Impact of pre-fermentation maceration techniques on yeast populations and color of Pinot noir wine

by

Molly L. Zook

A PROJECT

Submitted to

Oregon State University

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Abstract approved: _____
James P. Osborne

The quality of red wine is primarily determined by the flavor, aroma, and color. A common wine making technique used to improve these characteristics is the pre-fermentation maceration. In this study two pre-fermentation maceration techniques were used, one was a traditional cold soak and the second involved addition of dry ice to the grapes. Samples were taken daily and yeast viable cells were assessed by growth on WL media. This also enabled the major species of yeast to be identified. The two major yeast species seen throughout the pre-fermentation maceration were *Pichia membranaefaciens* and *Kloeckera apiculata*. Yeast population in both pre-fermentation macerations increased during the maceration period. However, after three days the population in the cold soak treatment was 1 log or greater than the yeast population in the dry ice treatment. This difference remained for the rest of the maceration. After wines were produced their color and phenolic contents were analyzed. Total color, polymeric pigments and monomeric anthocyanins in wines that underwent a pre-ferementation maceration were not significantly different than the control. However, there was a significant difference in the amount of tannins between the dry ice and cold soak treatment to the control.

Key Words: pre-fermentation maceration, dry ice, red wine color, non-*Sacchromyces*

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Committee Member, representing Food Science and Technology

Chair, Department of Food Science and Technology

Dean, University Honors College

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

Molly L. Zook, Author

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Impact of pre-fermentation maceration techniques on yeast populations and color of Pinot noir wine.

INTRODUCTION

The quality of a red wine is usually determined by the sensory attributes color, aroma, flavor, and mouthfeel. These attributes can be influenced by many different processes in the vineyard and in the winery although the most important factor determining the quality of a red wine is the quality of the grapes themselves. Grape quality is primarily influenced by specific vineyard practices, climate, and grape varietal (Jackson and Lombard, 1993). However, although the quality of the starting material is very important, the winemaking process can have a large influence on the final attributes of a red wine as the winemaker has many different tools and practices at their disposal with which to alter the final properties of the wine (Jackson, 2000).

Red winemaking begins when grapes are harvested and processed at the winery. Processing usually includes de-stemming where the grape berries are removed from the rachis, and crushing, where the berry is split causing separation of the pulp from the skin. After crushing, grapes may begin alcoholic fermentation immediately or may undergo a pre-fermentation maceration step often called a cold soak.

The alcoholic fermentation is most commonly performed by the yeast *Sacchromyces cerevisiae*. Often the grape must is inoculated with a commercial yeast starter culture but in some cases a natural fermentation is allowed to occur where yeast naturally present on the grapes or in the winery perform the fermentation. During the alcoholic

fermentation the grape sugars, glucose and fructose, are converted to ethanol and carbon dioxide. Yeast also produce compounds such as esters and higher alcohols that contribute to the aroma and flavor of the wine. Once the wine has completed fermentation it is then pressed to separate the wine from the skins and seeds (Jackson, 2000).

After pressing, wines may also undergo a secondary fermentation known as the malolactic fermentation (MLF). During this process lactic acid bacteria, usually *Oenococcus oeni,* convert malic acid to lactic acid. This process lowers the acidity of the wine and is often performed on red wines. In particular, it is used on wines made from grapes grown in cool climates, such as Oregon, where grapes often contain high concentrations of malic acid. The MLF may also modify other sensory characteristics of the wine such as aroma and mouthfeel. At the completion of the MLF SO_2 is usually added to the wine to prevent microbial spoilage and oxidation.

Aside from conducting a MLF there are many other post fermentation processes that the wine maker can do to further improve wine quality. These can include pH adjustments, sugar adjustments, and blending of the wine. Protein and tartrate stabilization as well as clarification of the wine may also occur at this point. Fining of the wine can be done to remove bitter or astringent phenolic compounds and can also eliminate off odors and flavors such as hydrogen sulfide (rotten egg smell). The final step in wine making is the aging process which usually takes place in oak barrels. Many wines can benefit from aging but the time in which ageing actually improves

the wine is different for each individual wine. The oak barrel is the most common way to store red wines during this aging process due to the oaks ability to impart flavor and aroma to the wine. After a suitable aging period the wine may be filtered before it is bottled and packaged ready for sale (Jackson, 2000).

Although the basics of red winemaking are the same from one winery to another, there are a number of practices that some winemakers employ to alter the characteristics of the final wine. One of these processes is the pre-fermentation maceration. Winemakers choose to employ this process for two major reasons: to improve the color of the wine and/or to modify the flavor and aroma of the wine. The color of a red wine is primarily driven by the concentration of anthocyanins present in the grape skins. These compounds are water-soluble pigments that are extracted into the wine during fermentation (Zoecklein, 1995). In Pinot noir malvidin-3-glucoside is the predominate anthocyanin often accounting for greater than 80% of the total anthocyanin content (Dicey, 1995). Because anthocyanins are water soluble, it is proposed that the pre-fermentation maceration results in greater extraction of these compounds from the grape skins thus improving wine color (Zoecklein, 1995; Alvarez et al. 2006).

Red wine color is not due just to the concentrations of anthocyanins in the wine. Once anthocyanins have been extracted into the wine they rapidly form co-pigmentation complexes (Boulton, 2001) and begin undergoing numerous other reactions resulting in a number of new pigmented compounds (Bakker and Timberlake, 1997; Fulcrand

et al., 1998) categorized as polymeric pigments. These new color compounds are often much more stable and produce greater color than would be expected from their concentrations in the wine (Sacchi et al., 2005). Therefore, a second proposed mechanism for increased color due to pre-fermentation maceration is increased extraction of compounds involved in co-pigmentation and polymeric pigment formation (Kennedy and des Gachons, 2003).

Co-pigmentation involves the association of the anthocyanin with a non-colored organic compound creating more color than the unbound pigment. The major co-pigments in wine include *p*-coumaric acid, caffeic acid, catechin, and quercetin (Brouillard et al., 1989; Boulton, 2001). The concentration of these compounds in a wine can determine the color of a red wine color regardless of the concentration of anthocyanins (Boulton, 2001). Polymeric pigment formation is influenced by the concentration of anthocyanins as well as tannins, acetaldehyde, and pyruvic acid. These compounds are more resistant to SO_2 bleaching than monomeric anthocyanins and are also resistant to oxidation (Bakker and Timberlake, 1997) making them an important component of red wine color and color stability. The concentration of compounds such as tannins and *p*-coumaric acid, caffeic acid, catechin, and quercetin, may change during pre-fermentation maceration and in turn alter the final color of the wine. For example, Heatherball et al. (1997) reported that pre-fermentation maceration increased wine phenolic and anthocyanin content and also enhanced the color intensity in treatments with high SO_2 additions. In contrast, Watson et al.

(1995) noted that Pinot noir that underwent a pre-fermentation maceration was lower in anthocyanins and color intensity than the control.

Aside from color changes, winemakers may choose to perform a pre-fermentation maceration to improve the flavor and aroma of the resulting wine. Changes in aroma and flavor during the pre-fermentation maceration may be due to either extraction of specific compounds from the grape skins or pulp or through the action of yeast that are present during the maceration (Zoecklein et al., 1997; Gardner et al., 2011). There are many species of yeast that are found on the grape berry naturally including many non-*Saccharomyces* species and sometimes low levels of *Saccharomyces* yeasts. Non-*Sacchromyces* yeasts species that are commonly found on the grapes are *Candida, Kloeckera,* and *Pichia*. These yeast are generally more cold tolerant than *Saccharomyces* species (Egli et al., 1998) and so winemaking practices such as a pre-fermentation maceration may encourage their growth relative to *Saccharomyces* populations. A number of studies have shown that non-*Saccharomyces* yeast species can impact the concentration of specific flavor and aroma compounds (Zoecklein et al., 1997; Romano et al. 2003) that are important in wine such as esters and terpenes. Non-*Sacharomyces* yeast generally die off once alcoholic fermentation begins and ethanol levels increase. However, if they are present in sufficient initial quantities they can still impact the final aroma and flavor of the wine (Mora, 1992).

The conditions of the pre-fermentation can significantly impact the growth and survival of the yeast present. Winemakers use cold temperatures (typically 8-12°C), SO_2, and a low oxygen environment to control yeast growth. This is typically done by cooling the grapes in a temperature controlled tank (jacketed) with an addition of 30-50 mg/L of SO_2 and blanketing the grapes with inert gas (Zoecklein et al 1995; Jackson 2000). An alternative method that can be employed is the addition of dry ice to rapidly decrease the temperature of the grapes and produce a high CO_2 environment. Both of these methods will create different environments and challenges for the growth and survival of yeast present on the grapes. However, little is known about the impact of these practices on yeast populations and diversity and specifically the consequences of dry ice addition. If different pre-fermentation techniques impact yeast populations then this may result in changes in the final aroma and flavor of the wine. Therefore, the objective of this research was to investigate the impact of two common pre-fermentation maceration techniques (dry ice addition and standard cold soaking) on yeast populations. In addition, the impact of pre-fermentation maceration on the color and tannin content of the wines was also examined.

MATRIALS AND METHODS

Winemaking:

Pinot noir grapes were harvested from the Oregon State University vineyard (Alpine, OR), transported to the Oregon State University research winery, pooled, and then destemmed. Basic grape parameters (pH, titratable acidity (TA), °Brix) were measured using standard methods (Zoecklein et al., 1996) while yeast assimilable nitrogen (YAN) was calculated as the sum of α-amino nitrogen measured by NOPA (Dukes and Butzke, 1998) combined with ammonia measured by enzymatic test kit (R-Biopharm, Darmstadt, Germany). The juice parameters were pH 3.39, TA 7.2 g/L tartaric acid equivelents, 22.5 °Brix, and 272 mg/L nitrogen (YAN).

After destemming an addition of 50 mg/L SO_2 was made to the grapes before approximately 60 L of grape must was placed into each of six 100 L stainless steel tanks. Two of the tanks were layered with dry ice at approximately 3 kg dry ice per 100 kg grape must (dry ice treatment). This was done by alternating layers of pelleted dry ice with grape must. After dry ice addition the tanks were sealed with lids and kept at room temperature. Two other tanks were blanketed with argon gas, sealed with lids, and immediately placed in a cold room at 7.2°C (standard pre-fermentation maceration treatment). The final two tanks were inoculated with *S. cerevisiae* RC212 to induce the alcoholic fermentation following the manufacturers recommended rehydration protocol and inoculation rate (30 g/hL) (control). These tanks were placed in a temperature controlled room at 27°C.

Grapes undergoing a pre-fermentation maceration remained in the cold room (7.2°C) for 7 days. Grapes treated with dry ice were originally kept at room temperature. However, after 48 hrs the temperature of the grape must in the dry ice treated tanks had risen dramatically and so the tanks also were placed in a cold room at 7.2°C to prevent alcoholic fermentation from occurring. During this seven day period tanks were sampled daily after mixing. After sampling grapes were blanketed with argon gas and lids were placed back on the tanks. Tanks were removed from the cold room after seven days and placed in the 27°C room. After 12 hours at this temperature the tanks were inoculated with *S. cerevisiae* RC212 to induce the alcoholic fermentation following the manufacturers recommended rehydration protocol and inoculation rate (30 g/hL).

During the alcoholic fermentation the fermentations were mixed twice daily by punching down the cap. °Brix and temperature were measured using a digital density meter (DMA 35N, Anton Paar). When the fermentations were dry as assessed by Clinitest™ (< 0.5 g/L sugar), the wines were pressed using a small Willmes bladder press. The same pressing times and pressures were used for all the treatments. After 48 hrs settling the wines were racked into 20 L carboys with airlocks, placed in a temperature controlled room at 20°C and MLF was induced by the direct addition of *O. oeni* VFO at approximately 1 x 10^6 cfu/mL. At the completion of the MLF (malic acid measured by enzymatic test kit (R-Biopharm)), 50 mg/L SO_2 was added to the wines and the wines were placed in a cold room at 8°C to promote settling. Wines were filtered through 2-3 μm filter pads before being passed through a 0.6 μm filter

cartridge and finally a sterile 0.45 μm membrane filter. Wines were then bottled in 750 mL glass bottles under a screw-cap closure. Samples were taken at this point and stored at -80°C until needed for analysis.

Microbial monitoring:

During the pre-fermentation maceration as well as the dry-ice treatment samples were aseptically taken daily and yeast viable cells were assessed by plating. Samples were plated onto WL media after appropriate dilutions (0.5% peptone) and incubated at 25°C for 2-3 days. WL media was used as the majority of yeast species typically found in wine fermentations can be distinguished on the basis of colony color and/or morphology during growth on this media (Pallmann et al., 2001). After incubation, plates were counted and colonies examined on WL media in order to identify unique colony types based on color, shape, consistency, and size.

Color and phenolic analysis:

Wines were analyzed for total color at 520 nm, color due to copigmentation, and color due to polymeric pigment as outlined by Boulton et al. (1999). Monomeric anthocyanin concentrations were determined by high-performance liquid chromatography (HPLC) based on Cortell et al. 2007. In brief, anthocyanins were analyzed using a Hewlett-Packard/Agilent Series 1100 (Palo Alto, CA) equipped with HP ChemStation software and photodiode-array detector (DAD). The column used was a LiChroSpher reverse-phase C_{18} (4 x 250mm, 5mm particle size) (Merck, Darmstadt, Germany) held at 30°C. Gradients of solvent A (water/formic acid,

90:10, v/v) and solvent B (methanol) were applied as follows: 5 to 35% B linear (1.0 mL/min) from 0 to 15 min, static at 35% B (1.0 mL/min) from 15 to 20 min, 35 to 80% B linear (1.0 mL/min) from 20 to 25 min, then 5% B (1.0 mL/min) from 25 to 32 min to re-equilibrate the column to initial conditions. Anthocyanins were detected by scanning from 190 to 700nm. Quantification was performed against an external standard at 520 nm and expressed as a function of malvidin-3-glucoside concentration. Wine samples were filtered (0.45 um) before 20 μL samples were injected into the HPLC. Tannins were measured using the Harberston-Adams assay (Adams and Harberston, 1999) and quantified as catechin equivalents.

Statistical Analysis

Statistical analysis was performed using the student T-test to determine significant differences between means at the $p<0.05$ level.

RESULTS

During the pre-fermentation maceration period total viable yeast counts were taken as well as temperature readings. The results for the cold soak treatment can be seen in Figure 1. Grapes underwent either a pre-fermentation maceration in a cold room or were treated with dry ice. For temperature, there was an initial rise from 8 to 14°C after one day, and then a decrease to between 8 to 10°C for the remainder of the pre-fermentation maceration until the tanks were warmed at day seven. Yeast viable cells were initially at approximately 1.6 $x10^4$ CFU/mL and slowly increased over the pre-fermentation maceration period reaching a maximum of $4x10^5$ CFU/mL at day six. After warming at day seven the total yeast population reached $5x10^5$ CFU/mL.

For the dry ice treatment the temperature was initially much colder than the cold soak treatment (4°C) due to the addition of dry ice. However, after one day the temperature rapidly rose to 12°C and the tanks were subsequently moved into a cold room at 8°C to prevent alcoholic fermentation (Figure 2). For the remainder of the pre-fermentation maceration the temperature remained between 8-10°C. The initial total yeast viable cell counts was approximately 6.5 $x10^4$ CFU/mL but decreased after three days to 4 $x10^4$ CFU/mL and increased from there to a final count of 5 $x10^4$ CFU/mL at day six (Figure 2).

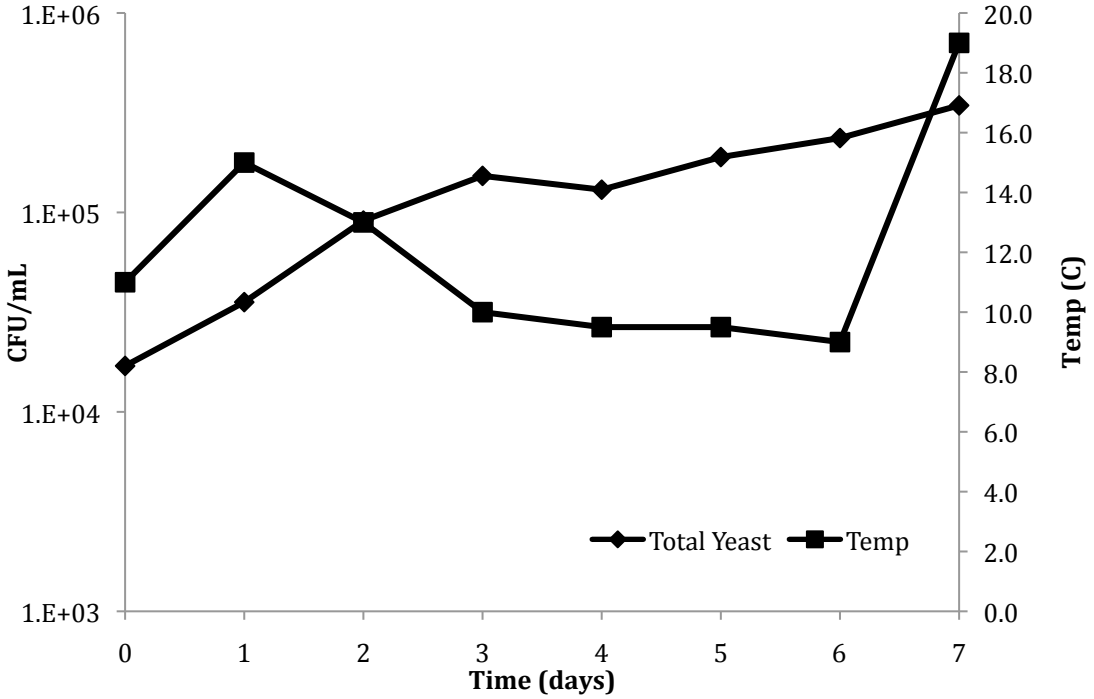

Figure 1. Total yeast viable cells and temperature during pre-fermentation maceration of Pinot noir grapes in the cold soak treatment.

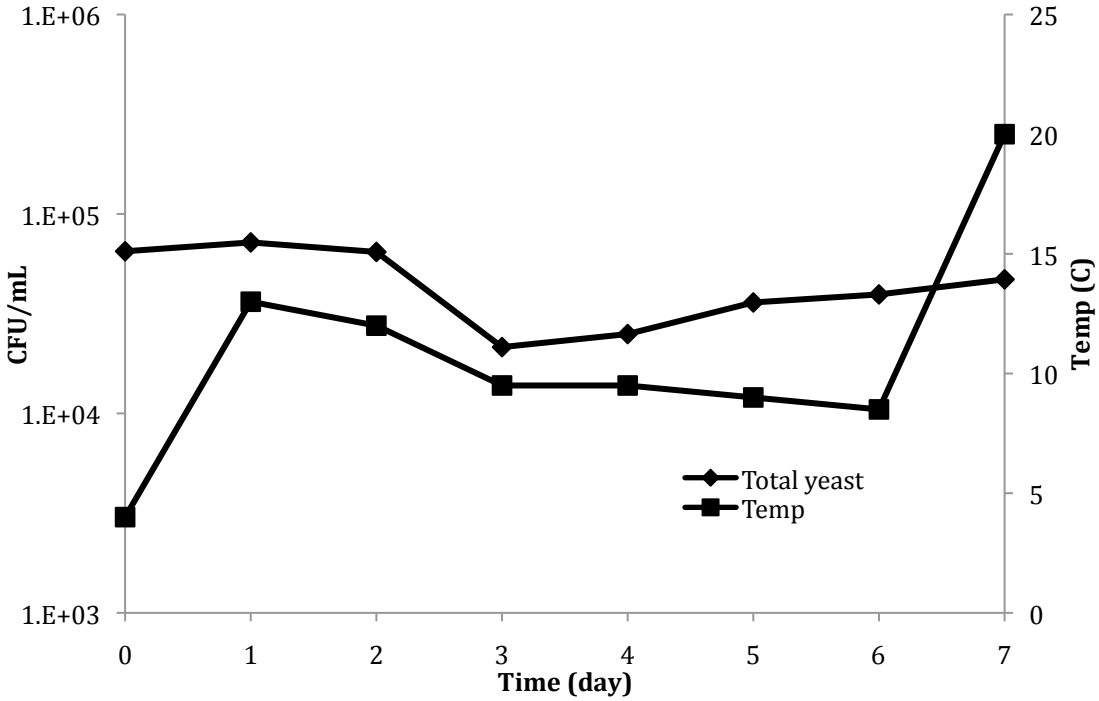

Figure 2. Total yeast viable cells and temperature during pre-fermentation maceration of Pinot noir grapes treated with dry ice.

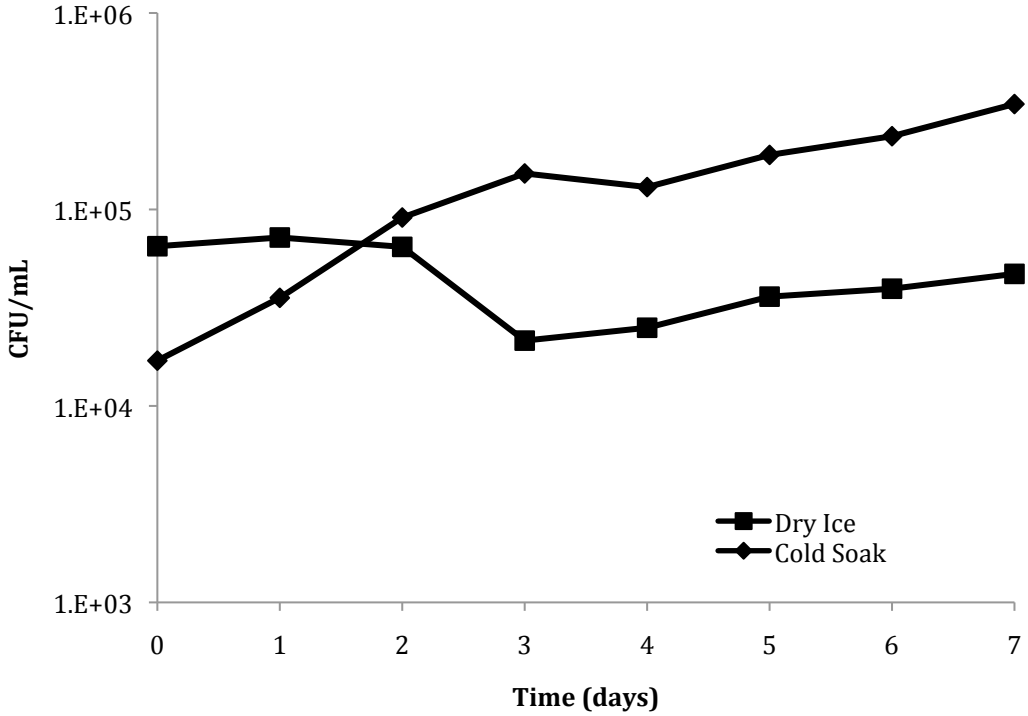

Figure 3. Total yeast viable cells during pre-fermentation maceration of Pinot noir grapes in both the dry ice and cold soak treatments.

When comparing the total yeast populations present during the cold soak versus the dry ice treatment (Figure 3) the yeast population was initially higher in the dry ice treatment (6.5×10^4 CFU/mL compared to 1.6×10^4 CFU/mL) but it decreased below the cold soak yeast population after three days. For the remainder of the pre-fermentation maceration the total yeast population in the dry ice treatment remained lower than in the cold soak treatment with a difference of about one log being observed.

Two dominant yeast species were present during the pre-fermentation maceration. The yeast that was present in the highest numbers was *Kloeckera apiculata* and its

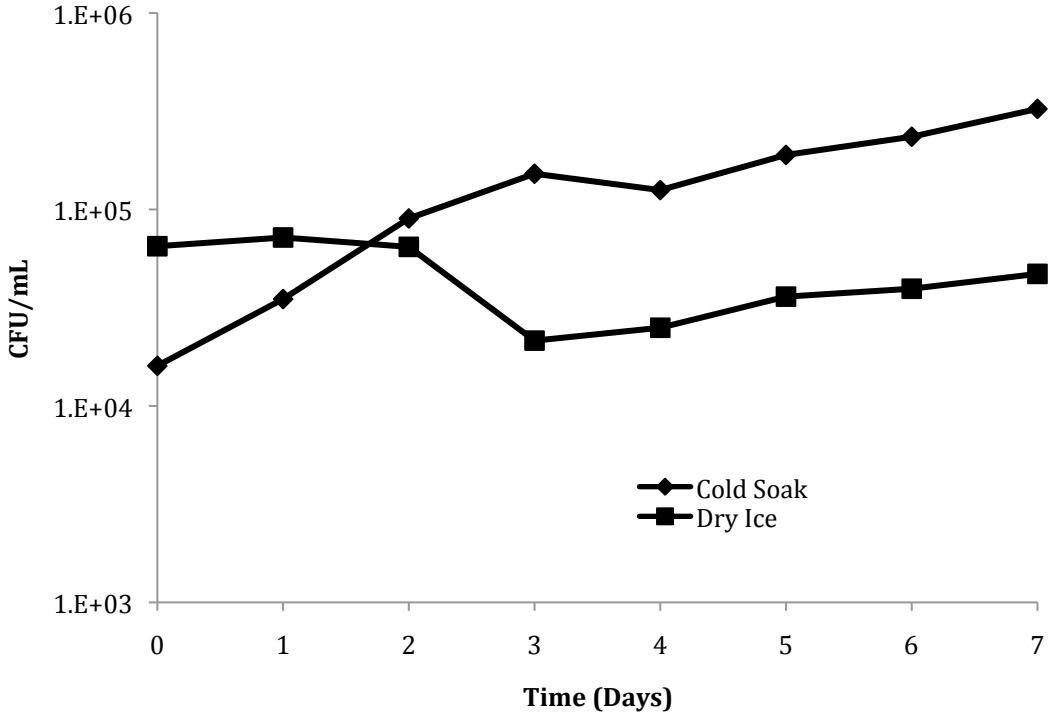

Figure 4. Total yeast viable cells of *Kloeckera apiculata* during pre-fermentation maceration of Pinot noir grapes under two different maceration conditions.

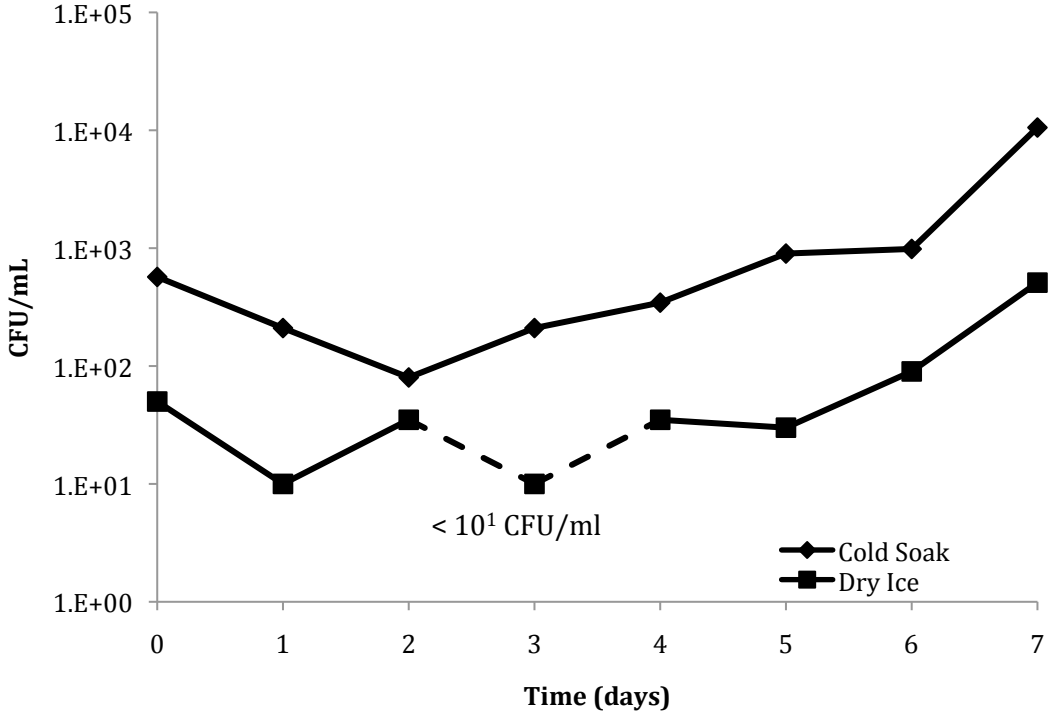

Figure 5. Total yeast viable cells of *Pichia membranaefaciens* during pre-fermentation maceration of Pinot noir grapes under two different maceration conditions.

growth is shown in Figure 4. Yeast viable cells started around 6 $x10^4$ CFU/mL for the dry ice treatment and 1.5 $x10^4$ CFU/mL for the cold soak treatment. The yeast population slowly increased over the course of the pre-fermentation maceration in the cold soak treatment reaching a maximum of 3 $x10^5$ CFU/mL. The dry ice treatment had a decrease in *K. apiculata* population at day 3 and then increased to 4 $x10^4$ CFU/mL at the end of pre-fermentation maceration. The other major yeast species present was *Pichia membranaefaciens* (Figure 5). This yeast was initially present at approximately 9 $x10^1$ CFU/mL for the dry ice treatment and 9 $x10^2$ CFU/mL for the cold soak treatment. Populations slowly increased over the course of the pre-fermentation maceration reaching maximums of 5 $x10^2$ CFU/mL and 2 $x10^4$ CFU/mL for dry ice and cold soak respectively. The dry ice treatment had a drop in *Pichia membranaefaciens* population at day 3 with $<10^1$ CFU/mL being present.

The results for the alcoholic fermentation of all treatments can be seen in Figure 6. All treatments underwent an alcoholic fermentation induced by *S. cerevisiae* RC212. After inoculation fermentations proceeded normally with a rapid drop in Brix 24-48 hrs after yeast inoculation. Although all fermentations were completed by 144 hrs, the dry ice and control treatments fermented slightly slower than the cold room treatment and completed the fermentation about 24 hrs later.

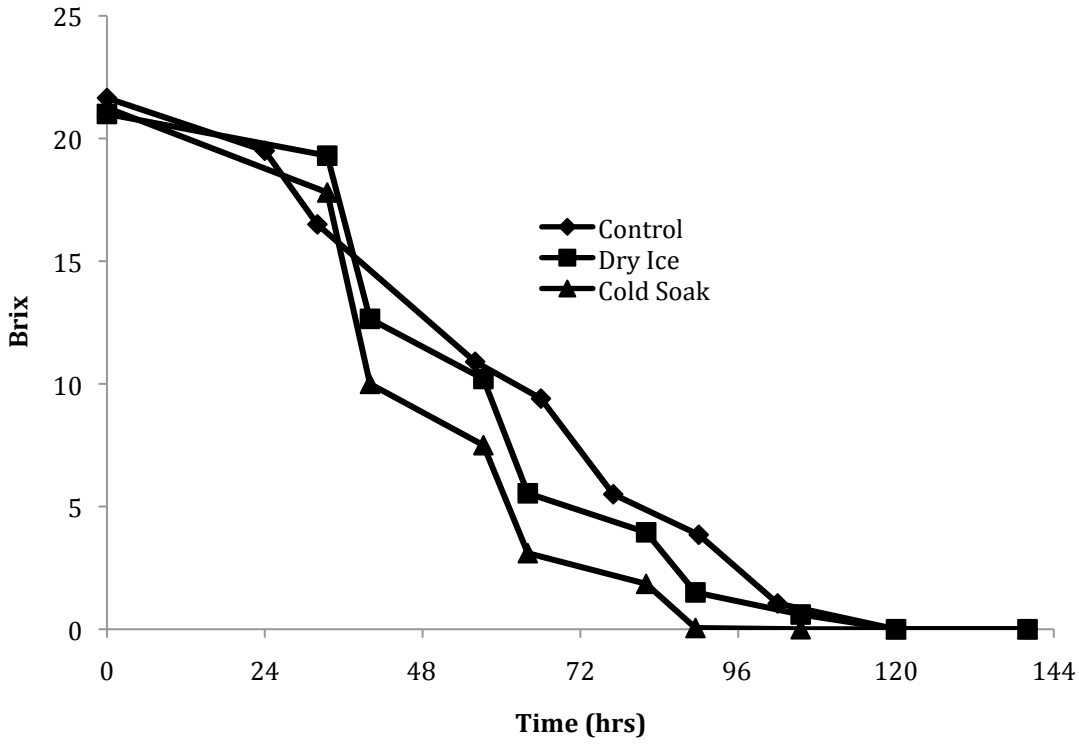

Figure 6. Brix during fermentation of Pinot noir grapes that had (dry ice and cold soak) or had not undergone a pre-fermentation maceration.

After malolactic fermentation, SO_2 addition, filtering and bottling, the wines were analyzed for a number of color parameters. For A520, hue, co-pigmentation, anthocyanins, and polymeric pigments there was no significant differences between wines made from grapes that had not undergone a pre-fermentation maceration (control) and wines made with a cold soak or dry ice treatment (Table 1). For total monomeric anthocyanin as measured by HPLC the means for cold soak and dry ice treatments were higher than the control but these differences were not significantly different at the $p<0.05$ level (Table 2). However, wines made with a pre-fermentation maceration (cold soak or dry ice) contained significantly higher tannin than the control (Table 2).

Table 1. Color at A520, hue, co-pigmentation, anthocyanins, and polymeric pigment of Pinot noir wines produced from grapes that had (Dry Ice, Cold Soak) or had not (Control) undergone a pre-fermentation maceration. Values are means of duplicate fermentations.

	Color A520	Hue	Co-pigmentation	Anthocyanins	Polymeric Pigments
Control	2.04±0.01[a]	0.87±0.01[a]	1.64±0.05[a]	0.96±0.03[a]	0.55±0.02[a]
Dry Ice	2.21±0.02[a]	0.84±0.02[a]	1.62±0.11[a]	1.15±0.09[a]	0.58±0.03[a]
Cold Soak	2.20±0.02[a]	0.84±0.01[a]	1.57±0.09[a]	1.19±0.12[a]	0.58±0.02[a]

[a-b] Values with different letters in same column are significantly different at the $p<0.05$ level.

Table 2. Concentrations of monomeric anthocyanins and tannins in Pinot noir wines produced from grapes that had (Dry Ice, Cold Soak) or had not (Control) undergone a pre-fermentation maceration. Values are means of duplicate fermentations.

	Monomeric Anthocyanins (mg/L M-3-G eq.)	Tannin (mg/L catechin eq.)
Control	131.74 ±3.08[a]	204.68 ±12.86[a]
Dry Ice	148.01 ±12.08[a]	246.82 ±30.18[b]
Cold Soak	147.58 ±16.29[a]	230.27 ±16.79[b]

[a-b] Values with different letters in same column are significantly different at the $p<0.05$ level.

DISCUSSION

Pre-fermentation macerations are often undertaken to improve the color or flavor and aroma of a red wine. In this study the impact of two types of pre-fermentation macerations on yeast populations and final red wine color was investigated. For both pre-fermentation maceration techniques the total yeast populations increased during the six to seven days of maceration despite the cold temperatures. Pre-fermentation maceration is usually conducted at cold temperatures (typically between 8-13°C) so as to prevent yeast growth and alcoholic fermentation. However, many non-*Saccharomyces* species that can be present on the grapes or winery equipment are more cold tolerant than *S. cerevisiae* and can survive and grow at typical pre-fermentation temperatures (Egli et al., 1998). This was observed in the present study where only non-*Saccharomyces* species were detected during pre-fermentation maceration. In particular, *K. apiculata* was identified as the major yeast population in both the dry ice treatments and cold soak treatments. In a study done in an apple juice fermentation *K. apiculata* was shown to have enhanced growth and survival at the lower temperature of 10°C than the higher temperature of 25°C (Bilboa et al., 1997).

Yeast populations differed between the two pre-fermentation macerations. Although yeast populations were initially higher in the dry ice treatment, these populations decreased after three days and remained approximately one log lower than the yeast population in the cold soak treatment. One proposed reason for this drop in the dry ice yeast population was the change in tank temperature from day one to three. After the initial addition of the dry ice the temperature was quite cold at 4°C. However, due to

the small size of the tank and the small mass of grapes the temperature did not remain low for very long. In fact, after three days the temperature was already above 12°C. At this point the tanks were placed in the cold room and the temperature of the grapes decreased to between 8-10°C. This drop in temperature corresponds with the decrease in yeast populations in the dry ice treatment. However, there was also a relatively similar decrease in the temperature of the cold soak treated grapes where temperatures dropped from a high of 15°C after one day to below 10°C after three days. In this case there was no subsequent decrease in yeast populations. Instead, the yeast population continued to increase. These results suggest that the temperature drop at day three of the dry ice treatment is likely not responsible for the decrease in the yeast population. Alternatively, the addition of dry ice to the grape must may have killed some of the yeast through direct contact with the extreme cold causing freezing of the cells. However, if this theory were true we would have expected to see the drop in total yeast population at day zero or one not later at day three.

Another possible explanation for the lower yeast populations in the dry ice treatment is the change in oxygen and carbon dioxide concentrations in the grape must due to the addition of dry ice. The addition of the dry ice would have turned the aerobic environment of the grape must into an anaerobic environment as the sublimination of the dry ice created an environment high in CO_2, dropping the O_2 concentration in the grape must. Each yeast species is different as to their survivability in aerobic or anaerobic environments. *Saccharomyces* yeast has been shown to survive in both aerobic and anaerobic environments while *K. apiculata*, the major yeast species in the

pre-fermentation maceration, prefers an aerobic environment for growth (Jolly et al., 2006). This preference of *K. apiculata* could explain the drop in yeast population in the dry ice treatment. The lack of oxygen in the grape must may have resulted in an initial lack of growth and then a decrease in viability by day three of the maceration. The slow increase in total yeast population after day three could be explained by introduction of oxygen to the system during daily mixing. However, because the CO_2 and O_2 concentrations were not measured during the pre-fermentation maceration we do not know whether changes in these compounds directly impacted the yeast populations. Future research should investigate the changes in the CO_2 and O_2 concentrations during a pre-fermentation maceration and whether or not the changes caused by dry ice additions impact yeast growth.

K. apiculata was identified as the major yeast population in both the dry ice treatments and cold soak treatments. Throughout the pre-fermentation maceration *K. apiculata* populations closely matched the total populations in both treatments. *Kloeckera apiculata* has also been identified as a spoilage organism in some fermentations and high populations may be unwanted. High numbers of *K. apiculata* have potential to lead to high acetic acid levels in the wine (Romano et al., 2003). The second major yeast species that was seen throughout the pre-fermentation maceration was *Pichia membranaefaciens.* The cold soak treatment had consistently higher populations of *Pichia membranaefaciens* than the dry ice treatment. *Pichia membranaefaciens* is also considered a spoilage organism as it causes film formation

on wine surfaces exposed to air. This exposure can lead to off flavors arising from increased acetic acid and ester production (Mora, 1992).

Aside from changes in flavor and aroma, pre-fermentation macerations are conducted to improve red wine color. This is probably the most commonly stated reason why a winemaker might choose to perform a pre-fermentation maceration. However, in this study there were no color differences between the wines that had or had not undergone a pre-fermentation maceration. A study by Heatherball et al. (1996) also showed no long-term color differences between the control and the cold soak treatment in Pinot noir. Furthermore, Watson et al. (1995) saw that Pinot noir undergoing a pre-fermentation maceration had lower anthocyanins and color intensity than the control. The lack of color differences between the wines may have been due to the relatively young age of the wines. Color analysis was performed at the completion of malolactic fermentation (approximately 30 days post-pressing) and it would be interesting to see if there were any color differences when the wines are maybe 1 year old. Many previous studies that investigated pre-fermentation maceration did not measure polymeric pigment content. These compounds are important for stable, long-term color compound in red wines. The young Pinot noir wines had relatively low polymeric pigment content and correspondingly high monomeric anthocyanin content. But again, there were no significant differences for these parameters when comparing the wines.

Although there were no differences in color between the wines, there was a significant difference in tannin concentration between wines made with grapes that underwent a pre-fermentation maceration compared to the control. Because tannins are located in the skins and seeds of a grape there may have been an increase in the amount of tannin extracted with the extended skin and seed contact that the two pre-fermentation maceration treatments received (Jackson, 2000). Another possible explanation for the increased tannin extraction in the dry ice treatment is the technique of must freezing. Must freezing freezes the grapes, rupturing the cell walls and membranes, potentially releasing more tannins in to the wine. It has been shown that wines with frozen musts had 52% more tannin than the control wines (Sacchi et al., 2005).

Conclusions:

The yeast populations in both the cold soak and dry ice treatments increased during the pre-fermentation maceration. There was a drop in yeast population after three days in the dry ice treatment which then remained below the yeast population of the cold soak treatment for the duration of the pre-fermentation maceration. In both treatments *K. apiculata* was the dominant yeast species. As *K. apiculata* and *P. membranaefaciens* have both been identified as spoilage microorganisms high populations may be unwanted. The dry ice treatment shows lower populations of both yeast species possibly making it an ideal pre-fermentation maceration technique for a winemaker concerned about high acetic acid and ester levels. Neither of the pre-fermentation maceration treatments impacted the polymeric pigment content of the

wine when compared to the control treatment which indicates that possibly there will be no differences in color after aging. A winemaker concentrating on improving the color of the wine may want to employ another technique in order to improve color. But more research needs to be done on the impact of pre-fermentation macerations on long-term color. Both the wines made from grapes that underwent the pre-fermentation maceration had significantly higher tannin concentration that the control wine.

Further research that could be done in this area would include how dry ice addition changes the environment of the grape must, (CO_2 and O_2 concentrations) and the impact of direct dry ice contact to the yeast cell wall. It would be interesting to see the effects of the different pre-fermentation maceration techniques on wine color after aging. Little research has been done on the long-term effects of pre-fermentation maceration on color and polymeric pigment formation. In addition, analysis on flavor and aroma compounds in the wines that underwent the pre-fermentation maceration compared to the control. In regards to the specific yeast species, interesting research would be to determine the exact flavors and aromas that each species may impart to the wine.

BIBLIOGRAPHY

Álvarez, I., Aleixandre, J.L., García, M.J., Lizama, V. 2006. Impact of prefermentative maceration on the phenolic and volatile compounds in Monastrell red wines. Analytica Chimica Acta. 563:109–115.

Adams, D.O. and Harbertson, J.F. 1999. Use of alkaline phosphatase for the analysis of tannins in grapes and red wines. Am. J. Enol. Vitic. 50:247-252.

Bilbao, A., Irastorza, A., Dueñas, M., Fernandez, K. 1997. The effect of temperature on the growth of strains of *Kloeckera apiculata* and *Saccharomyces cerevisiae* in apple juice fermentation. L. Appl. Microbiol. 24:37–39.

Boulton, R.B., Neri, R., Levengood, J., Vaadia, M. 1999. Copigmentation of anthocyanins in Cabernet Sauvignon and Merlot wines from the Napa valley of California. In: Lonvaud-Funel, A. (ed). Proc. 6th Symposium International d' Oenologie, Tec. & Doc. Publ., France.

Boulton, R. 2001. The copigmentation of anthocyanins and its role in the color of red wine: A critical review. Am. J. Enol. Vitic. 52:67-87.

Brouillard, Mazza, G., Saad, Z., Albrecht-Gary, A.M., Cheminat, A. 1989. The copigmentation reaction of anthocyanins: A microprobe for the structural study of aqueous solutions. J. Am. Chem. Soc. 111:2604-2610.

Charoenchai, C., Fleet, G.H., Henschke, P.A., Todd, B.C. 1997. Screening of Non-Saccharoses Wine Yeast for the Presence of Extra cellular Hydrolytic Enzymes. Aust. J. Grape Wine Res. 3: 2-8.

Cortell, J.M., Halbleib, M., Gallagher, A.V., Righetti, T.L., Kennedy, J.A. 2007. Influence of vine vigor on grape (*Vitis vinifera* L. Cv. Pinot noir) anthocyanins 2. Anthocyanins and pigmented polymers in wine. J. Agric. Food Chem. 55:6585-6595.

Dicey, M. 1995. The Effect of Cold Maceration With and Without Sulphur Dioxide on Pinot Noir Wine. M. Appl. Sci. Thesis, Lincoln University, New Zealand.

Egli, C.M., Edinger, W.D., Mitrakul, C.M., Henick-Kling, T. 1998. Dynamics of indigenous and inoculated yeast populations and their effect on the sensory character of Riesling and Chardonnay wines. J. Appl. Microbiol. 85:779-789.

Fleet, G. H., Lafon-Lafourcade, S., Ribereau-Gayon, P. 1984. Evolution of yeasts and lactic acid bacteria during fermentation and storage of Bordeaux wines. Appl. Environ. Microbiol. 48:1034-1038.

Fulcrand, H., Benabdeljalil, C., Rigaud, J., Cheynier, V., Moutounet, M. 1998 A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. Phytochem. 47:1401-1407.

Gardner, D.M., Zoecklein, B.W., Mallikarjunan, K. 2011. Electronic Nose Analysis of Cabernet Sauvignon (Vitis vinifera L.) Grape and Wine Volatile Differences during Cold Soak and Post Fermentation. Am. J. Enol. Vitic. 62: 81-90.

Heatherbell, D., Dicey, M., Goldsworthy, S., Vanhanen, L. 1997. Effect of prefermentation cold maceration on the composition, color and flavor of Pinot noir wine. In: Henick-Kling, T., Wolf, T. E. & Harkness, E. M. (eds). Proc. 4th Int. Symp. Cool Climate Vitic. & Enol., 16-20 July 1996, Rochester, USA. pp. VI 10-1.

Jackson and P. B. Lombard. 1993. Environmental and management practices affecting grape composition and wine quality: A Review. Am. J. Enol. Vitic. 44:409-430.

Jackson, R.S. *Wine Science, Principles, Practice, Perception.* 2nd ed. Orlando, Florida: Academic Press, 2000.

Jolly N.P., Augustyn, O.P.H., Pretorius, I.S. 2006. The role and use of non-*Saccharomyces* yeasts in wine production. S. Afr. J. Enol. Vitic. 27:15-39.

Kennedy, J.A., Peyrot des Gachons, C. 2003. Phenolic extraction during red wine production. Practical Winery and Vineyard. July/August:38-46.

Mora, J., Rossello, C. 1992. The growth and survival of *Pichia membranaefaciens* during fermentation of grape juice. Am. J. Enol. Vitic. 43:329-332.

Romano, P., Fiore, C., Paraggio, M., Caruso, M., Capece, A. 2003. Function of yeast species and strains in wine flavor. Inter. J. Food Microbiol. 86:169-180.

Pallmann, C.L., Brown, J.A., Olineka, T.L., Cocolin, L., Mills, D.A., Bisson, L.F. 2001. Use of WL medium to profile native flora fermentations. Am. J. Enol. Vitic. 52:198-203.

Sacchi et al. 2005. A Review of the Effect of Winemaking Techniques on Phenolic Extraction in Red Wines. Am. J. Enol. Vitic. 56: 197-206.

Timberlake, C.F., and Bridle, P. 1976. Interactions between anthocyanins, phenolic compounds, and acetaldehyde and their significance in red wines. Am. J. Enol. Vitic. 27:97-105.

Watson, B.T., Price, S.F., and Valladao, M. 1995 Effect of fermentaion practices on anthocyanin and phenolic composition of Pinot noir wines. Abstr. Am. J. Enol. Vitic. 46:404.

Zoecklein, B.W., Marcy, J.E., Williams, J.M., Jasinski, Y. 1997. Effect of native yeast and selected strains of *Saccharomyces cerevisiae* on glycosyl glucose, potential volatile terpenes, and selected aglycones of white Riesling (*Vitis vinifera* L.) wines. J. Food Comp. Anal. 10:55-65.

Zoecklein, B.W., Fugelsang, K.C., Gump, B.H., Nury, F.S. *Wine Analysis and Production*, New York, Chapman and Hall. 1995.