Chemical Additives to Reduce Browning in White Wines

VASSILIKI PANAGIOTAKOPOULOU and J. R. MORRIS

Abstract. Juices from two white grape cultivars, Aurore and Cayuga, received 13 different wine treatments to determine effects on browning in wine. The chemicals, used alone and in combination, were ascorbic acid, hypophosphorous acid, thiodipropionic acid, Trolox-C, stannous chloride, Sporix, and sulfur dioxide. All chemicals, except SO₂, were added to wine made from nitrogen-sparged juice. One lot of the wine received no treatment. All treatments were stored at 20°C and 37°C for nine months and exposed to air after three and six months storage to accelerate browning. The use of SO₂ during bottling resulted in the least browning, and the use of SO₂ during crushing resulted in the most browning. Wine treated with the other chemicals had less browning than the wine produced from the nitrogen-sparged juice but had more browning than the untreated wine. The addition of ascorbic acid and most of the ascorbic acid combinations reduced browning to a greater extent than any of the other chemicals or combinations.

Oxidation (browning) can be a problem in the production of white wines. Results of extensive research on browning of both musts and wines indicate that phenolic substances are the major cause of this browning (2, 22, 24, 25, 26). The quantity and kind of the phenolic substances are important because they affect the color and flavor of wine (22, 26).

Sulfur dioxide (SO₂) is the most important and widely used chemical for the protection of wine from oxidation. However, SO₂ has been reported to cause headaches, coughing, asthma attacks, loss of consciousness, and anaphylactic shock (14, 20). Thus, a growing public awareness regarding the use of SO₂ in wines has created the need for its reduction or replacement.

The primary objective of this study was to evaluate seven chemical compounds (SO₂, ascorbic acid, thiodipropionic acid, hypophosphorous acid, stannous chloride, Sporix, and Trolox-C) in various combinations to determine the effect on the browning of wine made from the white grape cultivars Aurore and Cayuga following different storage treatments. A secondary objective was to evaluate the effectiveness of small amounts of SO₂ at bottling of wine as compared to using large amounts of SO₂ during the crushing of grapes. Also, a suitable method for objectively evaluating the browning of white wines was examined.

Materials and Methods. Two white grape cultivars, Aurore (high browning potential) and Cayuga (low browning potential) were hand-harvested from the University of Arkansas vineyard in Fayetteville.

Juice preparation and analysis: Grapes from each cultivar were divided into three lots, washed, crushed, destemmed, and pressed. To one lot, 70 mg/L of SO₂ was added during crushing. In another lot, a sparging stone was used to drive out existing oxygen with nitrogen gas. The remaining lot received no treatment. All juice was held for 24 hours at 2°C to settle insoluble solids.

The lab analysis of the juice measured pH, total acidity, and percent soluble solids. These analyses indicated that no significant differences existed among the various lots. The average values for the Aurore cultivar were as follows: pH, 3.20; total acidity, 1.04; and percent soluble solids, 15.5. The average values for the Cayuga cultivar were: pH, 3.16; total acidity, 0.92; and percent soluble solids, 15.9. The initial browning of the juice after cold settling was measured with the Gardner Color Difference Meter (CDM) standardized to a white plate (L = 92.4, a = -1.0, b = 1.0); absorbance was measured by a Bausch & Lomb Spectronic 20 spectrophotometer at 420 nm. Prior to measurement with the spectrophotometer, the juice was centrifuged for 10 minutes at 15 000 rpm.

Wine preparation: After 24 hours at 2°C, the juice was racked and filtered through a 0.45-pm (nominal) cartridge filter. The soluble solids were adjusted to 20% by the addition of sugar, and 0.26 g/L of yeast (Saccharomyces cerevisiae) was added. All batches of juices were fermented at 20°C and racked two or three times at weekly intervals in the absence of oxygen.

Fermentation was completed after 20 days, and the wine was racked and tested for percentage of alcohol, pH, total acidity, residual sugars, and SO₂ content (33). The results of percentage of alcohol, pH, total acidity, and SO₂ indicated that no difference existed among the various lots of wine. The average values for the Aurore cultivar were as follows: percent alcohol, 11.95; pH, 3.12; and total acidity, 0.84. For the Cayuga cultivar, the average values were as follows: percent alcohol, 12.00; pH, 3.02; and total acidity, 0.85. No residual sugars or free SO₂ were found in any of the wines (33), but for each cultivar the lots of wine treated with 70 mg/L of SO₂ at crushing had a bound SO₂ level of 38 mg/L as determined with the aeration oxidation procedure for SO₂ (33).

The wines were cold stabilized at 20°C for three weeks. After cold stabilization, the pH and total acidity values had changed for both cultivars. For the Aurore cultivar, the pH was 3.15 and the total acidity was 0.82; for the Cayuga cultivar, the pH was 3.04 and total acidity was 0.79.

Addition of chemicals to wine: Six chemicals and their combinations were added to the wine produced from the nitrogen-sparged juice of both cultivars. The selection of these chemicals was based upon the antioxidant activities they have exhibited on wine, ascorbic acid (1, 8, 11, 20, 32), or other food and drinks: thiodipropionic acid (11), stannous chloride
In addition to these chemicals used alone, combinations of Sporix and ascorbic acid, Sporix and hypophosphorous acid, thiodopropionic acid and ascorbic acid, and TroloxC and ascorbic acid were used. Each compound was used in a concentration of 200 mg/L except for stannous chloride at 20 mg/L and Sporix at 500 mg/L. All of the chemicals, except Trolox-C, were soluble in wine. TroloxC was dissolved in 30 mL of 95% ethanol before its addition to the wine. Hypophosphorous acid lowered the pH of the wine. Ten mL of 10 N NaOH in 3.8 L of wine was required to titrate the wine to its original pH value. The wine that received no treatment from each cultivar was separated into two portions. To one portion of the untreated wine 10 mg/L of SO2 was added.

All of the wines were bottled. All bottles were purged with nitrogen to remove the air immediately before filling. Also, the headspace air in the filled bottles was replaced with nitrogen before capping (21).

**Storage and evaluation of wine:** Half of the bottles from each treatment and cultivar were stored at 20°C, and the other half were stored at 37°C in a dark room. After nine months storage at 20°C, bottles from each treatment and cultivar were opened for off-odor evaluation.

After three months in storage at 37°C, bottles from each treatment and cultivar were opened, and a 50-mL sample of wine was removed for objective browning evaluation. These same bottles were closed after introducing a 50-cc headspace of air and returned to storage at 37°C. Three months later, the same bottles were reopened and another 50-mL sample of wine was removed for objective and subjective browning evaluation. These bottles were again closed with a 100-cc headspace of air and returned to storage at 37°C. After an additional three months, these bottles were again reopened, and a 50-mL sample of wine was removed for both objective and subjective browning evaluation. The bottles that had been opened twice were given a total headspace of 100 cc for the final three months of storage at 37°C. The last
sampling represented the most abusive of all the storage treatments, and the effects of the chemical compounds on browning were expected to be maximized.

**Subjective evaluation:** Panels for subjective evaluation of browning of samples stored at 37°C were held after six months and nine months in storage. Ten trained panelists from the Department of Food Science were used for visual and off-odor evaluation. Thirteen samples per cultivar were evaluated for degree of browning. Off-odor evaluation was determined on samples stored at 20°C for nine months. Each treatment for both cultivars was poured into wine glasses and covered with watch glasses. The panelists were asked to rate the samples on a 9-cm bar with anchor terms labeling the ends. The panelists' ratings for visual evaluation were plotted with the percent transmission readings and L values of the samples to determine the correlation of subjective and objective ratings.

The data from each evaluation of browning were analyzed by cultivar and treatment for each time, storage temperature, and headspace condition. The SAS procedure GLM was used to test mean values for significance at a 5% level. To compare the instrument evaluations with the visual rankings, correlation regression analyses were run to compare visual observations and CDM L and absorbance values for wines exposed to air headspaces for six and nine months at 37°C.

**Results and Discussion**

**Cultivar effect:** As expected, the Aurore wine had a higher browning potential (lower Gardner CDM L values and higher absorbance values) than the Cayuga wine (Fig. 1). This result is in agreement with Lee and Jarwoski (15,16) who have shown that the Aurore cultivar has a relatively high content of phenolic substances and, consequently, a high browning potential. Furthermore, research with the Cayuga cultivar at the University of Arkansas Experimental Wine Cellars has shown that this cultivar has a low browning potential.

**Comparisons of instrument values to visual ranking:** There was a high correlation among visual ranking for browning and the Gardner CDM L values and the absorbance values for wine stored at 37°C for six months and then exposed to a headspace of air after three months of storage (Fig. 1). Both Gardner CDM L values and absorbance proved to be adequate methods to evaluate browning of these two white wines.

**Effects of chemical treatments:** The effects of chemical compounds on visual browning, Gardner CDM L values, and absorbance of Aurore and Cayuga wines stored at 37°C for three, six, and nine months and exposed to different air headspaces during storage are shown in Tables 1, 2, 3, 4, and 5. The visual browning differences among the wines were easily distinguished by a trained panel. Also, the effects of the chemical compounds on Gardner CDM L values and absorbance values were easily distinguished after storage treatments. The panel, as well as the Gardner CDM L values and absorbance readings of both cultivars, indicated that treating the wine with SO₂ immediately before bottling prevented browning better than any other treatment. The use of SO₂ during crushing of grapes produced wines with the lowest Gardner CDM L values, the highest absorbance, and the highest visual browning.

A possible explanation for the results obtained when the wine was treated with SO₂ during crushing is the binding of SO₂ with phenolic substances, oxidative enzymes, acetaldehydes, and other carbonyl compounds (keto acids like pyruvate and a-ketoglutarate) (1,2,3,12,17,20,26,30,31). This binding left no free sulfur dioxide to protect the wine after fermentation.

The wine treated with SO₂ at bottling had received no other treatment throughout the experiment. Both the enzymatic oxidation and the reactions leading to Grape Reaction Products (GRP) (4,5,6,19,25,27,28) probably reduced the wine's phenolic content after fermentation and racking (17). Also, some enzymatically oxidized phenolic substances might have formed brown polymers, which then interacted with proteins or adsorbed onto the surfaces of the yeast cells and precipitated (18,23). The oxygen content of wine, after the above enzymatic reactions, would have been reduced. Thus, the wine that had received no treatment other than the addition of SO₂ at bottling probably had a lower phenolic and oxygen
content than the wine treated with SO₂ during crushing. Furthermore, the addition of SO₂ at bottling gave the wine protection against the nonenzymatic oxidation of phenols that survived fermentation and racking.

<table>
<thead>
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<th>Chemical treatments</th>
<th>3 mon</th>
<th>6 mon</th>
<th>9 mon</th>
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<td>28.5h</td>
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1Means within a column with like letters are not significantly different at the 5% level.

<table>
<thead>
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<th>6 mon</th>
<th>9 mon</th>
</tr>
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<tbody>
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<td>Thiodi. acid</td>
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<td>0.52c</td>
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<td>Stannous Chloride</td>
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<td>0.60a</td>
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1Means within a column with like letters are not significantly different at the 5% level.
The second best treatment for reducing browning was the untreated wine. The proposed explanation concerning the removal of the phenolic substances or their transformation to GRP in the wine that received no treatment is supported by the results obtained from the untreated wine in both cultivars. However, browning of the untreated Aurore wine was not significantly different from that in wine treated with ascorbic acid or ascorbic acid combinations. Also, for the Cayuga wine, results from no treatment were not significantly different from those with ascorbic acid, ascorbic acid-Sporix, and ascorbic acid-trolox-C. Although the initial steps of oxidation were the same for wines treated with SO₂ at bottling and for the untreated wine, the phenolic substances on the untreated wine started to polymerize and form brown compounds during storage. The free SO₂ in the wine treated with SO₂ at bottling reacted with the phenolic substances, the hydrogen peroxide, and the oxygen dissolved in the wine during storage, preventing the wine from browning.

The wine that was sparged with nitrogen and received no other treatment had a surprisingly high level of browning after the most abusive storage treatment as indicated by all values. The sparging of musts with nitrogen was a way to remove dissolved oxygen in musts (1,13,21). In this way, the enzymatic oxidation was reduced since polyphenoloxidase required the presence of oxygen. Also, this would limit the reaction that leads to GRP. As a consequence, more phenolic substances survived fermentation and polymerized during storage.

Nitrogen sparging did not remove the dissolved oxygen completely from the must. Although oxygen would be present in small amounts, it is responsible for the oxidation of phenols and the production of hydrogen peroxide, a very potent oxidant, resulting in non-enzymatic oxidation during storage.

From the remaining six proposed antioxidant substances, the ascorbic acid gave the best results in both cultivars. Ascorbic acid tended to be a better treatment when used in combination with Sporix, Trolox-C, or thiodipropionic acid. Antioxidant values of these treatments were significantly different from the values of the nitrogen treated wine in both cultivars. But their contribution to wine protection against oxidation did not prove to be as effective as the SO₂ at bottling or even as effective as when the wine received no treatment. The only exception occurred for the Cayuga wine treated with only ascorbic acid. This treatment was expected to produce browner wine than the nitrogen treated wine, since oxidation of ascorbic acid produces dehydroascorbic acid and hydrogen peroxide (20). However, the results showed just the opposite. One explanation could be the small amount of ascorbic acid used; higher rates of ascorbic acid might have increased browning.

The rest of the chemical substances used in this study, Trolox-C, thiodipropionic acid, hypophosphorous acid, and stannous chloride did not show potential as antioxidants for wine. However, wines containing these materials were significantly less wines produced by the addition of SO₂ during crushing. The only exception was the high Gardner CDM L value of the thiodipropionic acid treatment in the Cayuga wine.

The off-odor evaluation showed that wine from both cultivars that received SO₂ during crushing had the highest score, although not significantly higher than many of the other treatments (Table 6). The most definite observation made by the panel was the repulsive off-odor in the wine treated with hypophosphorous acid and hypophosphorous acid-Sporix in both cultivars. This off-odor was detected by all panelists. Some of the panelists described the off-odor as a mercaptan aroma. For this reason, the use of hypophosphorous acid is not recommended as an antioxidant substance in wines at the
concentration of 200 mg/L, even though the results showed that the hypophosphorous acid treated Aurore wine resisted browning.

Conclusions

Addition of SO$_2$ at bottling of wines proved to be the best treatment for reducing the browning reactions, but addition of SO$_2$ at crushing of grapes proved to be the worst treatment. In most wines, the second best results against browning reactions were obtained when the wine received no treatment. Ascorbic acid and its combinations tended to reduce browning of wines to a greater degree than any of the other chemicals and their combinations, except the addition of SO$_2$ of bottling.

The best protection against wine browning is provided when the enzymatic oxidation before and during fermentation is not prevented by sparging the juice with nitrogen or adding SO$_2$ at crushing. In this way, the phenolic substances are polymerized or transformed to GRP. The precipitate can be removed from the wine after the fermentation is complete. Addition of SO$_2$ at bottling will protect the wine from browning during storage by preventing the polymerization of the phenolic substances that survive fermentation.

Wines with acceptable levels of browning and with low levels of SO$_2$ can be made from cultivars that have a low phenolic content able to resist the oxidation process during storage. Further protection from browning can be obtained by harvesting the grapes at a low pH (~3.0) and using a small amount of SO$_2$ (10 ppm) at bottling. Additional research is needed to evaluate the potential of using a small amount of ascorbic acid in combination with SO$_2$ at bottling to protect against browning of white wines.

Literature Cited