Project Title: Identification of Factors that Influence the Level of Large and Small Polymeric Pigments in Grapes and Wines.

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General Summary of Current Year's Results

During the 2001 season we set out to identify the most important factors that influence the level of large and small polymeric pigments in wines. Polymeric pigments are important because they are the stable color compounds that form during fermentation and wine aging. We found that Pinot noir wines made after cold soak had more polymeric pigment at the time of pressing but that they had less after a period of barrel aging. All wines in our experiments exhibited dramatic compositional changes from pressing until the first racking, showing a decline in tannin and a large increase in both large and small polymeric pigments. Experiments with maximum fermentation temperatures showed that temperature probably plays the biggest role in tannin extraction and polymeric pigment formation. Three temperatures were used, 25C, 30C and 33C. Tannin, large polymeric pigment and small polymeric pigment were all greater at 33C than 25C with 30C intermediate. In all cases there were declines in tannin levels during barrel aging but a concomitant increase in the amount of polymeric pigments that formed. It is clear that important compositional changes occur during barrel aging with regard to tannins and polymeric pigments. It will be important to follow all of the wines made this season through to the level of finished wine in order to adequately evaluate all of the treatment effects imposed on the experimental wines.

Preliminary work indicated that polymeric pigments bleach to some extent with SO2. It was important to determine the extent to which they bleach so that correction factors could be derived for calculating the actual amount of polymeric pigments in wines. This is particularly important in aged wines (3 years or greater) where nearly all of the pigments can be present in the polymeric form. Using aged Pinot noir and Cabernet Sauvignon wines (8 to 28 years old) that contained no monomeric pigments we performed experiments using column purified polymeric pigments to show that they were indeed bleached extensively by SO2. Using combined protein precipitation and SO2 bleaching we were able to derive correction factors that we can now use to more accurately calculate the amount of polymeric pigments present in grape extracts and wines.

Project Report

Viticulture Consortium
April 1, 2001 – March 31, 2002

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Objectives and Experiments Conducted to Meet Stated Objectives:

The objectives for the 2001 season were

- Determine if pre-fermentation treatments such as cold soak influence the amount of LPP and SPP in berry skins prior to fermentation.
- Study the effect of fermentation variables on extraction of SPP and formation of LPP during winemaking, with an emphasis on temperature.
- Determine the extent to which polymeric pigments bleach with SO_2, so that more accurate estimates of LPP and SPP can be made in grape extracts and wines.

Determine if pre-fermentation treatments such as cold soak influence the amount of LPP and SPP in berry skins prior to fermentation.

This experiment was done in conjunction with a study on extended maceration. All of the experiments were conducted on commercial Pinot noir fermentations so that results are meaningful for real scale fermentations. All of the fruit used in these experiments came from the same vineyard.
In this experiment, three 7-ton fermentations were compared:

1. Control: pressed at 2° Brix, no cold soak
2. Cold Soak: uninoculated under CO_2 for 7 days, pressed at 0.6° Brix
3. Extended Maceration: pressed 16 days after the last pumpover .

Samples for tannin and polymeric pigment analyses were collected at pressing and for the control and the cold soak treatment samples were also collected from barrels after the first racking. Data from the first racking for the extended maceration treatment are currently being run but were not yet available at the time of this report.

Figure 1 Tannin levels in cold soak and extended maceration study.

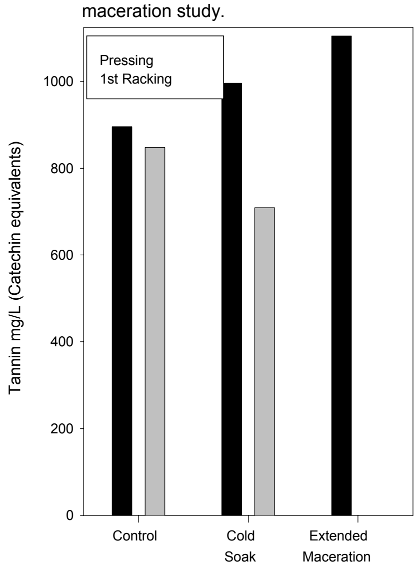


Figure 1 shows the tannin levels in each of the treatments at pressing and at the first racking for the control and the cold soak treatment. At pressing both the cold soak treatment and the extended maceration contained more tannin than the control. However, by the time of the first racking the tannin in the cold soak treatment had declined to below that of the control. The decline in tannin during barrel aging was a general phenomena we observed during this experiment (see below) and it will be important to continue to follow these experiments in order to see what the treatment effects are at the time of bottling.

Figure 2 shows the level of large polymeric pigments (LPP) in each of the treatments along with the control tank. At pressing both the cold soak and extended maceration had greater amounts of LPP than the control but changes during barrel aging seem to show a different effect. In the control there was a 3.2 fold increase in the level of LPP in the wine from pressing until the first racking. In the cold soak treatment there was also an increase in the amount of LPP in the wine, but it was much smaller, so that by the time of the first racking the LPP in the control was higher than in the cold soak treatment. The increase in LPP is consistent with what we observed in all of our other experiments, but thus far the values for LPP are lower in the treatments than in the control tank. We have proposed to follow these samples until bottling in order to evaluate the effects of the treatments on the LPP in the finished wine.

Figure 2. LPP in cold soak and extended maceration study

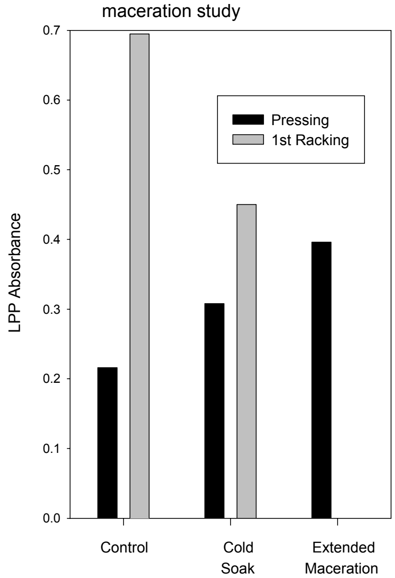


Figure 3 shows the level of small polymeric pigments (SPP) in the cold soak and the extended maceration experiment along with the control tank. The results with SPP are very similar to the pattern seen with LPP in this experiment. At pressing both the cold soak and the extended maceration treatments showed greater amounts of SPP than the control. However, once again, changes during barrel aging were greater in the control than in the cold soak, so by the time of the first racking the control had

Figure 3. SPP in cold soak and extended maceration study

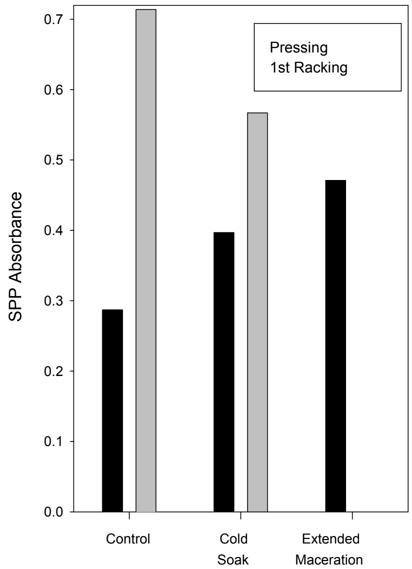
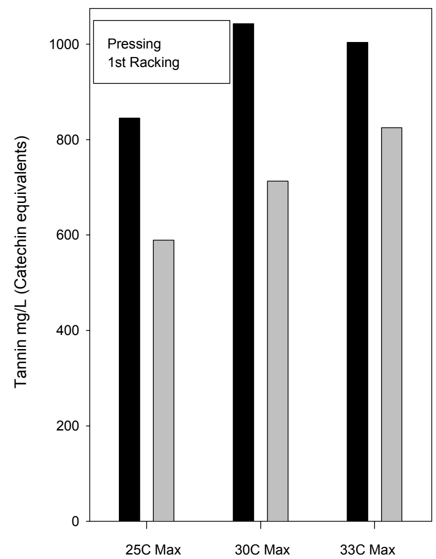


Figure 4. Tannin in fermentations with different maximum temperatures



more SPP than the wine from the cold soak treatment. It will be important to see if the differences observed at the first racking persist to the level of finished wine.

Study the effect of fermentation variables on extraction of SPP and formation of LPP during winemaking, with an emphasis on temperature.

In this experiment, grapes were crushed and distributed evenly among 3 tanks of 5 tons each. The hose from the crusher was moved from one tank to the next in one-ton increments to assure similarity in must composition. Peak fermentation temperature was then controlled using a computerized glycol system. The highest-temperature fermentation proceeded without cooling and reached a peak of 33°C. Thus the three maximum temperatures used were 25 C, 30 C and 33C.

Figure 4 shows the tannin levels in each of the wines at pressing and at the time of the first racking. At pressing the 25 C fermentation showed less tannin than either of the other two temperatures. By the time of the first racking the tannin levels in all three treatments had declined. The decline in tannin during barrel aging was greater for the 25 C treatment and in the samples from the first racking the 33 C maximum had 40% more tannin than the 25 C. It will be important to see how the tannin levels in these wines differ by the time the wines are finished and ready for bottling.

Figure 5 shows the LPP levels in each of the wines at pressing and at the time of the first racking. At the time of pressing the amount of LPP in the wines reflected the maximum

Figure 5. LPP in fermentations with different maximum temperatures

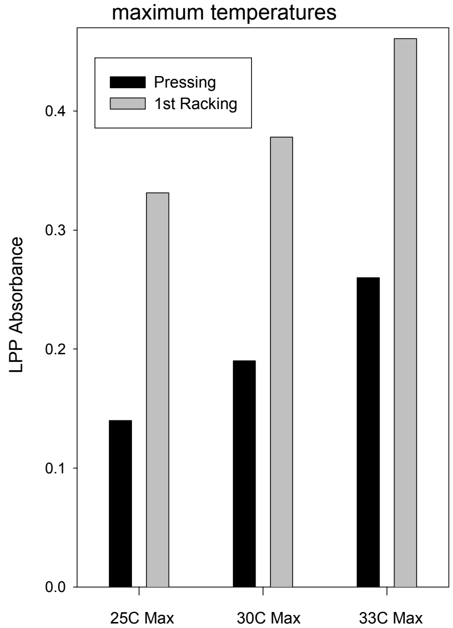
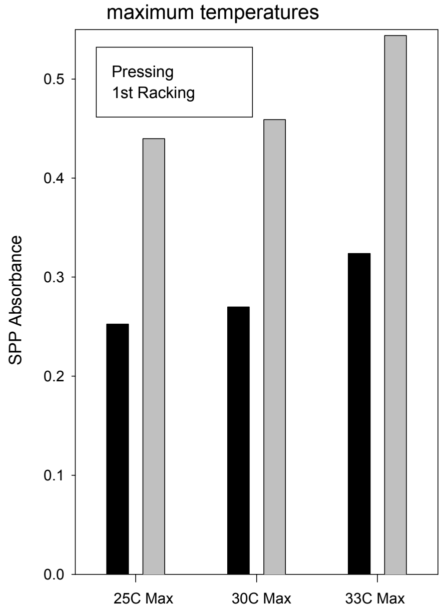


Figure 6. SPP in fermentations with different maximum temperatures



fermentation temperature with 25 having the least amount and 33 having the highest. In each case there was a dramatic increase in the amount of LPP in the wines from the time of pressing to the first racking. Indeed, the amount of LPP that formed during barrel aging was nearly identical in each of the three wines. Thus, the differences in LPP observed at the time of first racking were already present in the wines at pressing. Note that we see LPP formation occurring while the total tannin in these wines is declining. This is particularly interesting because LPP is formed by reaction of monomeric anthocyanins with tannins.

Figure 6 shows the SPP levels in the wines from each of the three maximum temperature treatments at pressing and at racking. SPP levels at pressing showed less difference than LPP levels and only the highest temperature appears to have a higher level of SPP at pressing. In all three wines there was an increase in SPP during barrel aging. As was the case for LPP, the amount of SPP that formed was nearly the same for each of the wines.

The effect of temperature on tannin extraction is perhaps what could have been expected. The higher the maximum temperature during fermentation the greater the amount of tannin extracted. The tannin values obtained at pressing were higher than those found in the wine at the time of first racking. The exact opposite effect is seen with both LPP and SPP. During barrel aging the LPP increased in each of the wines to nearly the same extent. Likewise, there was an increase in SPP in each of the wines. The results show that wines allowed a higher fermentation temperature maximum form greater amounts of LPP during fermentation and the differences persist at least until the first racking. SPP shows the same pattern as LPP but the magnitude is somewhat reduced.

The temperature effects documented in this experiment are interesting, but we are surprised by the changes observed during barrel aging. In our proposal for the 2002 season we propose to continue to follow these wines through barrel aging to the level of finished wine. It will be very important to measure tannin and polymeric pigments in each of the finished wines in order to properly evaluate the treatment effects in all of the experiments

Determine the extent to which polymeric pigments bleach with SO_2, so that more accurate estimates of LPP and SPP can be made in grape extracts and wines.

Several wines were selected for analysis. A set of Cabernet Sauvignon and Pinot noir wines from the cellar at U.C. Davis were obtained and the absence of monomeric anthocyanins was verified by HPLC. Thus, in these wines the pigments were all polymeric pigments and the absence of monomeric anthocyanins was verified. The Cabernet Sauvignon wines were from 1973, 1974, 1976 and 1980 and the Pinot noir wines were 1985, 1990, 1991, and 1992

A Toyopearl HW-40(F) column (30 mm x 350 mm) was employed to separate the monomeric phenols from the tannins. For each of the eight wines the ethanol was first removed from a 5 ml sample using a rotary evaporator. The Toyopearl HW-40(F) column was equilibrated with de-ionized water for 20 minutes at 2 ml/min and then the dealcoholized wine was loaded onto the column. Water was used to elute any residual sugars (50 ml). Two 100 mL fractions were then collected. The first fraction was collected using ethanol/water/trifluoroacetic acid (55:45:0.005) and the second fraction with acetone/water (3:2). The solvents were removed using a rotary evaporator and the water was removed by freeze-drying. This process was repeated for all eight wines.

The freeze-dried column chromatographed wine afforded two fractions. One from the ethanol/water/TFA elution (ethanol fraction) and one from the acetone/water elution (acetone fraction). Each fraction was taken up in 2 mL of model wine solution and the samples were centrifuged at 11,000 g for 3 minutes to remove any insoluble material prior to analysis.

For each fraction in model wine solution, two 500 μL aliquots were put into separate analysis tubes. One of the 500 μL aliquots was diluted in 1 ml of acetate buffer and the other aliquot diluted in 1 ml of a model wine. After a 10 minute incubation the total absorbance was recorded at 520nm. Then 80 μL of 250 g/L sodium bisulfite solution was added and after a 5 minute incubation period the absorbance was recorded at 520nm. From these readings the degree of bleaching for each fraction was calculated.

Figure 7 shows the percentage of the polymeric pigments bleached in the ethanol fraction (mostly SPP) and the acetone fraction (mostly LPP) for each of the four Cabernet Sauvignon wines examined. Figure 8 shows the percentage of the polymeric pigments bleached in the ethanol fraction and the acetone fraction for each of the Pinot noir wines studied. The results clearly confirmed our preliminary studies that indicated that polymeric pigments bleached in the presence of bisulfite. It also shows that the polymeric pigments from these two different varieties bleach similarly.

The results from Figures 7 and 8 confirm bleaching of polymeric pigments by bisulfite, but our objective was to obtain bleaching values that could be used to correct values that we obtain using our combined protein precipitation/ SO_2 bleaching assay. In order to derive percentages that would be useful for that purpose we performed our standard tannin/SO_2

Figure 7. Bleaching of Polymeric Pigments in Cabernet Sauvignon Wine Fractions

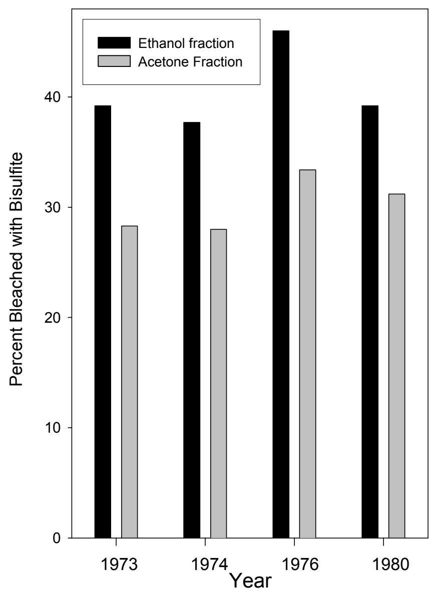
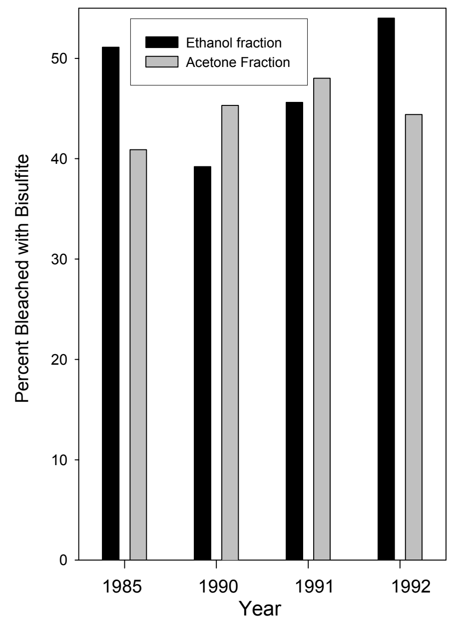


Figure 8. Bleaching of Polymeric Pigments in Pinot noir Fractions



bleaching assay to give polymeric pigment values for each of the eight wines listed above. Since we had demonstrated that these wines contained no monomeric pigments, the values from the assay could be used to calculate the degree of polymeric pigment bleaching in our standard assay.

The assay was conducted with a 200 µL aliquot of wine diluted appropriately with a model wine solution. A 0.5 mL sample of the diluted wine was added to 1 mL of acetate buffer pH 4.9 containing 1 mg/L BSA protein. The solution was allowed to incubate for 10 minutes. The protein tannin precipitate was collected by centrifugation at 11,000 g for 3 minutes and then 1 mL of the supernatant was transferred to a 2 mL cuvette and the remainder of the supernatant was discarded. The pellet was washed with fresh acetate buffer (250 µL) and then dissolved in 100 µL of SDS/TEA buffer and allowed to incubate at room temperature for 10 minutes. After the pellet was dissolved 1400 µL of acetate buffer was added to the dissolved pellet solution. The absorbance at 520 nm was recorded, then 80 µL of 5% bisulfite solution was added and after a 5 minute incubation a second reading at 520 nm was taken. An absorbance reading was obtained for the supernatant and then 80 µL of 5% bisulfite solution was added and a second reading recorded after a 5 minute incubation. Since these wines contained no monomeric anthocyanins the bleaching observed on addition of SO_2 must have been due to bleaching of SPP and LPP. Comparing the reading of the pellet and the supernatant before and after bleaching with bisulfite allowed us to calculate the percentage that LPP and SPP bleached in our assay.

Figure 9. Bleaching of SPP and LPP in aged Cabernet Sauvignon wines by bisulfite.

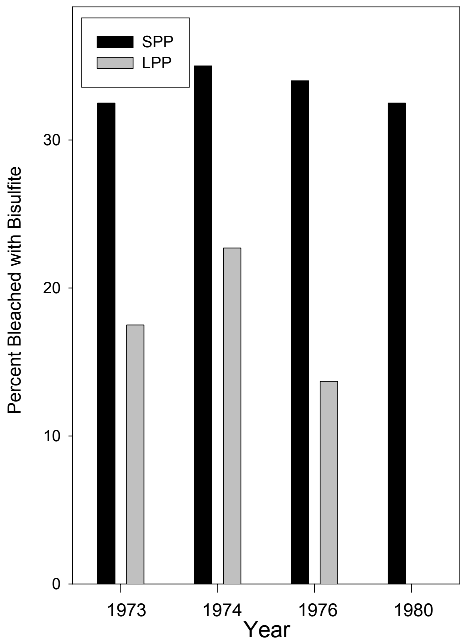


Figure 10. Bleaching of SPP and LPP in aged Pinot noir wines by bisulfite

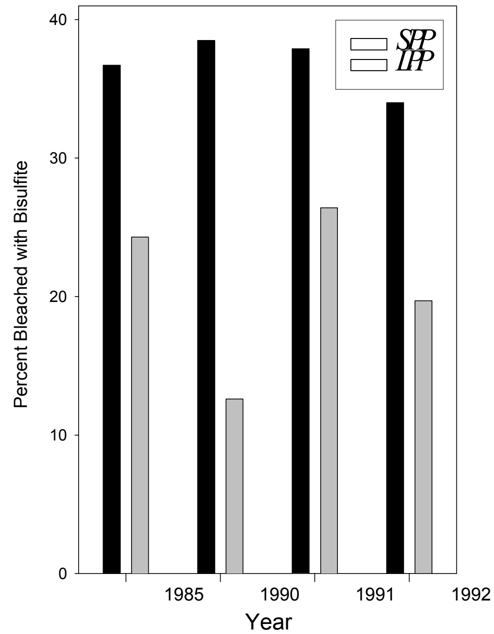


Figure 9 shows bleaching of SPP and LPP in the old Cabernet Sauvignon wines by bisulfite in our standard tannin assay. Figure 10 shows bleaching of LPP and SPP in the old Pinot noir wines by bisulfite under the same conditions. By taking means of these values we have derived average percentages by which LPP and SPP are bleached. By using those values we can now routinely calculate the actual amount of large and small polymeric pigment present in wines when using our combined protein precipitation/ SO_2 bleaching assay. This is particularly important in the case of old wines where all of the pigments are present in the polymeric form (as shown by HPLC). In old wines the standard Somers assay for polymeric pigments shows values close to 20% as monomeric anthocyanins where in fact no monomeric anthocyanins are present. Using the values obtained in this study to correct for bleaching of polymeric pigments by bisulfite gives values for polymeric pigments in old wines close to zero. This should provide a significant improvement in the analysis of polymeric pigments in aged wines in all assays where bisulfite bleaching is used to differentiate monomeric from polymeric pigments.

Summary of Research (Major Accomplishments and Results) by Objective:

Determine if pre-fermentation treatments such as cold soak influence the amount of LPP and SPP in berry skins prior to fermentation.

We found that the amount of polymeric pigments in fruit subjected to cold soak was not different prior to fermentation. At pressing there was more LPP in wine from fruit that had undergone cold soak but during barrel aging there was much greater formation of LPP in the control wine than the one from the cold soak. This shows that all of the treatment effects may not develop until wines begin to undergo the compositional changes that occur during aging.

Study the effect of fermentation variables on extraction of SPP and formation of LPP during winemaking, with an emphasis on temperature.

The temperature effect on tannin extraction and polymeric pigment formation is large and important. The higher the maximum temperature during fermentation the greater the amount of tannin extracted from the fruit, the greater the amount of LPP formed and the greater the amount of SPP extracted from the fruit or formed during fermentation. We also observed important compositional changes in wines during barrel aging. The amount of tannin declined whereas the amount of LPP and SPP increased dramatically.

Determine the extent to which polymeric pigments bleach with SO_2, so that more accurate estimates of LPP and SPP can be made in grape extracts and wines.

Using aged Cabernet Sauvignon and Pinot noir wines where all of the pigments were either present as SPP or LPP (i.e. no monomeric anthocyanins) we were able to confirm that polymeric pigments are bleached by SO2. This is significant because all current assays for polymeric pigments rely on SO2 bleaching to differentiate monomeric from polymeric pigments and polymeric pigments are taken to be non-bleaching. Using column purified fractions as well as determinations in our standard precipitation/SO2 bleaching assay for polymeric pigments we derived correction factors so that more accurate estimates of polymeric pigments can be made in grape extracts and wines.

Research Success Statements.

We have identified many of the important fermentation parameters that influence tannin extractions and polymeric pigment formation. Among the factors we studied, temperature seems to play the largest role. We have observed important compositional changes that occur in wines as they age and have provided the industry with the tools to quantify those compositional changes with a simple inexpensive assay for tannins and polymeric pigments.

We have shown that polymeric pigments bleach with SO2 and have conducted experiments to measure the degree to which they bleach. This research has provided vintners/growers with information that can be used to derive much more accurate values for wine pigments, especially the polymeric pigments which are responsible for permanent color in red wines.

Funds Status

We are continuing to monitor the wines that were made during the 2001 season to see how the wine composition changes during barrel aging. By the end of the grant period all of the funds allocated for salary will be expended and we anticipate that funds in other categories will be used in support of the ongoing research.