. The two vineyard treatments, +SoilN and +Nutriferm, were not found to be significantly different from each other. There were

no significant different between the vineyards treatment and winery treatments when compared to each other.

2018 CH Triangle Tests

In 2018 CH no significant differences were found between any of the treatments except the control and +Nutrifemr treatments (Table 3-2).

2016 CH Mouthfeel Napping®

MFA incorporated the spatial Napping® data (Figure 3-9). In total F1 and F2 account for 42.71% of the total variance (F1-24.65%, F2-20.06%). Most treatment replicates were grouped near each other. Replicates of treatment A were grouped away from all other treatments but spatially separated due to variance in F2. Replicates of treatment B and C were both spatially separated due to variance in F2 and F2 respectively. Treatment D replicates were grouped close, but still spatially separated due to variance in F1.

Correspondence analysis utilized the UFP data (Figure 3-10). The first 2 factors incorporated 51.54% of the total variance (F1-32.85%, F2-18.69%). In UFP the panelists struggled to agree on terms for most treatments. Treatment A replicates were spatially separated due to variance in the F1 axis. The replicates of both treatments B and C were spatially separated along the F2 axis. Treatment D replicates were grouped tightly together. Each treatment was characterized with mouthfeel descriptors as follows: treatment A was soft, medium mouthfeel, medium freshness, nice mouthfeel, low mouthfeel, and sharp; treatment B was full-bodied, phenolic, fresh, mineral, hot, and zingy; treatment C was balanced, persistent, astringent, sharp, and low mouthfeel; treatment D was rich, ripe, fruit, light, sweet, and round.

2017 CH Mouthfeel Napping®

MFA incorporated the spatial Napping® data (Figure 3-11). In total F1 and F2 account for 42.72% of the total variance (F1-20.71%, F2-22.01%). Most of the replicated treatments were paired somewhat well. Replicates of treatment A and C both were each grouped well. Replicates of treatment B and D were grouped away

from all other treatments and both treatments had spatial separation of replicates due to variance in F2. Treatment E replicates were spatially separated to variance in F2.

Correspondence analysis utilized the UFP data (Figure 3-12). The first 2 factors incorporated 63.61% of the total variance (F1-43.70%, F2-20.21%). In UFP some of the terms used to describe mouthfeel moved treatments that had been poorly grouped in Napping® closer together. Treatment A replicates were group well and separated from all other treatments. The replicates of both treatments B and E were grouped well together. Treatment C replicates were spatially separated long the F2 axis. Treatment D replicates were grouped well. Each treatment was characterized with mouthfeel descriptors as follows: treatment A was tart and had high astringency; treatment B was thin, round, smooth, medium bodied, and mildly astringency; treatment C was full, smooth, fleshy and hot; treatment D was bitter, sharp, and fleshy; treatment E was thin, creamy, medium bodied, and mildly astringent.

2018 CH Mouthfeel Napping®

MFA incorporated the spatial Napping® data (Figure 3-13). In total F1 and F2 account for 36.01% of the total variance (F1-18.78%, F2-17.23%). Most of the replicated treatments were somewhat well paired. Replicates of treatment A were grouped close together but separated across the upper quadrants to due to variance in F1. Replicates of treatment B, C, D, and E were all spatially separated due to variance in F1.

Correspondence analysis utilized the UFP data (Figure 3-14). The first 2 factors incorporated 45.58% of the total variance (F1-23.31%, F2-22.27%). In UFP some of the terms used to describe mouthfeel moved treatments that had been poorly grouped in Napping® closer together, but also moved some that were grouped well further apart. Treatment A and B replicates were spatially separated due to variance in F2. The replicates of both treatments C and D were grouped near each other, but with some spatial separation due to variance in F2. Treatment E replicates were spatially separated due to variance in F1. Each treatment was characterized with mouthfeel descriptors as follows: treatment A was less sweet, sour, and mid acid; treatment B

was long finish, dry and astringent (medium); treatment C was low acid, astringent (high), and soft; treatment D was shorter finish, thin, and watery; treatment E was mid sugar, thin, light, and acid.

2016 CH Aroma Napping®

MFA incorporated the spatial Napping® data (Figure 3-15). In total F1 and F2 account for 49.17% of the total variance (F1-32.90%, F2-16.27%). All of the replicated treatments were paired well together. Replicates of treatment A were grouped well within the lower right quadrant. Replicates of treatments B and C were grouped well within upper right quadrant, although C replicates were more tightly grouped and B had some variation within the quadrant due to variance in F2. Replicates of treatment D were grouped close together, but did not fall within the same quadrant due to variance in F2.

Correspondence analysis utilized the UFP data (Figure 3-16). Panelists had been able to spatially group the replicates based off similarities using Napping®, and they were somewhat in agreement over the aromatic terms as shown in UFP. The replicates of treatment A were grouped well. Treatment B and C replicates were tightly grouped and near each other. The replicates of treatment D were grouped near each other, but there was some separation due to variance in F2. Each treatment was characterized with aroma descriptors as follows: treatment A was fresh, peach, lemon, and clean; treatment B was light, green, and reduced; treatment C was low fruit, sulfite, neutral, earthy, funk, and apple, and sweet; treatment D was tropical, fruity, and ripe.

2017 CH Aroma Napping®

MFA incorporated the spatial Napping® data (Figure 3-17). In total F1 and F2 explain 42.72% of the total variance (F1-22.01%, F2-20.71%). Some of the replicated treatments paired well with each other while others did not. Treatment A replicates were grouped well within the left quadrants. Treatment B and C and D replicates were all grouped near each other, and all replicates were slightly separated

due to variance in F2. Replicates of treatment E were tightly grouped in the lower right quadrant.

Correspondence analysis utilized the UFP data (Figure 3-18). While panelists had mostly been able to spatially group the replicates based off similarities using Napping®, they struggled over the some of aromatic terms shown in UFP. Replicates of both treatment A and B were grouped well. Treatment C replicates were relatively close, but had some spatial separation due to variance in F2. The replicates of treatment D were spatially separated due to variance in F1. Treatment E replicates were found to vary in spatial positioning the most due to variance in F1. Each treatment was characterized with aroma descriptors as follows: treatment A was oxidized, closed/tired and had mild fruit; treatment B was fresh fruit and tropical; treatment C was mild fruit, floral, closed/tired, and sweet; treatment D was tropical, fresh fruit, orange, and floral; treatment E was citrus, green apple, and oxidized.

2018 CH Aroma Napping®

MFA incorporated the spatial Napping® data (Figure 3-19). In total F1 and F2 explain 36.81% of the total variance (F1-21.57%, F2-15.24%). All replicated treatments except for treatment E were in separate quadrants. Treatment A, C and D were separated across the left and right quadrants due to variance in F1. Replicates of treatment B were spatially separated to due variance in F1. Treatment E replicates were grouped in the upper left quadrant, with some separation due to variance in F1.

Correspondence analysis utilized the UFP data (Figure 3-20). While panelists had struggled to spatially group the replicates based off similarities using Napping®, most of the replicate were well described using the aromatic terms shown in UFP. The replicates of treatment A were separated between due to variance in F1. Treatment B, C, and D replicates were each grouped well. Treatment E replicates were spatially separated due to variance in F2. Each treatment was characterized with aroma descriptors as follows: treatment A was peach, mild, and intense aroma; treatment B was sweet/candied, floral, and fruity; treatment C was peach, stone fruit, and pungent;

treatment D was tropical, strong, and sulfur; treatment E was oxidized, strong, vegetal, and spice.

Table 3-1 Binomial distribution of triangle test data for the 2016 and 2017 Pinot noir, significant findings for single comparisons within a year are denoted with asterisks. (A = Control, B = +DAP in winery, C = +Nutriferm in winery, D = +SoilN, E = +FoliarN).

Comparison	P-Value	
	2016	**2017**
A v B	0.29	0.45
A v C	0.02*	0.46
A v D	0.03*	0.24
A v E	N/A	0.14
B v C	0.28	0.24
B v D	0.00***	0.18
B v E	N/A	0.01**
C v D	0.21	0.45
C v E	N/A	0.05*
D v E	N/A	0.42

*P-values $\leq \alpha$ 0.05 **P-values $\leq \alpha$ 0.01 *** P-values $\leq \alpha$ 0.001

Table 3-2 Binomial distribution of triangle test data for 2016-2018 Chardonnay, significant findings for single comparisons within a year are denoted with asterisks. (A = Control, B = +DAP in winery, C = +Nutriferm in winery, D = +SoilN, E = +FoliarN).

Comparison	**P-Value**		
	2016	**2017**	**2018**
A v B	0.30	0.00***	0.23
A v C	0.17	0.02*	0.04*
A v D	0.00***	0.00**	0.06
A v E	N/A	0.01**	.089
B v C	0.2119	0.05*	0.060
B v D	0.00***	0.17	0.40
B v E	N/A	0.05	0.12
C v D	0.01*	0.17	0.16
C v E	N/A	0.25	.27
D v E	N/A	0.33	0.33

*P-values $\leq \alpha$ 0.05 **P-values $\leq \alpha$ 0.01 *** P-values $\leq \alpha$ 0.001

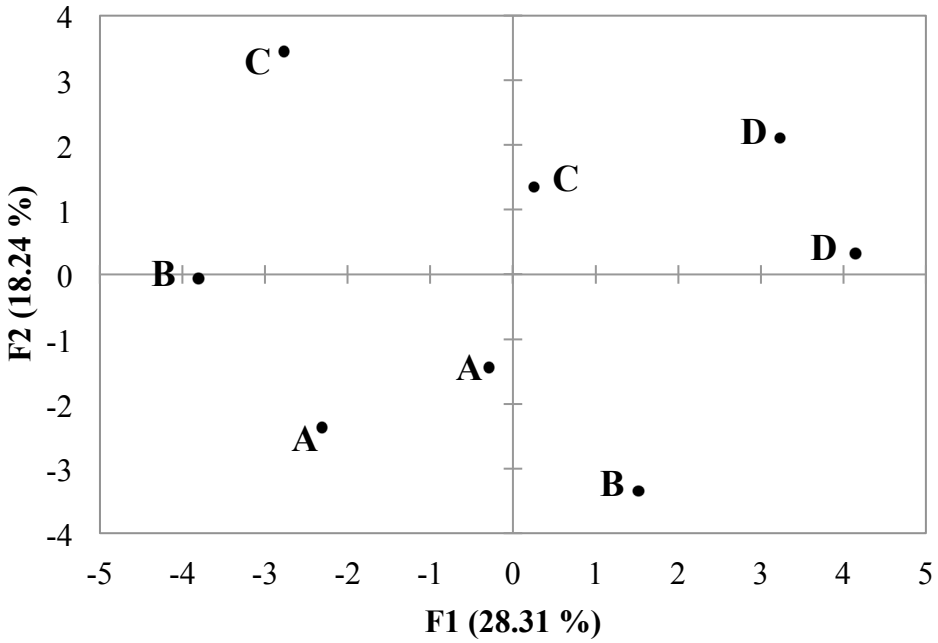

Figure 3-1 Multiple Factor analysis of mouthfeel Napping® results of 2016 Pinot Noir wines made with different nitrogen applications (A = Control, B = +DAP in winery, C = +Nutriferm in winery, D = +SoilN).

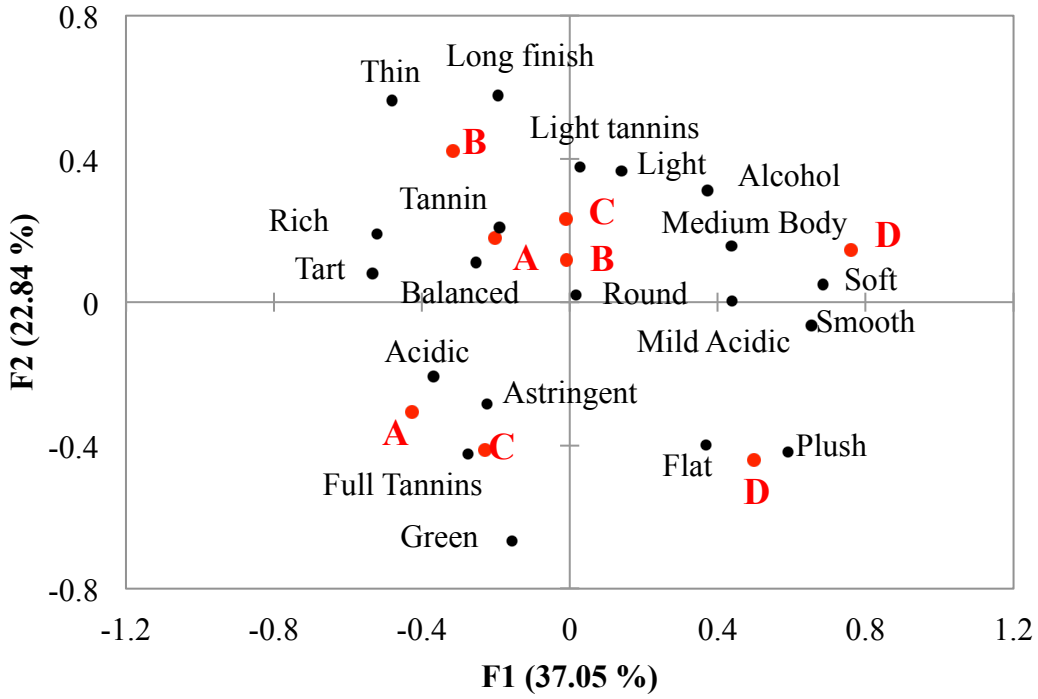

Figure 3-2 Correspondence analysis of mouthfeel terms used for UFP analysis of 2016 Pinot Noir wines (A = Control, B = +DAP in winery, C = +Nutriferm in winery, D = +SoilN).

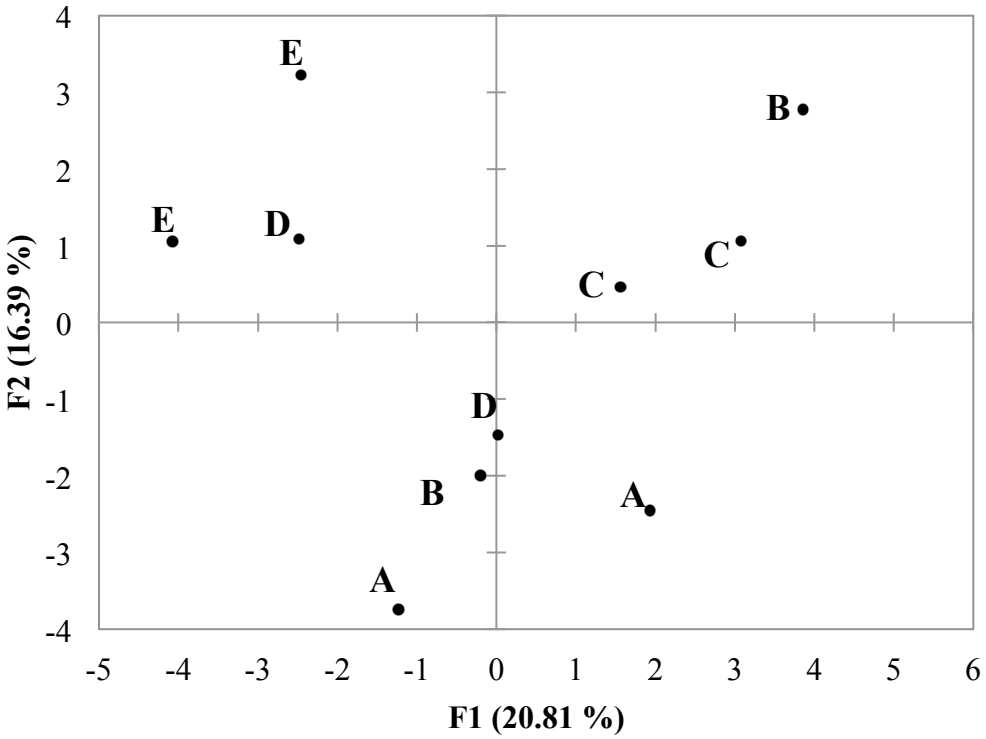

Figure 3-3 Multiple Factor analysis of mouthfeel Napping® results of 2017 Pinot Noir wines made with different nitrogen applications (A = Control, B = +DAP in winery, C = +Nutriferm in winery, D = +SoilN, E = +FoliarN).

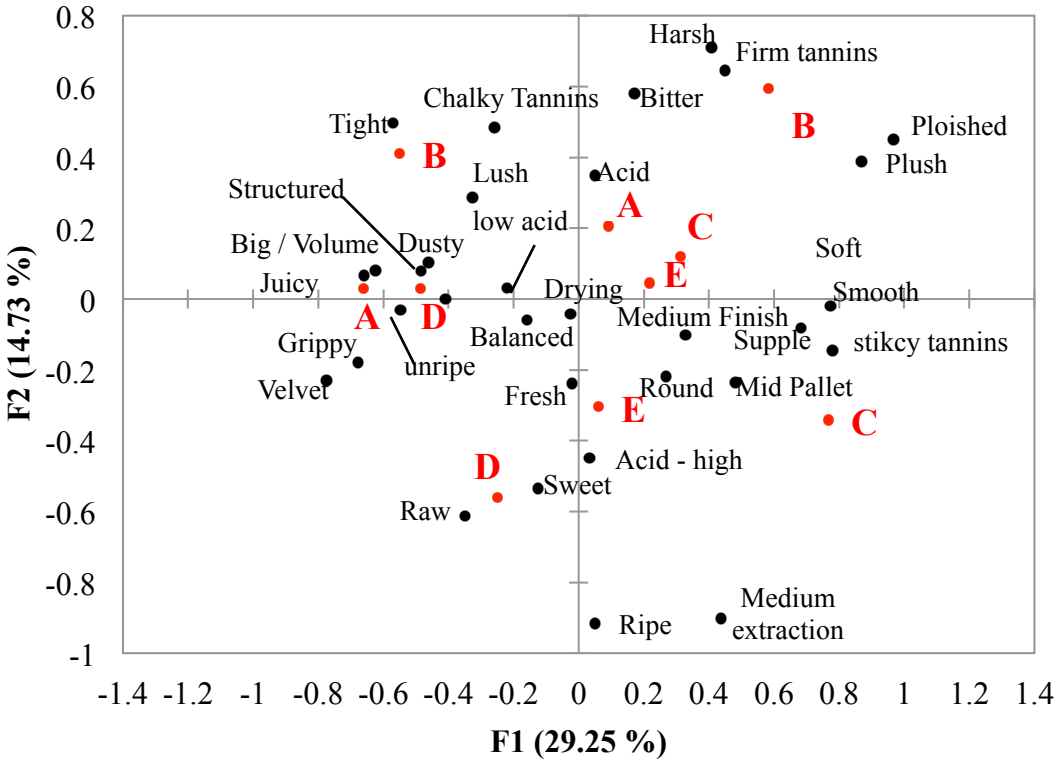

Figure 3-4 Correspondence analysis of mouthfeel terms used for UFP analysis of 2017 Pinot Noir wines (A = Control, B = +DAP in winery, C = +Nutriferm in winery, D = +SoilN, E = +FoliarN).

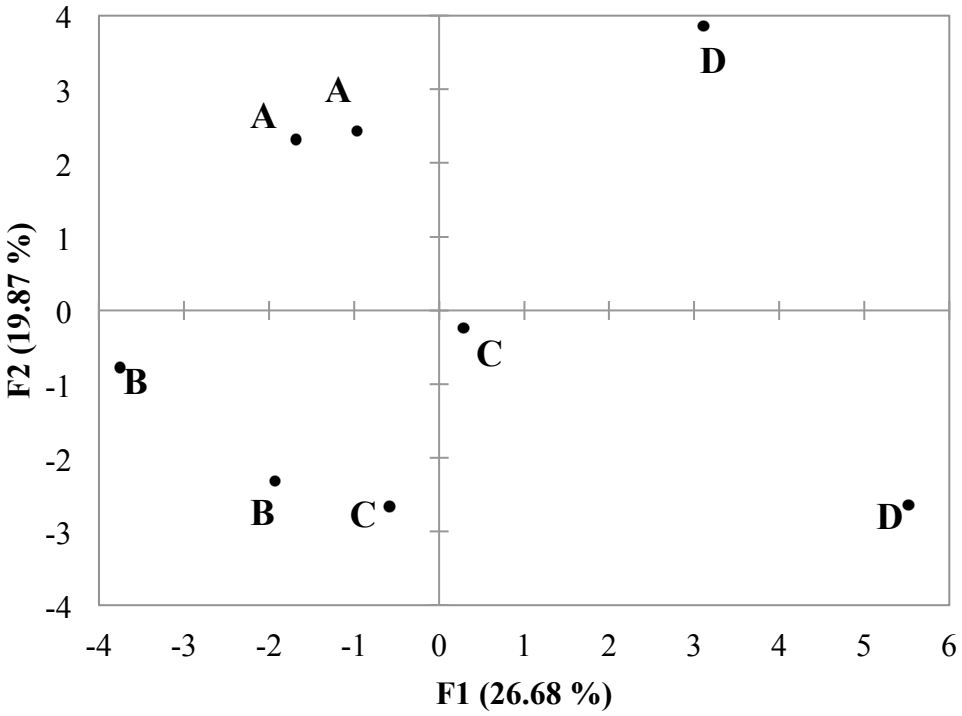

Figure 3-5 Multiple Factor analysis of aroma Napping® results of 2016 Pinot Noir wines made with different nitrogen applications (A = Control, B = +DAP in winery, C = +Nutriferm in winery, D = +SoilN).

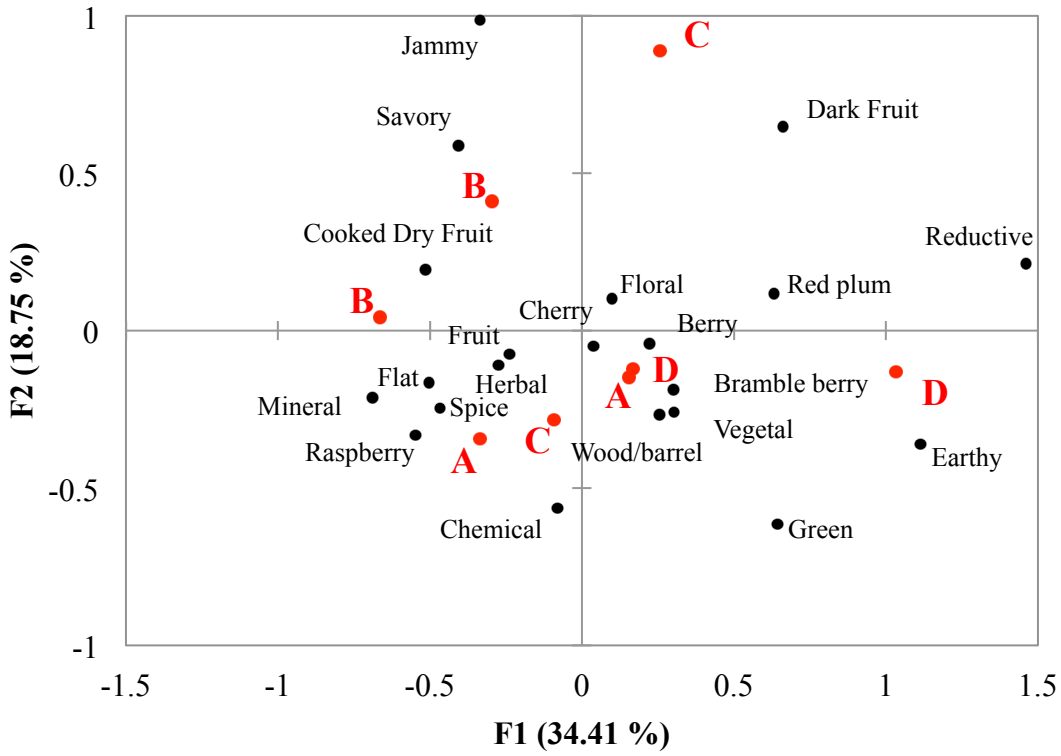

Figure 3-6 Correspondence analysis of aroma terms used for UFP analysis of 2016 Pinot Noir wines (A = control, B = inorganic nitrogen in winery, C = organic nitrogen in winery, D = vineyard soil fertigation nitrogen).

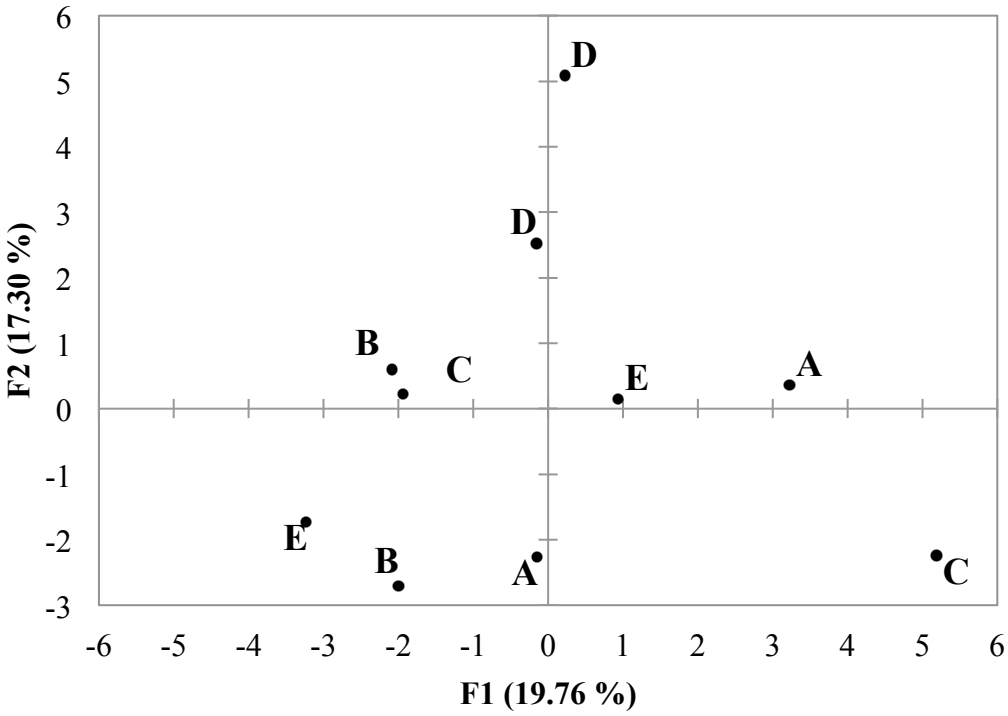

Figure 3-7 Multiple Factor analysis of aroma Napping® results of 2017 Pinot Noir wines made with different nitrogen applications (A = Control, B = +DAP in winery, C = +Nutriferm in winery, D = +SoilN, E = +FoliarN).

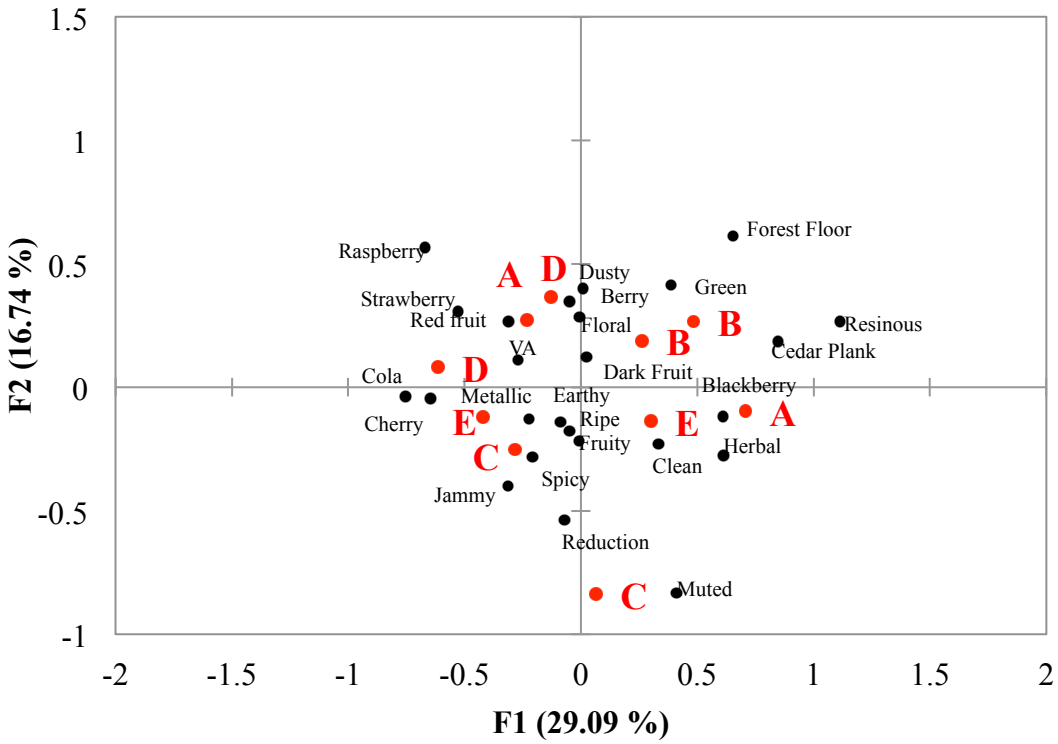

Figure 3-8 Correspondence analysis of aroma terms used for UFP analysis of 2017 Pinot Noir wines (A = Control, B = +DAP in winery, C = +Nutriferm in winery, D = +SoilN, E = +FoliarN).

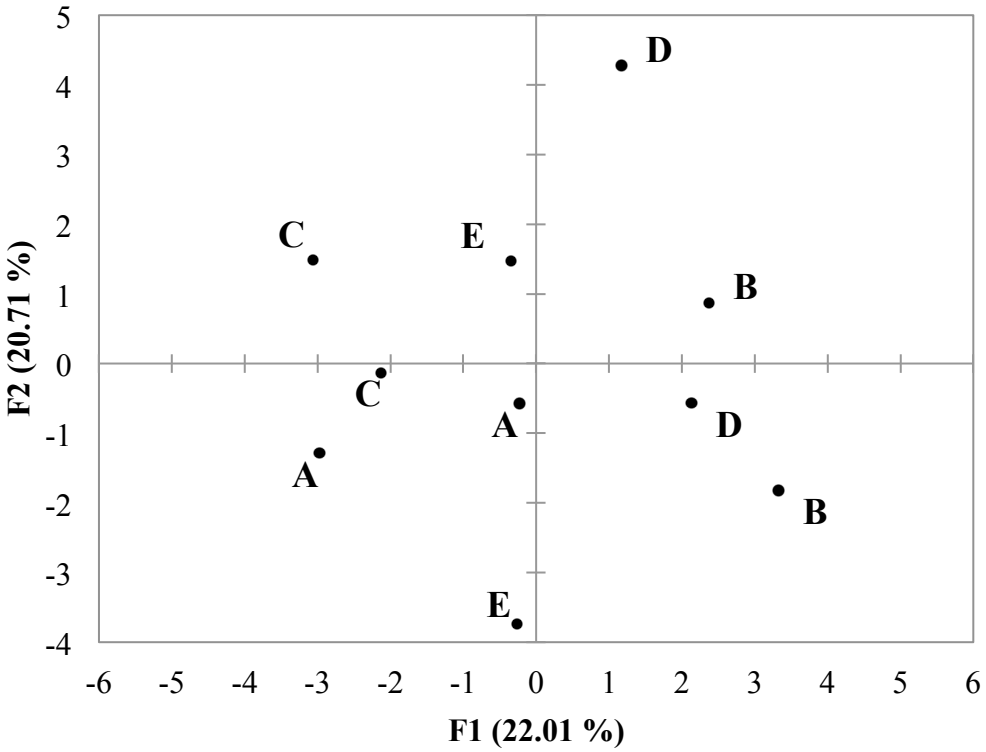

Figure 3-9 Multiple Factor analysis of mouthfeel terms used for UFP analysis of 2016 Chardonnay wines (A = Control, B = +DAP in winery, C = +Nutriferm in winery, D = +SoilN).

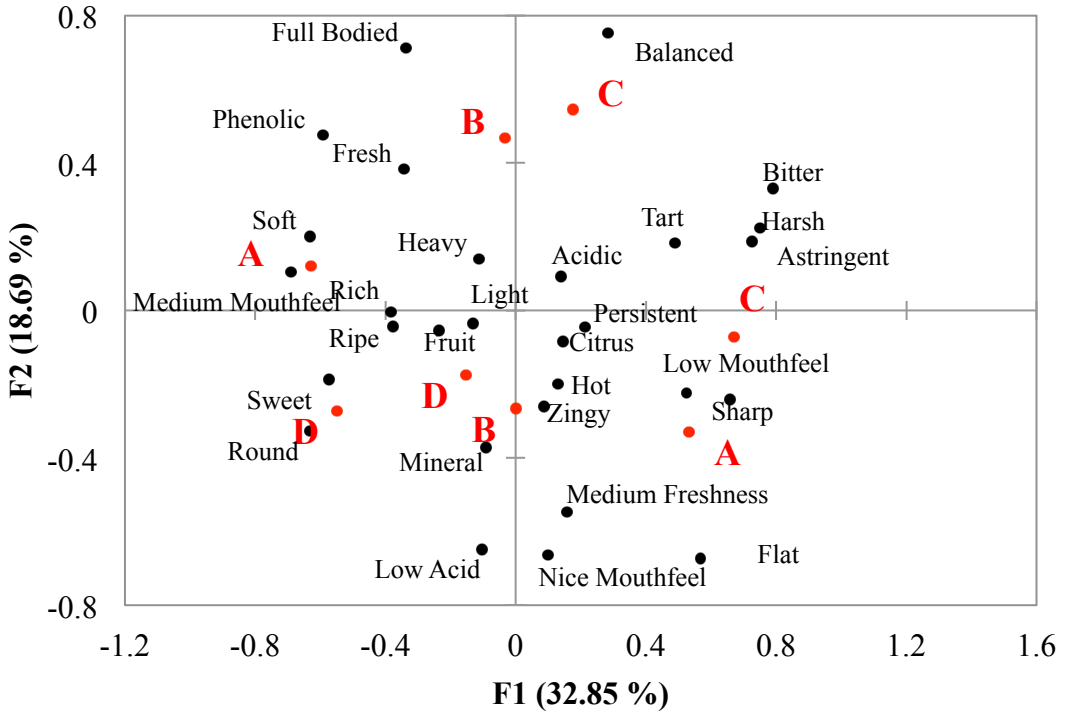

Figure 3-10 Correspondence analysis of mouthfeel terms used for UFP analysis of 2016 Chardonnay wines (A = Control, B = +DAP in winery, C = +Nutriferm in winery, D = +SoilN).

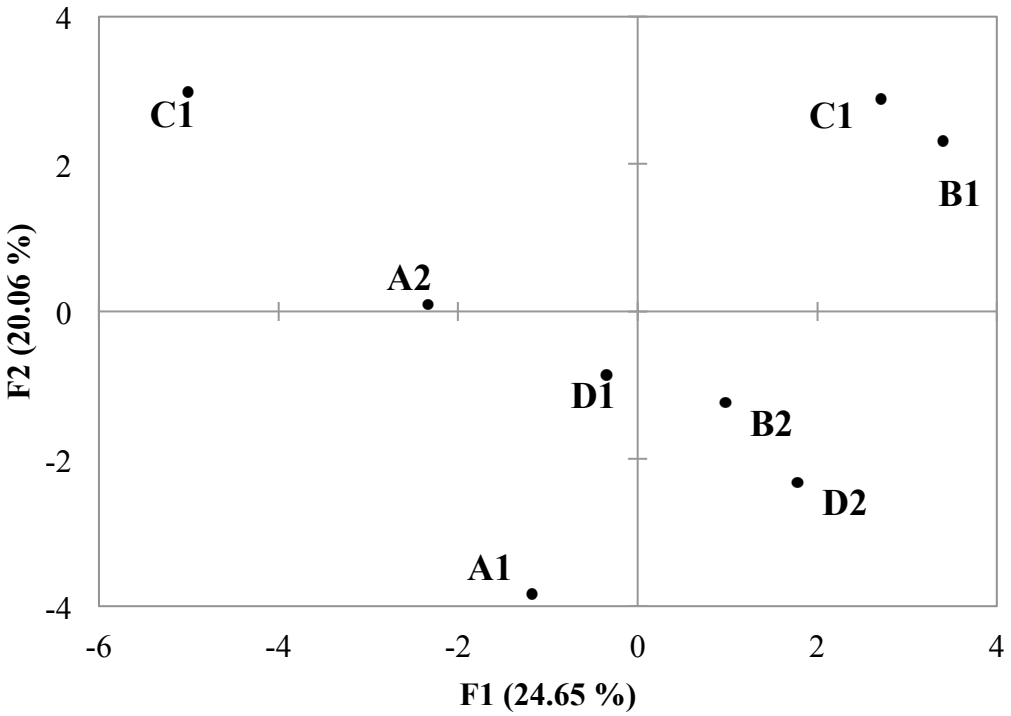

Figure 3-11 Multiple Factor analysis of mouthfeel terms used for UFP analysis of 2017 Chardonnay wines (A = Control, B = +DAP in winery, C = +Nutriferm in winery, D = +SoilN, E = +FoliarN).

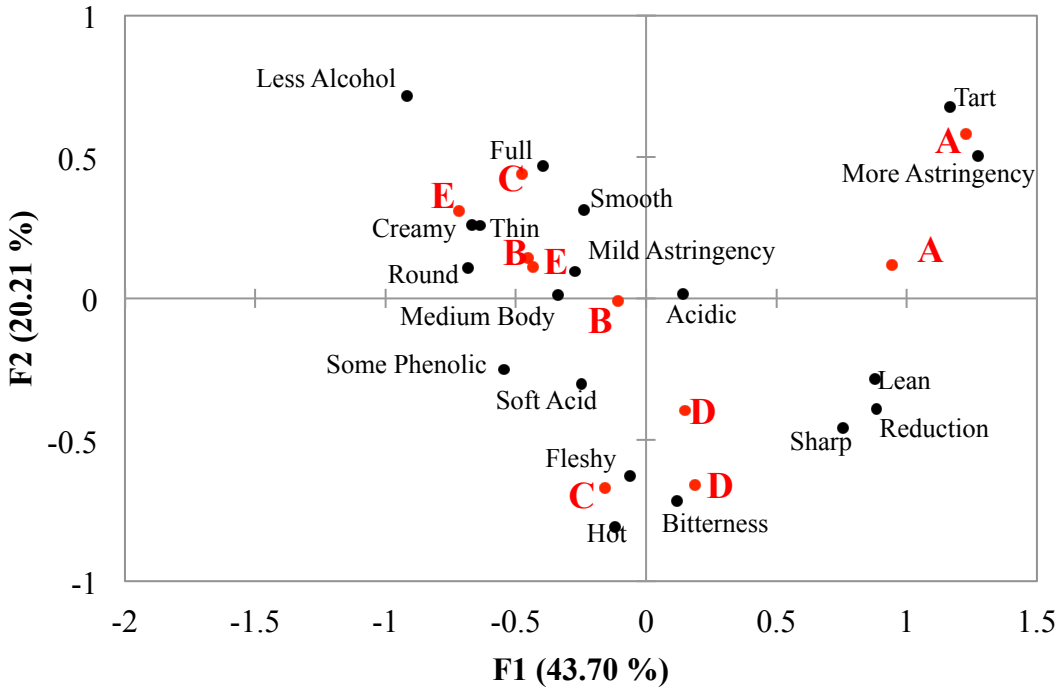

Figure 3-12 Correspondence analysis of mouthfeel terms used for UFP analysis of 2017 Chardonnay wines (A = Control, B = +DAP in winery, C = +Nutriferm in winery, D = +SoilN, E = +FoliarN).

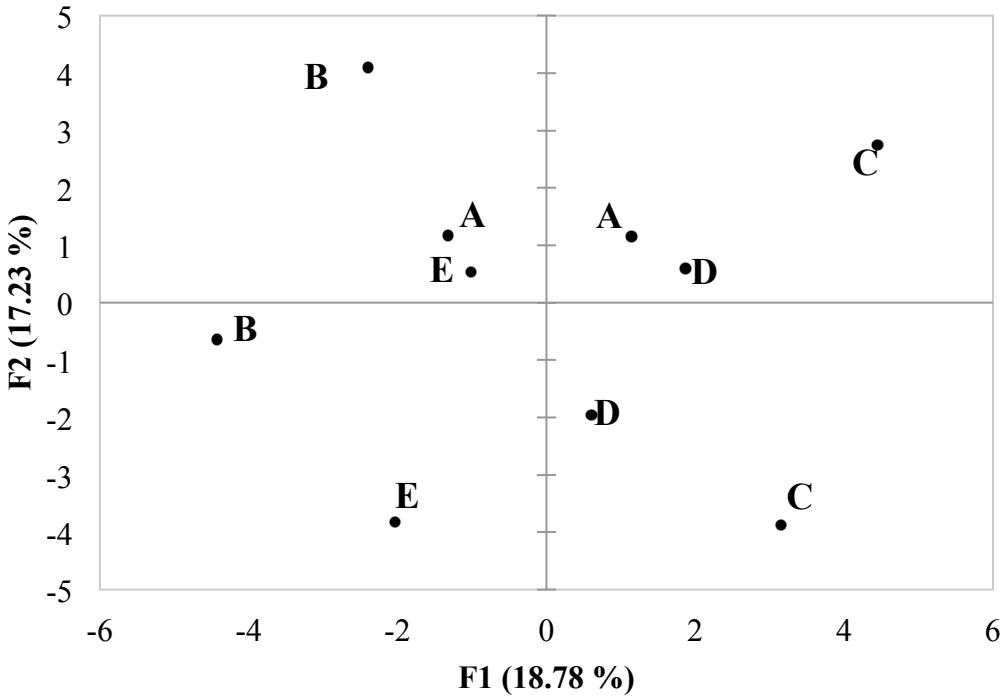

Figure 3-13 Multiple Factor analysis of mouthfeel terms used for UFP analysis of 2018 Chardonnay wines (A = Control, B = +DAP in winery, C = +Nutriferm in winery, D = +SoilN, E = +FoliarN).

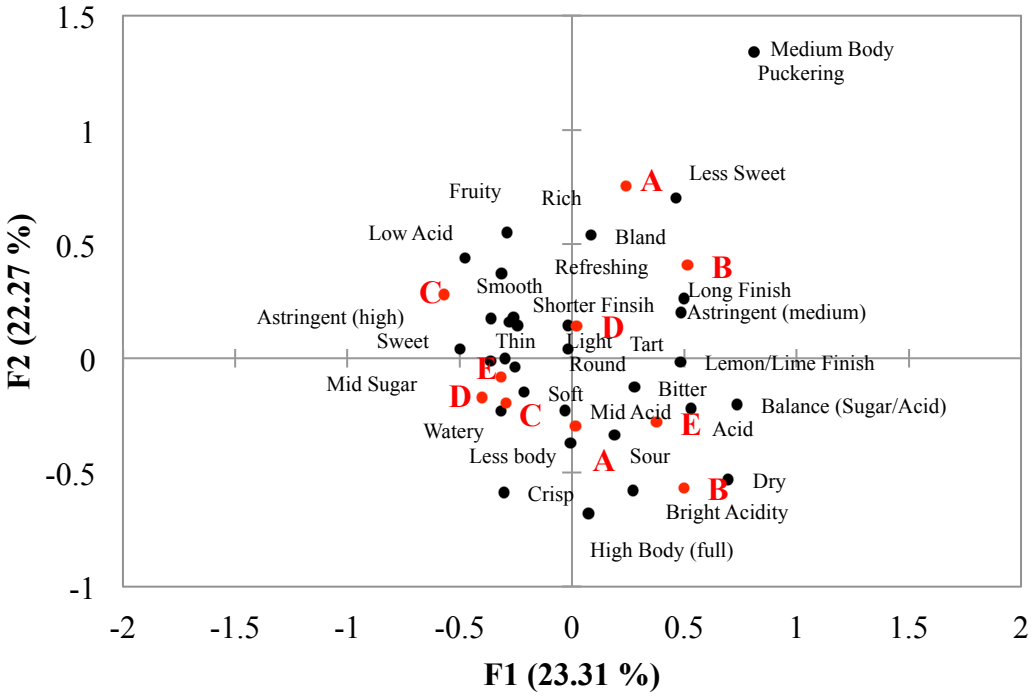

Figure 3-14 Correspondence analysis of mouthfeel terms used for UFP analysis of 2018 Chardonnay wines (A = Control, B = +DAP in winery, C = +Nutriferm in winery, D = +SoilN, E = +FoliarN).

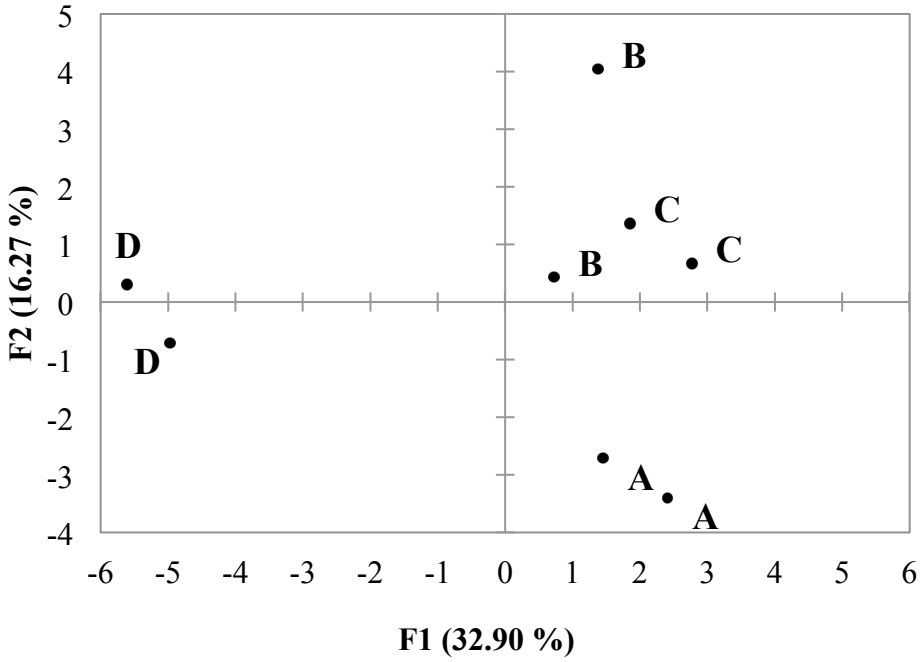

Figure 3-15 Multiple Factor analysis of aroma Napping results of 2016 Chardonnay wines analyzed (A = Control, B = +DAP in winery, C = +Nutriferm in winery, D = +SoilN).

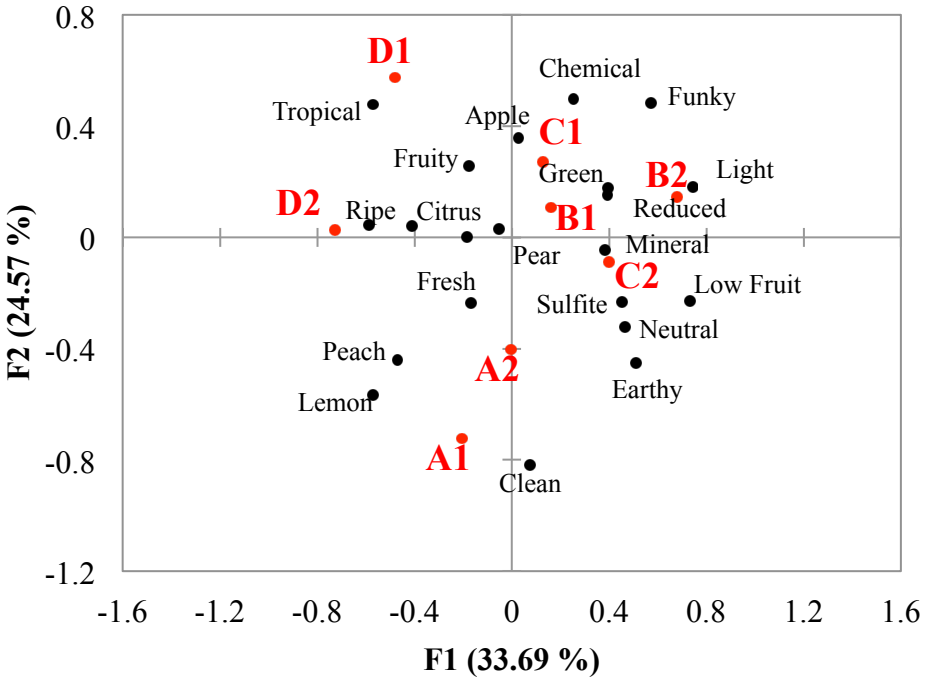

Figure 3-16 Correspondence analysis of aroma terms used for UFP analysis of 2016 Chardonnay wines (A = Control, B = +DAP in winery, C = +Nutriferm in winery, D = +SoilN).

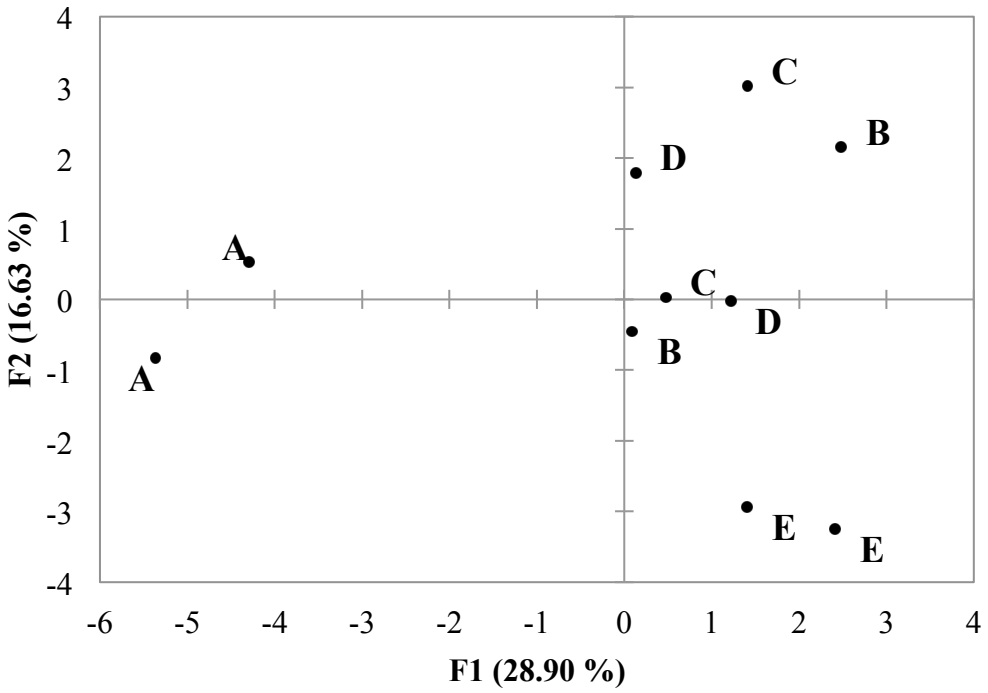

Figure 3-17 Multiple Factor analysis of aroma Napping results of 2017 Chardonnay wines analyzed (A = Control, B = +DAP in winery, C = +Nutriferm in winery, D = +SoilN, E = +FoliarN).

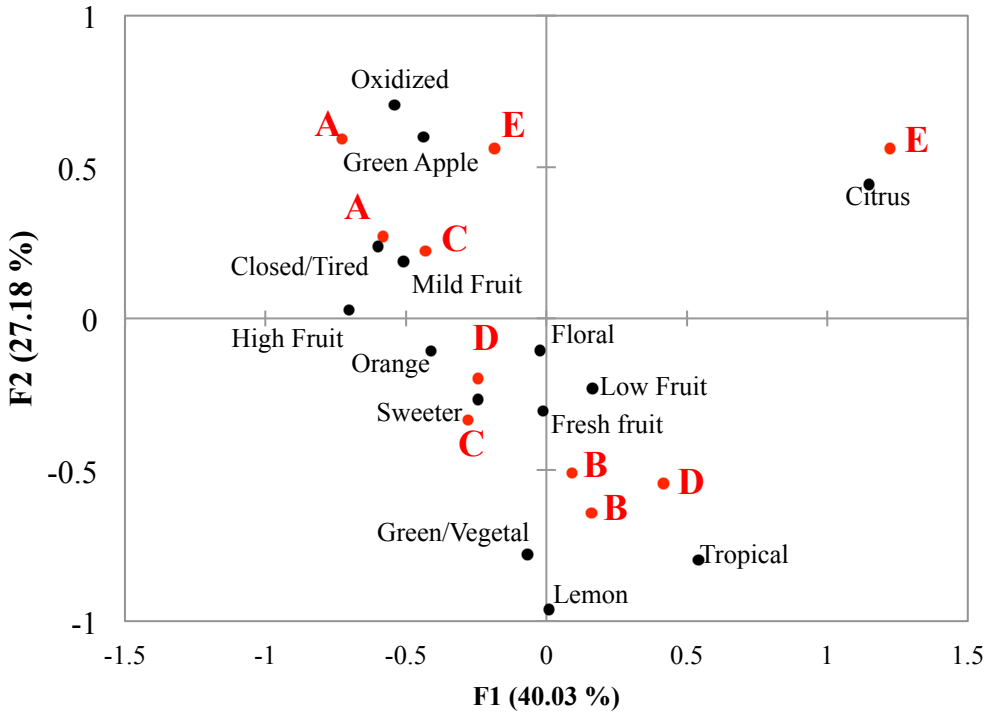

Figure 3-18 Correspondence analysis of aroma terms used for UFP analysis of 2017 Chardonnay wines (A = Control, B = +DAP in winery, C = +Nutriferm in winery, D = +SoilN, E = +FoliarN).

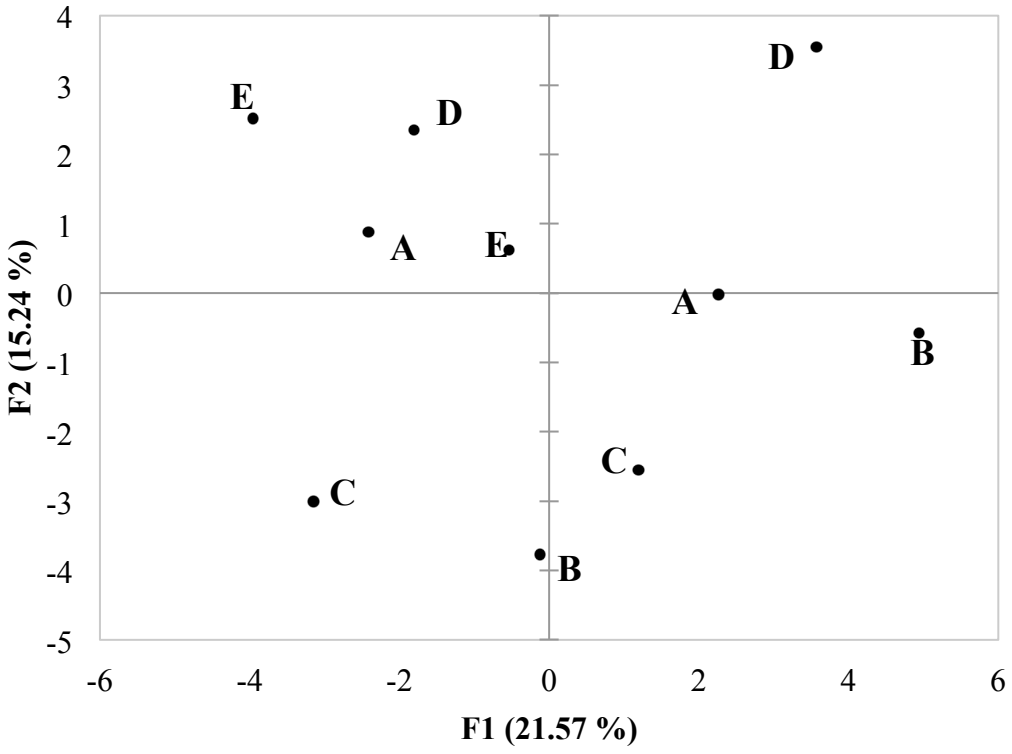

Figure 3-19 Multiple Factor analysis of aroma Napping results of 2018 Chardonnay wines analyzed (A = Control, B = +DAP in winery, C = +Nutriferm in winery, D = +SoilN, E = +FoliarN).

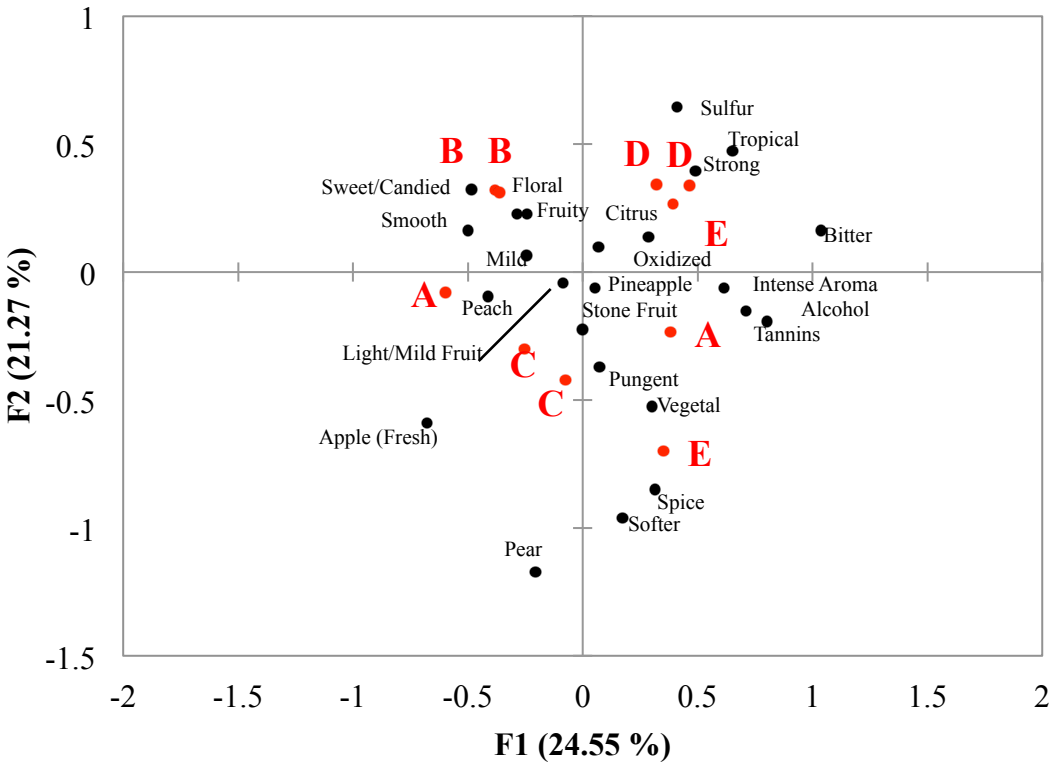

Figure 3-20 Correspondence analysis of aroma terms used for UFP analysis of 2018 Chardonnay wines (A = Control, B = +DAP in winery, C = +Nutriferm in winery, D = +SoilN, E = +FoliarN).

Discussion

Pinot Noir

There has been much research on how the amount and timing of nitrogen additions affect wine quality. However, it is unclear how the source of nitrogen, vineyard or winery, can impact wine quality. To date, studies have shown that increased vineyard nitrogen reduces total polyphenolics, and that decreased vineyard nitrogen increases berry and wine polyphenolics (Hilbert et al. 2015, Keller et al. 1999, Schreiner et al. 2014, Yuan et al. 2018). Sensory tests were not conducted in these studies, but lower polyphenolics are known to impact wine mouthfeel (Treeby et al. 2000). Research in the winery using amino acid based supplements has been shown to improve volatile aroma production, but again these studies do not include sensory information (Ugliano et al. 2008). Even though researchers show changes in volatile compound production this does not indicate perceivable changes to sensory attributes (Styger et al. 2011). Therefore in this study we conducted sensory tests, and found that different nitrogen treatments and concentrations have a minor impact on wine quality as related to mouthfeel and aroma in Pinot noir.

Triangle test results show that the different sources and concentrations of nitrogen tested did not significantly impact Pinot noir wine sensory attributes. Despite these non-significant differences the results of the mouthfeel and aroma Napping® with UFP do show some trends based off the nitrogen treatments. The conflicting results between Napping® and triangle tests were not surprising as panelists are better able to discriminate difference when specific questions are asked, versus the more generic questions given in triangle tests (Lawless and Heymann 2010). The type of cognitive processing required for discrimination testing versus descriptive testing is well known and utilize different cognitive processes, which is why the same samples can have different results based on the given test or question (Gridgeman 1970). Therefore it was anticipated that even with non-significant results for triangle tests, differences would be found using napping and UFP data.

Some of the significant differences found in some of the triangle test results were also expressed in the mouthfeel multiple factor analysis (MFA) data. For example, panelists were able to differentiate the +SoilN treatment from the control and +DAP treatment in both the triangle test data and mouthfeel MFA data in 2016 (Figure 3-1). This impact to wine mouthfeel was unexpected as nitrogen is not known to have a direct effect on wine mouthfeel. Much research has focused on nitrogen impacts to aroma. Little information on the impact of nitrogen on mouthfeel parameters is known.

The major components that influence red wine mouthfeel include the phenolics, flavonoids and non-falvonoids and nitrogen is not found in the structure of these compounds (Gawel 2008, Vidal et al. 2004a, Waterhouse 2002). With this knowledge it was anticipated that panelists would struggle to group the different nitrogen treatments based off mouthfeel differences. However, we found that panelists could group wines based on nitrogen treatment. In the 2016 MFA data panelists were able to separate the vineyard treatments as different from each other and all other treatments (Figure 3-1). In contrast, the 2017 MFA data showed that many of the replicate groupings for each treatment overlapped with one another (Figure 3-3), with no noticeable differences between treatments. The only exception to this was the two vineyard treatments. These treatments were, again, somewhat separated from all other treatments. Even though the 2016 and 2017 vintages are from two different blocks, it was interesting that the vineyard treatments showed mouthfeel differences.

While there is not yet an understanding of how nitrogen may impact the metabolism of mouthfeel compounds, previous studies have explored the correlation between phenolics and nitrogen application in the vineyard (Bell and Henschke 2008a, Schreiner et al. 2014). Specifically studies have explored how nitrogen impacts growth and therefore indirectly impacts grape components (Keller and Hrazdina 1998). Studies to date are conflicting, as some show that increasing nitrogen, increases phenolics and other work shows that increasing nitrogen results in

decreasing phenolics (Gutiérrez-Gamboa et al. 2017, Portu et al. 2015). While these results are conflicting, they do show that in some way the application of nitrogen in vineyard could influence wine mouthfeel. This provides a possible explanation for the differentiation of the vineyard fertilizer treatments from the other treatments in both vintages. However, the exact mechanism regarding how PN mouthfeel is impacted by nitrogen is unclear.

The mouthfeel descriptors attached to each treatment may provide some information about how nitrogen can impact wine composition. It is important to know some of the challenges associated with mouthfeel descriptive analysis. Traditionally there is little consensus on the usage of mouthfeel terms as mouthfeel training is difficult (Gawel et al. 2008). There are no tasting standards currently available for the majority of mouthfeel terms. Panelists are also known to have different sensitivities to mouthfeel components which can influence mouthfeel descriptors (McRae and Kennedy 2011, Pickering and Robert 2006). Many studies have highlighted differences in panelist sensory perception due to unique variations between individuals. For example, researchers have found the formation of salivary proteins in individuals is unique and can greatly affect the way panelists perceive astringency (Dinnella et al. 2009, Kallithraka et al. 2001). The variation of sensitivity between panelists may explain how in the 2016 MFA results one panelist called the control treatment balanced, while another called it astringent and acidic.

The wide array of terms used to describe wine astringency demonstrates the complexity of wine mouthfeel. Thus, when examining the correspondence analysis from both 2016 and 2017 it was expected that panelists would not be successful at enriching the differences shown in MFA with descriptive terms (Figure 3-1, 3-3). An example of how panelists struggled to describe mouthfeel is shown in the correspondence analysis (CA) for the 2016 PN (Figure 3-2). In this figure the +Nutriferm treatment was described as both astringent and tannic. The use of these two terms highlights the wide variation in mouthfeel descriptors because astringency is a sensation that is known to be caused by tannins (Kennedy et al. 2006). Thus,

despite the use of these two different words it is highly likely panelists were describing the same mouthfeel sensation. Additionally, differences in terminology knowledge and sensitivity may have accounted for the wide number of terms used, as in the 2017 CA, the +SoilN replicates had five different terms (dusty, structured, balanced, raw, sweet, and unripe) assigned to them to describe mouthfeel. In conclusion, it is still unclear how nitrogen in the vineyard or winery can directly affect mouthfeel. While differences in mouthfeel were found it is unclear if it is due to nitrogen or other environmental factors like location and climate. Overall, the results of the 2016 and 2017 MFA (Figure 3-1, 3-3) did display some mouthfeel differences based on nitrogen concentration and source, but the descriptive terms in the CA were variable and inconsistent (Figure 3-2, 3-4). The source and concentration of nitrogen supplementation may have subtly impacted the mouthfeel of Pinot noir wine. However, larger more significant differences were not seen.

Unlike wine mouthfeel, aroma compound development is known to be influenced by nitrogen (Bell and Henschke 2008a). YAN is comprised of ammonia and primary amino acids, and work regarding the metabolism of ammonia and primary amino acids has shown the direct and indirect role that ammonia and amino acids play in the production of volatile compounds (Styger et al. 2011, Waterhouse et al. 2016). Studies that have investigated ammonia (added to grape must in the form of DAP) have shown an increase in acetic acid/solvent character or esters (Torrea et al. 2011, Ugliano et al. 2008). Studies that investigate amino acids have found a number of amino acids (isoleucine, leucine, and phenylalanine) to be linked to desirable fruity aroma compounds (Styger et al. 2011). Other research has shown that an amino acid based supplement will produce more esters than an ammonia based supplement in wine (Guitart et al. 1999, Hernandez-Orte et al. 2006, Torrea et al. 2011). These past results are somewhat anticipated as it is well understood that a variety of amino acids are transformed into volatile compounds through the Ehlrich pathway (Styger et al. 2011, Waterhouse et al. 2016). Based on these studies it seems clear that ammonia based nitrogen supplements and amino acid based nitrogen supplements should significantly alter wine aroma in a different manner. Therefore, it was anticipated

that the +DAP and +Nutriferm treatments in this study would have noticeably different aromas.

When examining the 2016 MFA results the panelists were able to separate the two winery treatments, +DAP and +Nutriferm, but they were not as successful in 2017 (Figure 3-5, 3-7). Additionally the terms used to describe the winery treatments were similar as they were both dominated by red fruit descriptors in each vintage (Figure 3-6, 3-8). The fact that panelists could not separate the treatments was unexpected. However, Gonzalez-Marc et al (2010) found that if the amount of nitrogen is already sufficient in grape juice, the addition of amino acids does not improve the volatile composition of the wine. Therefore it is likely that the winery treatments were not separated well based on aroma due to the initial YAN levels in the grape must (Table 2-7, 2-8).

Despite the lack of separation between the +DAP and +Nutriferm treatments, the +SoilN treatment was noticeably different from all other treatments in both the 2016 and 2017 MFA (Figure 3-5, 3-7). The +SoilN differentiation may have been due to differences in grape amino acid profiles, or the concentration of various aroma precursor compounds that are formed during grape ripening. The addition of urea fertilizer to a Tempranillo vineyard showed a significant increase in the concentration of total YAN and amino acids when compared to the control (Garde-Cerdán et al. 2014). Thus, it is possible that the amino acids were significantly different in the +SoilN treatment compared to the winery and control treatments lead to a unique volatile compound profile.

Other researchers have found that aroma is heavily dependent on rootstock and not nitrogen supply (Treeby et al. 2000). With the information from this study it was likely that +FoliarN and +SoilN treatments would be grouped near each other. This grouping would be anticipated because they both had the nitrogen raised in the vineyard, and were grown with the same rootstock. However, the +FoliarN treatment was not grouped near the +SoilN treatment, rather it was grouped close to the winery

and control treatments (Figure 3-7). In conclusion, the results of this study show that aroma of Pinot noir is significantly altered by various sources of nitrogen, although in a different way than initially anticipated. This is because it was initially anticipated that the winery treatments would be separated from each other and they were not, and that the vineyards treatments would be grouped near each other when they were in fact separated.

Chardonnay

We know that nitrogen impacts grape varieties differently, so we also looked at Chardonnay how it was impacted compared to Pinot noir. From the results of the triangle tests it appears that the different sources and concentrations of nitrogen could significantly impact Chardonnay wine sensory attributes, but this impact may be dependent on vintage. In 2017 the control treatment was found to be significantly different than all other treatments, but in 2016 and 2018 it was only different from the +SoilN and +Nutriferm treatments respectively (Table 3-2). We anticipated the control would be different from all other nitrogen boosted treatments because numerous studies show that low levels of YAN can significantly alter wine sensory characteristics (Bell and Henschke 2008a, Jiranek et al. 1995a, Mendes-Ferreira et al. 2009). Together these results seem to indicate that the concentration of YAN may significantly change wine quality, but the extent of change is dependent on other factors like vintage.

Interestingly, the triangle tests data did not find the +Nutriferm and +DAP treatments to be significantly different from each other in 2016 and 2018 (Table 3-2). This result was surprising as previous work has shown ammonia and amino acid based supplements alter the formation of volatile aroma compounds which can affect sensory perception (Bell and Henschke 2008a, Styger et al. 2011, Torrea et al. 2011). Finally, the triangle test results showed no significant differences between the two vineyard treatments, +SoilN and +FoliarN, in any year. Despite the lack of significant differences in the triangle test results, the mouthfeel and aroma Napping® with UFP do show some significant differences based off the nitrogen treatments. As

mentioned earlier the different types of sensory tests conducted can explain how the results vary between the triangle and Napping® tests.

In contrast to red wine, white wine mouthfeel is less understood. While there are many phenolic compounds present in red wine there, there are much less in white wine (Waterhouse et al. 2016). The major phenolic compounds in white wine are hydroxycinnamates, which are linked to browning, and not astringency or bitterness (Vèrette et al. 1988). Gallic acid is one of the few phenolic compounds that may influence flavor or mouthfeel in white wine, but it is derived from oak and is not naturally present in the grape berry (Ibern-Gómez et al. 2001). Finally, the phenolic compounds that may influence mouthfeel in white wine do not contain nitrogen (Waterhouse 2002). Thus, based off what little is understood about white wine mouthfeel, it is unlikely that a change in nitrogen concentration or source would alter white wine mouthfeel.

As expected there were no noticeable trends across vintages that showed the nitrogen treatments significantly altering Chardonnay mouthfeel. Although, in 2016 and 2018 almost all treatments were well separated in MFA (Figure 3-9, 3-13). These distinct treatment separations were surprising, as previous research does not provide reasoning for nitrogen impacting white wine mouthfeel. Therefore, it was thought that the separations could be due to differences in final wine chemistry. However, the separated treatments in the 2016 and 2017 MFA were not clustered well based off titratable acidity, alcohol, phenolics, or residual sugar, all of which are known to influence mouthfeel perceptions (Gawel et al. 2014) (Table A-3, A-4). Despite the lack of alignment in the 2016 and 2017 sensory data compared to the chemistry data, the 2018 MFA sensory clusters did match some wine chemistry parameters. In the 2018 there were two clusters found, cluster one contained the +Nutriferm and +SoilN wines and cluster two continued the Control, +DAP, and +FolairN wines (Figure 3-13). Cluster one was had low alcohol and high residual sugar, while cluster two had high alcohol and low residual sugar (Table A-4). Even though only the 2018 MFA

was well explained by the chemical components of the wine, the use of terms in the CA can help explain the unique groupings in the 2016 and 2017 MFA.

In 2016 and 2017 the panelists were only asked to refrain from smelling the wines and were not required to wear nose clips when evaluating the mouthfeel of the wine. The lack of nose clips may have resulted in groupings and descriptions being influenced by retronasal aroma, because previous research has suggested that some mouthfeel terms can be influenced by the volatile fraction of wine (Sereni et al. 2016). Therefore, based off the terms used in 2016 and 2017 it is likely that retronasal aroma was influencing mouthfeel terminology. For example, in 2016 the usage of the terms fruit, fresh, and citrus are all known to dependent on the volatile fraction of wine. Furthermore, retronasal aroma influence on mouthfeel can account for the unique MFA treatment groupings in 2016 and 2017 (Figure 3-9, 3-11).

In addition to the influence of retronasal aroma on mouthfeel terminology, wine chemistry also played a role in term assignment. Certain wine components like pH, sugar, alcohol, and phenolics have been demonstrated to affect certain mouthfeel parameters. Viscosity is a mouthfeel parameter known to be influenced by sugar, polysaccharides, ethanol, and glycerol (Vidal et al. 2004b, Walker and Prescott 2000). Astringency and bitterness are linked to pH, and the presence of certain tannins and phenolic compounds (Gawel et al. 2014). Finally, titratable acidity in wine is well studied and it is known that wines with higher acidity can be described as more tart, sharp, and sour (Pickering and Demiglio 2008). These known relationships can account for the descriptive terms attached to the +SoilN treatment in 2017 and to the +Nutriferm treatment in 2018 (Figure 3-12, 3-14), as these treatments had the highest amount of total phenolics in each year and were described as “bitterness” and “astringent: (high)” respectively. While these terms were used to describe two different sensations, they are often used interchangeably (Breslin et al. 1993, Ma et al. 2014). Therefore, it is likely that panelists in each vintage were describing the same mouthfeel sensation elicited by the increased content of phenolics. Finally, in 2017 and 2018 the use of the term tart, and sour lined up well with wines that had greater

amounts of titratable acidity and lower residual sugar. In conclusion, panelists did a better job separating and describing Chardonnay wines based off differences in mouthfeel than they did in Pinot noir. However, the groupings and separations of the Chardonnay wines did not appear to be linked to the source or concentration of nitrogen treatment, but rather small differences in final wine chemistry.

Unlike white wine mouthfeel, white wine aroma was impacted by nitrogen concentration and source. As mentioned earlier there are clear connections between wine nitrogen and volatile compound formation. It was noted that the two winery treatments, +DAP and +Nutriferm, were expected to produce wines with noticeably different aromas. However, similar to the Pinot noir results, panelists were not able to clearly separate the Chardonnay wines supplemented with DAP or Nutriferm Arom Plus in any vintage based off aroma in MFA (Figure 3-15, 3-17, 3-19). Both ammonia and amino acids can donate nitrogen during anabolic and catabolic metabolism for the synthesis of desired amino acids and specific higher alcohols (Waterhouse et al. 2016). Therefore, even though the wines had different nitrogen supplements and amino acid profiles it is possible that they were utilized in a similar manner by the yeast resulting in similar aroma compounds.

Additionally, it was noted in chapter 1 the +DAP and +Nutriferm treatments did have some individual primary amino acid concentrations in common (Table 1-10, 1-11, 1-12). Thus, it is possible that these similarities allowed for common volatile compounds to be produced across the two treatments, resulting in similar aroma profiles. Finally, these treatments had similar YAN values and some studies have shown that regardless of nitrogen source, grape juice with similar YAN levels will produce similar volatile compounds, given that the level of YAN is adequate for fermentation (Bell and Henschke 2008a).

To date there has not been a lot of research regarding the impact of vineyard fertilizers on the aroma of Chardonnay wine. However, studies on Sauvignon blanc have found that nitrogen application in the vineyard can increase the concentration of

volatile thiol precursors in grapes and thus volatile thiols in finished wines (Lacroux et al. 2008). Research conducted on Riesling found that monoterepene concentrations are altered by the application of nitrogen fertilizers causing noticeable differences during sensory testing (Webster et al. 1993). These studies support the separation of the +SoilN wine and unique descriptors attached to it every year. In 2016-18 the +SoilN wine was described as being tropical, (Figures 3-16, 3-18, 2-30) and it was well separated from most other treatments in the 2016 and 2018 MFA (Figure 3-16, 3-18). This supports the results found earlier in the Pinot noir data that vineyard fertilizers can influence varietal aroma. Although, it is not presently clear how the nitrogen source for the +SoilN treatment produced this distinct tropical aroma, it could be related to the initial amino acid profile. As noted in chapter 1, a few primary amino acids present in the +SoilN juice were found in significantly greater concentrations than other nitrogen supplemented treatments. Thus, it is possible that one or more of these amino acids is allowing for the production of this unique aroma profile, although the mechanism for this direct relationship is not currently known. In conclusion, these results indicate that vineyard fertilizers can significantly alter Chardonnay wine aroma.

Conclusions

Overall, the nitrogen treatments tested are impacting the sensory attributes of Pinot noir and Chardonnay. As anticipated aroma perception of both were effected but only the mouthfeel perception of Pinot noir, and not Chardonnay, was altered. While some similarities in Pinot noir mouthfeel and aroma are seen across vintages, it is important to recall, from chapter one, that each vintage is from a different block, so specific patterns should not be drawn across years. Furthermore, at this time it is not currently clear how the different nitrogen treatments change specific Pinot Noir mouthfeel sensations or aroma compounds. In contrast, the Chardonnay aroma differences were better defined by descriptive terms, indicating that nitrogen has a more distinct influence on the aroma of Chardonnay than Pinot Noir. Furthermore, in the triangle tests consumers were able to differentiate treatments based of YAN concentration better in Chardonnay than Pinot Noir. In conclusion, the impact of nitrogen on Pinot noir wine mouthfeel and wine aroma on are small, meaning they are

very subtle and likely undetectable to most Pinot noir consumers. However, the impact of nitrogen on Chardonnay aroma is noticeable to both experts and consumers. Therefore, Chardonnay producers should carefully consider where and how they are adding nitrogen supplements, where as Pinot noir producers have some flexibility in how much nitrogen they use, and where they apply nitrogen supplements.

General Summary and Conclusions

Yeast assimilable nitrogen, YAN, is a major nutrient required for yeast to complete fermentation. While it is understood that YAN is needed for fermentation winemakers are not in agreement over how much YAN is adequate, and where YAN should be added. The results of our study show that YAN can be boosted successfully in the vineyard and the winery for both Chardonnay and Pinot Noir, and that the location of YAN boost does not impact fermentation rate. In this study both Pinot Noir and Chardonnay were able to finish fermentation with less than 150 mg N/L with out issue. Overall, the amount of YAN required for successful fermentation appears to be lower than initially thought. However, it was seen that yeast strain and type of nitrogen could significantly impact fermentation rate. It is also important to note that YAN concentration and composition were affected by the choice of YAN supplement, and changes to YAN composition could have impacts on wine quality.

In this study the amino acid profiles of Chardonnay grape juice from the various nitrogen treatments showed that different nitrogen sources inconsistently change individual amino acid concentrations. Volatile aroma compounds in wine have been linked to the presence of specific amino acids, so future work should continue with volatile aroma analysis to better understand how changes to YAN composition may impact wine aroma. Even though no volatile data was collected sensory studies were run to learn how the nitrogen treatments could be affecting wine quality. From the sensory studies it was apparent that the nitrogen treatments impacted aroma, but not mouthfeel. Panelists were able separate and describe Chardonnay wines that had nitrogen added in the vineyard through the irrigation system each year. However, Pinot noir panelists were only able to separate, not describe, the nitrogen treatments based on aroma. From these results it is evident that nitrogen source and concentration do influence wine aroma, but this impact is variable based on grape variety.

In conclusion, these results suggest that Chardonnay producers should carefully consider where they add nitrogen and how much nitrogen they add as it can

noticeably impact wine aroma, but Pinot noir producers have more flexibility in where they boost YAN. Future work should continue to explore how YAN composition changes wine aroma. Additionally, future studies should also continue to investigate the interaction between yeast strain and nitrogen source. Together these would be useful tools for wine producers, allowing them to potentially predict volatile compound formation based off YAN source, and yeast strain.

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Appendices

Appendix A

Table A.1 Basic wine parameters for the 2016 Pinot Noir treatments, where YAN was adjusted prior to fermentation in the winery with DAP (+DAP) or Nutriferm Arom Plus (+Nutriferm), or in the vineyard (+SoilN), or no adjustments were made (Control).

Wine Parameter	Control	+DAP	+Nutriferm	+SoilN
pH	3.82 (0.00)	3.82 (0.00)	3.87 (0.01)	3.98 (0.00)
Titratable Acidity (g/L)	5.50 (0.15)	5.29 (0.16)	5.28ab (0.14)	4.93 (0.22)
Residual Sugar (g/L)	0.32 (0.00)	0.22 (0.00)	0.42a (0.05)	0.38 (0.02)
Alcohol %	13.9 (0.20)	13.4 (0.12)	14.0 (0.12)	13.6 (0.12)
Acetic Acid (g/L)	0.18 (0.11)	0.27 (0.01)	0.25a (0.01)	0.12 (0.04)
Phenolics (mg/L)	2600.0 (70.1)	2635.7 (114.2)	3250.0 (20.2)	3200.0 (90.9)

Table A.2 Basic wine parameters for the 2017 Pinot Noir treatments, where YAN was adjusted prior to fermentation in the winery with DAP (+DAP) or Nutriferm Arom Plus (+Nutriferm), or in the vineyard (+SoilN, +FoliarN), or no adjustments were made (Control).

Wine Parameter	Control	+DAP	+Nutriferm	+SoilN	+FoliarN
pH	3.56 (0.01)	3.50 (0.01)	3.58 (0.01)	3.68 (0.01)	3.54 (0.01)
Titratable Acidity (g/L)	5.82 (0.22)	6.08 (0.10)	4.66 (0.05)	5.02 (0.03)	5.09 (0.16)
Residual Sugar (g/L)	0.21 (0.01)	0.17 (0.74)	0.21 (0.03)	0.24 (0.03)	0.13 (0.03)
Alcohol %	13.9 (0.00)	13.9 (0.00)	13.7 (0.15)	13.4 (0.17)	13.5 (0.00)
Acetic Acid (g/L)	0.99 (0.03)	0.79 (0.04)	0.79 (0.13)	0.90 (0.02)	1.01 (0.01)
Phenolics (mg/L)	1814.29 (207.6)	2400.00 (70.7)	1557.14 (111.1)	1671.43 (50.5)	1921.43 (415.5)

Table A.3 Basic wine parameters for the 2016 Chardonnay treatments, where YAN was adjusted prior to fermentation in the winery with DAP (+DAP) or Nutriferm Arom Plus (+Nutriferm), or in the vineyard (+SoilN), or no adjustments were made (Control).

Wine Parameter	Control	+DAP	+Nutriferm	+SoilN
pH	3.11 (0.01)	3.08 (0.01)	3.09 (0.01)	3.11 (0.01)
Titratable Acidity (g/L)	7.42 (0.33)	7.38 (0.20)	7.51 (0.22)	8.07 (0.24)
Residual Sugar (g/L)	6.13 (0.01)	4.61 (0.07)	3.92 (0.14)	3.80 (0.34)
Alcohol %	13.0 (0.12)	13.4 (0.06)	13.5 (0.00)	13.5 (0.00)
Phenolics (mg/L)	305.95 (18.86)	297.86 (27.22)	314.52 (8.37)	316.43 (22.18)

Table A.4 Basic wine parameters for the 2017 Chardonnay treatments, where YAN was adjusted prior to fermentation in the winery with DAP (+DAP) or Nutriferm Arom Plus (+Nutriferm), or in the vineyard (+SoilN, +FoliarN), or no adjustments were made (Control).

Wine Parameter	Control	+DAP	+Nutriferm	+SoilN	+FoliarN
pH	3.10 (0.00)	3.14 (0.01)	3.18 (0.00)	3.32 (0.00)	3.25 (0.00)
Titratable Acidity (g/L)	8.39 (0.14)	7.58 (0.09)	7.63 (0.13)	7.00 (0.35)	6.70 (0.10)
Residual Sugar (g/L)	4.20 (0.17)	4.46 (0.23)	4.34 (0.20)	2.98 (0.06)	6.78 (0.10)
Alcohol %	13.1 (0.06)	13.0 (0.06)	13.1 (0.00)	12.9 (0.06)	13.4 (0.06)
Phenolics (mg/L)	298.81 (12.31)	277.38 (2.18)	303.10 (9.07)	295.95 (20.02)	284.52 (22.78)

Table A.5 Basic wine parameters for the 2018 Chardonnay treatments, where YAN was adjusted prior to fermentation in the winery with DAP (+DAP) or Nutriferm Arom Plus (+Nutriferm), or in the vineyard (+SoilN, +FoliarN), or no adjustments were made (Control).

Wine Parameter	Control	+DAP	+Nutriferm	+SoilN	+FoliarN
pH	3.08 (0.02)	3.04 (0.02)	3.05 (0.03)	3.07 (0.02)	3.09 (0.03)
Titratable Acidity (g/L)	8.75 (0.29)	8.10 (0.20)	8.35 (0.12)	9.09 (0.21)	8.83 (0.08)
Residual Sugar (g/L)	6.83b (0.41)	8.04 (0.28)	11.00 (1.30)	10.20 (0.01)	9.48 (0.04)
Alcohol %	13.2 (0.17)	13.1 (0.00)	12.8 (0.06)	12.9 (0.00)	13.2 (0.06)
Phenolics (mg/L)	308.81 (47.84)	314.05 (10.82)	367.86 (12.70)	312.14 (10.79)	315.00 (7.42)