

## Effect of Ultrafiltration on Wine Quality and Browning

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**Abstract** A study was completed to investigate the potential of ultrafiltration (UF) to reduce browning and to serve as a replacement for sulfites in Seyval and Vidal wine. Wine samples received the following treatments: (1) nonfiltered, nonsulfited; (2) nonfiltered, sulfited; (3) pad filtered before fermentation; (4) ultrafiltration at four molecular weight cut-offs (MWCO); 500 000, 100 000, 50 000, and 30 000 either before or after fermentation. All wines were subjected to storage at three different headspaces. Ultrafiltration at 50 000 d MWCO before fermentation slowed oxidation in the Seyval wine, but not in the Vidal wine. Browning was greater with increased headspace. Storage temperature had less effect on browning than the varietal difference.

The oxidative browning of white wines during production and storage has long been considered a major problem in the wine industry (1,4,19). The browning is due to both enzymatic and nonenzymatic reactions (25). If must is oxidized before fermentation, the wine is less fruity and generally lower in quality; however, it is resistant to further browning (12).

Phenolic compounds are the primary source of color, flavor, and aromatic characteristics of wine, and they are the most important group of compounds distinguishing wine qualities (20,23). White wines require a small, but necessary, amount of phenolics to provide a desirable level of flavor, color, and aroma in the finished product (19,20). Phenolic quality and quantity varies with cultivar, vintage, and climate (9,19). Two basic classes of phenolic compounds are the acid phenols or the nonflavonoids from the grape pulp and the neutral or flavonoid phenols from the skins, seeds, and tissue (20,26). Many different phenols of each category are found in grapes (20,26), and they often exist as composites of phenolprotein-polysaccharide complexes (10). Although phenolic compounds are necessary for a good quality wine, they are also involved in the hazing and oxidative degradation of wine (7,8,20,25,26). The polymerization of phenolic compounds is generally considered the primary source of the characteristic brown color of oxidized wines (9,19,20,25). Flavonoid phenols have a tendency to brown more intensely than the nonflavonoids; the catechin fraction is considered a significant contributor to the flavor character of wine (4,26).

The vast majority of wines produced today contain sulfites that have antioxidant and antimicrobial properties (6,12,13,16, 18,25). As an antioxidant, SO<sub>2</sub> is effective in the prevention of both enzymatic and nonenzymatic oxidation (6,18,25) by inhibiting polyphenoloxidase activity as well as the polymerization of phenols (13). The SO<sub>2</sub> can also react with the products of phenolic oxidation, hydrogen peroxide and acetaldehyde (26), to prevent further conversions of H<sub>2</sub>O<sub>2</sub> to acetaldehyde and with acetaldehyde to form a stable bisulfite complex.

The presence of sulfites in foods has recently been controversial due to the reactions of some asthmatic individuals (2,24). This concern, coupled with a recent requirement by the FDA that foods containing more than 10 mg/L SO<sub>2</sub> (added or naturally occurring) must be so labeled (6), has given the wine industry incentive to find alternatives to SO<sub>2</sub> in wines (2).

One possible alternative to the use of SO<sub>2</sub> is ultrafiltration (UF). This method is used for the separation of compounds, the clarification and the concentration of nearly any liquid based on the molecular weight differences of its contents (5,8,11,14,15). Its variety of pore sizes (500 d to 500 000 d molecular weight cut-offs or MWCO) makes UF fairly versatile (3). It is theorized that through UF of juice prior to fermentation or filtration of the finished wine prior to bottling, certain browning precursors (unstable proteins, phenols, and polyphenoloxidase) could be removed (7,8,14) to reduce the oxidative potential of the wine during storage and to yield a sensory-acceptable product without the use of sulfiting agents.

Several studies have been conducted to determine whether UF would be an acceptable treatment in the production of wines (10,11,14,17,18,22). Matheis and Whitaker (10) showed that ultrafiltered wine was more resistant to oxidation than nonultrafiltered wine. Sims *et al.* (18) found that wines produced from ultrafiltered juice maintained a fruitiness in aroma and flavor that wasn't present in wines that were ultrafiltered after fermentation. Singleton *et al.* (22) reported that wine treated by post-fermentation UF was actually inferior to the standard wines, being unacceptably astringent and bitter, although it was chemically indistinguishable from the other wines produced in their study.

Padilla and McLellan (14) found that when apple juice was ultrafiltered a reduction in protein and phenolic content resulted. In similar studies by Sims *et al.* (17,18) involving grape juice and wines, a similar reduction of phenolic substances was found, which resulted in better color stability and quality of the final wine product. Sims *et al.* (18) showed that this color stability was due to the removal of colorless or nearly colorless polymers that would have later darkened considerably. One report also suggests that, because of the capability of sterile filtration, the removal of wild flora prior to fermentation and the ability to stop fermentation at the desired residual sugars could greatly improve the production and quality of wine (11). To date, most studies have dealt with wine quality as affected by the UF treatments. The objective of this study was to investigate the use of UF to reduce browning and to serve as a replacement for sulfites in wine.

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## Materials and Methods

**Cultivar selection and harvest:** The two cultivars used in this study, Seyval and Vidal, were hand harvested at the University of Arkansas vineyards in Fayetteville. Seyval was chosen for its moderate browning tendencies, and Vidal was chosen for its resistance to browning. Both cultivars were harvested at pH approximately 3.0.

**Juice preparation and analysis:** Juice from each cultivar was prepared in the following manner: (1) The grapes were washed, and extraneous material was removed. (2) They were crushed in a destemmer-crusher. (3) Grapes then were pressed in a Willmes bladder press yielding ca 300 L juice. (4) A pectinolytic enzyme was added at the rate of 1 g/20 L of juice. (5) A 19-L control sample was treated with 30 mg/L SO<sub>2</sub>. (6) All juice was placed in cold storage (2°C) overnight for settling of insoluble solids, (7) After settling, 19-L glass carboys of juice were separated from the large batch of juice for immediate filtration. (8) Approximately 130 L were racked into a 200-L container for immediate fermentation prior to filtration. (9) Laboratory analyses were conducted on the juice prior to fermentation including pH (Expandable Ion Analyzer EA-920), total acidity (titration with 0.01 N NaOH and expressed as mg tartaric acid/100 mL juice), and soluble solids (Reichert Abbe Mark II Refractometer) (10). Juice from both cultivars received five different filtration treatments: pad filtration (45-µm); ultrafiltration (UF) at 500,000 d MWCO; UF at 100,000 d MWCO; UF at 50,000 d MWCO; and UF at 30,000 d MWCO. For each cultivar, 19 L of juice was used per treatment.

**Wine preparation:** All juice was raised to 19% soluble solids with sucrose, and yeast supplement was added (15 g diammonium phosphate/19 L juice and 2.5 g yeast hulls/19 L juice). Yeast was added at a rate of 5.6 g/19 L juice after activation in 40°C water (~1 g yeast/10 mL water). The carboys were equipped with air locks, and all juice was fermented at 20°C to 0% residual sugars. After fermentation, each small batch of the SO<sub>2</sub>-treated control wine and the wines from filtered juice were racked into a clean 19-L carboy, topped with a N<sub>2</sub> head, capped, and placed in 2°C storage for cold stabilization. After about 14 days, these batches were reracked and returned to cold storage prior to bottling.

The wine in the 200-L container was racked into 19-L glass carboys and placed in 2°C storage for cold stabilization for 21 to 28 days. After this time, 19 L of this untreated wine was labeled as the untreated control, and the remaining untreated wine received the following filtration treatments yielding 19 L each: (1) UF at 500,000 d MWCO; (2) UF at 100,000 d MWCO; (3) UF at 50,000 d MWCO; and (4) UF at 30,000 d MWCO. At this point, each variety had received 11 different treatments:

1. Nonfiltered, nonsulfited
2. Nonfiltered, sulfited
3. Pad filtered before fermentation
4. Ultrafiltered at 500,000 d MWCO before fermentation
5. Ultrafiltered at 100,000 d MWCO before fermentation
6. Ultrafiltered at 50,000 d MWCO before fermentation
7. Ultrafiltered at 30,000 d MWCO before fermentation
8. Ultrafiltered at 500,000 d MWCO after fermentation
9. Ultrafiltered at 100,000 d MWCO after fermentation
10. Ultrafiltered at 50,000 d MWCO after fermentation
11. Ultrafiltered at 30,000 d MWCO after fermentation

**Bottling:** The first seven treatments were pad filtered (45-µm) prior to bottling; the remaining four were not pad-filtered after UF. Green wine cooler bottles having a total volume of 395 mL were filled with wine from each cultivar and treatment. Headspaces of 5 mL, 15 mL, or 25 mL were created by pipetting wine from the bottles, which were then capped and labeled. These procedures resulted in 33 treatments for each cultivar.

**Storage and evaluation:** The 66 bottled treatments were again divided and stored at either ambient temperature (22°C) or elevated temperature (38°C). After one year in storage, samples from each treatment received the following analyses: (1) Tristimulus color -Gardner Color Difference Meter (CDM) standardized to a white plate (L = 92.4, a = -1.0, b = 1.0). Samples of 50 mL were poured into an optical glass cup and covered with a white plate which reflected light back into the sample. The instrument was restandardized between samples. (2) pH - Expandable Ion Analyzer EA-920 standardized to buffers of pH = 4 and pH = 7. (3) Optical Density - measured as absorbance at 420 nm with a Model 340 Bausch and Lomb Spectrophotometer 20. A deionized water blank was used for standardization. (4) Titratable acidity - expressed as mg tartaric acid/100 mL wine. Five milliliters of the wine sample was mixed with 120 mL deionized water, and 0.01 N NaOH was used to titrate to a pH of 8.2. (5) Percent alcohol by an ebulliometer. (6) Sulfur dioxide by the Ripper method (26). (7) Phenolic content - according to method of Somers and Ziemelis (23) using Polyclar AT and spectral observation at 280 nm and 320 nm. Flavonoids were expressed as mg catechin/L wine, and nonflavonoids were expressed as mg caffeic acid/L wine. (8) Browning potential. Estimates were based on a modified method developed by Singleton and Kramling (21). One bottle of each treatment of the 5-mL headspace stored at room temperature was opened, and absorbance at 420 nm was recorded. The caps were placed on top of the bottles without sealing to prevent excessive evaporation, and the samples were left at room temperature for 40 days. The absorbance of each sample was again observed and recorded, and the wine was discarded at the twentieth and fortieth days after exposure. (9) Subjective evaluation of browning. Twenty trained panelists from the Department of Food Science were used for visual evaluation. Thirteen samples

were chosen from the total 132 treatments (including the headspace and temperature treatments) that represented the several different browning levels achieved during storage. These samples were selected using CDM 'L' value and absorbance values at 420 nm. The panelists were asked to rate the samples on a 10-cm bar against a 'lightest' and 'brownest' reference sample. The panelists' ratings vs. the absorbance and CDM 'L' values of the corresponding samples were plotted, and computer analyses were run to determine the correlation of the subjective and the objective ratings. (10) Data analyses between cultivars and within each cultivar. The SAS GLM procedure was used to test mean values for significance at the five percent level. Correlation analyses were conducted by using the SAS CORR procedure to compare the subjective mean rating as the objective lightness and absorbance values of the corresponding samples.

## Results and Discussion

The two cultivars generally responded differently to the filtration treatments. Data from the objective analyses were collected after a year in storage. Only the degree of browning was considered when comparing cultivars in both the objective and subjective analyses.

Comparison of objective and subjective ratings: The SAS CORR procedure was used to determine the degrees of correlation between the CDM 'L' values with the absorbance of light at 420 nm and between the visual ratings to each of these instrument readings. After storage, the CDM 'L' and absorbance values had correlation values of 0.95. The 'L' and visual ratings had a correlation coefficient of 0.94, and the absorbance and visuals were correlated at 0.96.

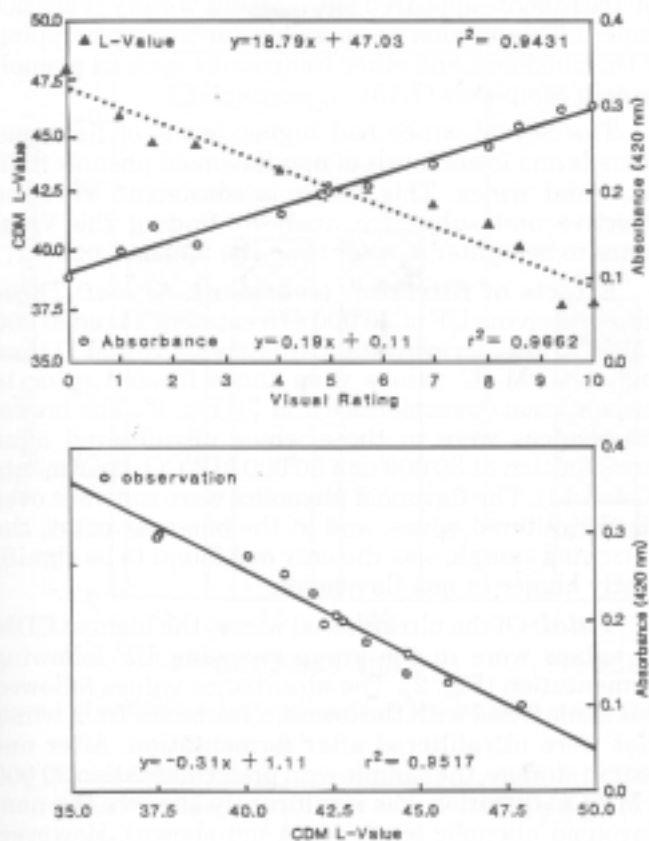


Fig. 1. Linear regression lines, formulas, and correlation values for the objective and subjective ratings on Seyval and Vidal wine samples after one year in storage.

These high correlations show that the instrument readings are adequate to evaluate browning in these white wine products. These relationships are shown in Figure 1.

**Cultivar effect on browning:** After one year in storage, the Vidal cultivar had a lower browning potential (higher CDM 'L' values, lower absorbance values) than the Seyval (Fig. 2). Although the Vidal was able to maintain a light, clear product, several panelists commented that those wines receiving the 30 000 d MWCO UF treatments appeared too light and 'watery' (data not shown). This opinion was possibly due to over-stripping of the phenolics and other compounds such as phenolprotein complexes (7,15). The Seyval wines had higher levels of flavonoid phenols and lower levels of non-flavonoid phenols than the Vidal wines. This result is consistent with the objective and subjective analyses finding the Vidal wines to be lighter in color than the Seyval.

**Effects of filtration treatment. Seyval:** Those wines receiving UF at 30,000 d (treatment 11) or 50,000 d MWCO after fermentation (treatments 10 and 11) had higher CDM 'L' values than those filtered prior to fermentation (treatments 6 and 7) (Fig. 2). The lowest absorbances were in those wines ultrafiltered after fermentation at 30,000 and 50,000 MWCO (treatments 10 and 11). The flavonoid phenolics were constant over all ultrafiltered wines, and at the one-year point, the untreated sample was the only one found to be significantly higher in nonflavonoids.

**Vidal:** Of the ultrafiltered wines, the highest CDM 'L' values were in the group receiving UF

following fermentation (Fig. 2). The absorbance values followed this same trend with the lowest absorbance from wines that were ultrafiltered after fermentation. After one year in storage, the sample with prefermentation 30,000 d MWCO filtration was significantly lower in the nonflavonoid phenolic levels (data not shown). However, while this same sample was also significantly lower in the flavonoid phenol level, the sample that was ultrafiltered after fermentation at 50,000 d MWCO was unexplainably lowest in flavonoid phenols (data not shown).

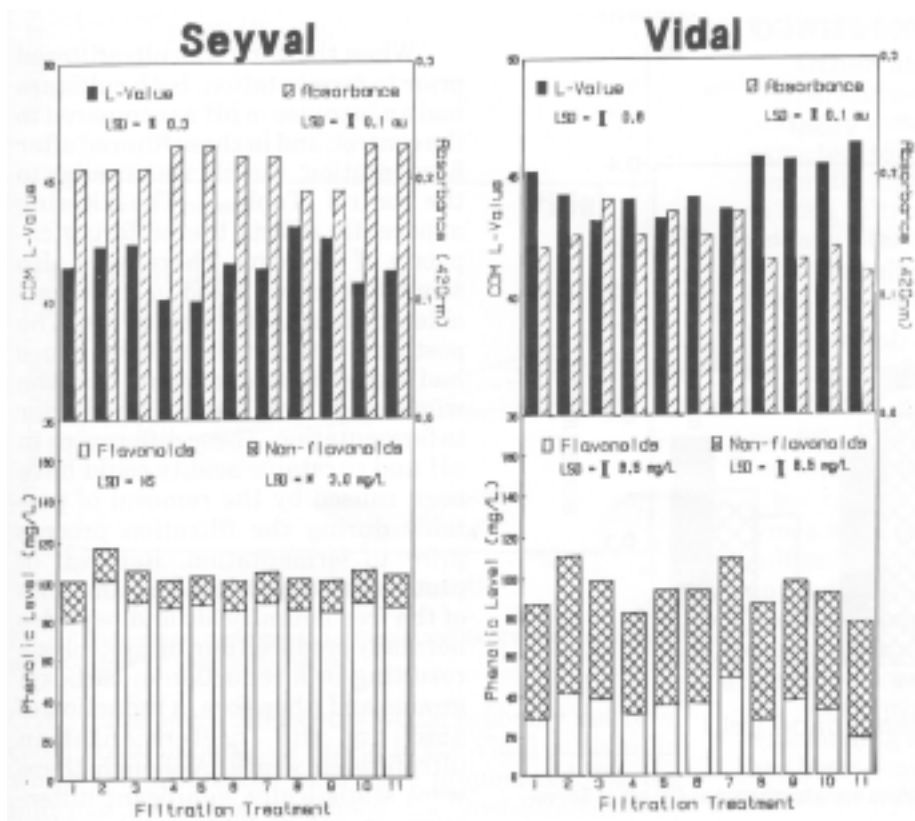


Fig. 2. The effect of filtration treatment on the CDM 'L' values, absorbances at 420nm, and phenolic levels of the Seyval and Vidal wines ( $p \leq 0.05$ ). Filtration treatments: 1=nonfiltered, nonsulfited; 2=nonfiltered, sulfited; 3= pad-filtered (45  $\mu$ m); 4=Ultrafiltered (UF at 30,000 d MWCO prior to fermentation; 5=UF at 50,000 d MWCO prior to fermentation; 6=UF at 100,000 d MWCO prior to fermentation; 7=UF at 500,000 d MWCO prior to fermentation; 8=UF at 30,000 d MWCO after fermentation; 9= UF at 50,000 d MWCO after fermentation; 10=UF at 100,000 d MWCO after fermentation; 11= UF at 500,000 d MWCO after fermentation

Table 1. pH and titratable acidities of Seyval and Vidal wines receiving filtration treatments.<sup>a</sup>

Treatment	Seyval		Vidal	
	pH	TA (% tart)	pH	TA (% tart)
1	2.99f	0.88a	2.80h	1.02a
2	3.02e	0.87b	3.08c	0.93e
3	3.09d	0.82d	3.09b	0.87f
4	3.11b	0.82d	3.07d	0.86g
5	3.11b	0.83c	3.09b	0.87f
6	3.12a	0.84c	3.11a	0.84h
7	3.10c	0.86b	3.11a	0.84h
8	2.99f	0.87b	2.81g	0.97c
9	2.98h	0.86b	2.82f	0.96d
10	2.98h	0.86b	2.84e	0.96b
11	2.99f	0.86b	2.84e	0.94c

<sup>a</sup>Treatments: 1 = non-filtered, non-sulfited; 2 = non-filtered, sulfited; 3 = pad-filtered (45- $\mu$ m); 4 = Ultrafiltered (UF) at 30 000 d molecular weight cut-off (MWCO) prior to fermentation; 5 = UF at 50 000 d MWCO prior to fermentation; 6 = UF at 100 000 d MWCO prior to fermentation; 7 = UF at 500 000 d MWCO prior to fermentation; 8 = UF at 30 000 d MWCO after fermentation; 9 = UF at 50 000 d MWCO after fermentation; 10 = UF at 100 000 d MWCO after fermentation, 11 = UF at 500 000 d MWCO after fermentation.

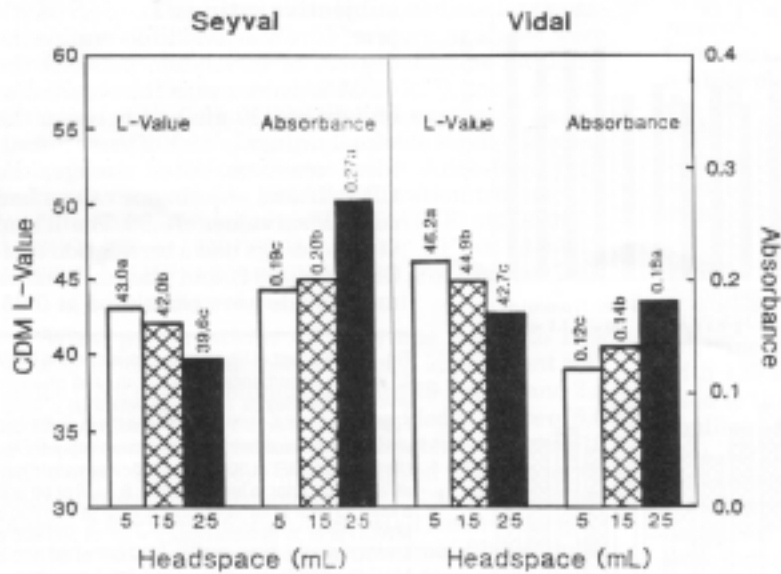


Fig. 3. The effect of storage headspace on the CDM 'L' values and absorbance at 420 nm of Seyval and Vidal wines ( $p \leq 0.05$ ).

When the wine was ultrafiltered prior to fermentation, both cultivars had an increase in pH as compared to the control, and in those filtered after fermentation, the pH was similar to the control (Table 1). The filtering apparently effects the buffering capacity of the wine. There were also some differences noted in the titratable acidities in both cultivars. The post-fermentation ultrafiltered wines had higher titratable acidity than the wines made from those filtered prior to fermentation. These differences in pH and titratable acidity could have been caused by the removal of proteins during the filtration process prior to fermentation. Removal of proteins would reduce the amounts of the free amino acids and peptides normally produced during proteolysis resulting in a reduction of carboxyl groups and, therefore, a reduction of acid in the pre-fermentation ultrafiltered wines. Although there were statistically significant differences in pH and titratable acidity (TA), these small differences would probably not be of commercial importance except in the Vidal cultivar, in which the differences were more pronounced. During informal tasting of the wines, the post-fermentation ultrafiltered wines were unacceptably acidic compared to the other wines produced in the study. This acidity could be linked to the pH and TA differences discussed above.

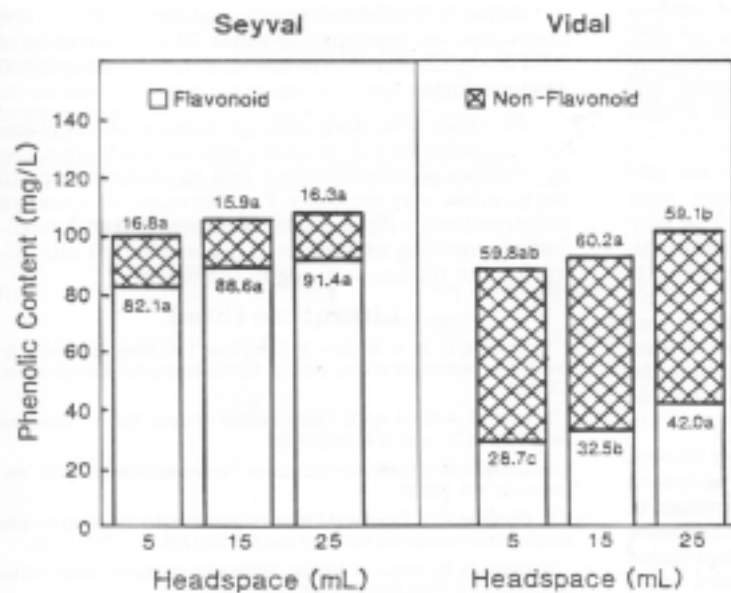


Figure 4: The effect of storage headspace on phenolic levels of Seyval and Vidal wines ( $p \leq 0.05$ ).

having only 5-mL headspaces. The phenolics increased with increased headspace (Fig. 4). Apparently, the polymerized phenols were detected more readily than the nonpolymerized phenols with the Somers and Ziemelis (23) methods of phenolic assay.

Also in both cultivars, the CDM 'a' and 'b' values (measuring the redness to greenness and yellowness to blueness, respectively) increased during storage and stabilized at less green and more yellow values (data not shown). These CDM changes are generally considered normal equilibration of the wine during the first six to twelve months of storage. Hue and chroma increased during storage as determined by CDM values (data not shown).

**Effects of bottle headspace:** In both cultivars, a greater degree of browning (lower CDM 'L' values) occurred in those bottles having more oxygen (Fig. 3). The 25-mL headspace allowed for excessive contact of the wine with oxygen and, therefore, the brownest wines were those that were stored with this headspace. The lower absorbances and the higher CDM 'L' values indicated lighter colored wines, and these were found in those bottles

**Other analyses.** Temperature: Since elevated temperatures have been found to encourage oxidation of juice and wines (14), low storage temperature would be expected to result in less oxidation and, therefore, generally higher CDM 'L' and lower absorbance values in both cultivars. In this study, temperature appeared to have little effect, and more differences were noted between cultivars than between storage temperatures (data not shown).

**Alcohol:** All wines tended to have alcohol levels ranging from 9.5% to 11%. There were no alcohol level differences due to treatment (data not shown).

**SO<sub>2</sub>** All wines, including the SO<sub>2</sub>-treated wines, had low levels of SO<sub>2</sub> present after storage (data not shown). Most ranged from 5 to 12 mg/L. The sulfite-treated wines were probably lower in SO<sub>2</sub> than expected since sulfites were not used at bottling. The SO<sub>2</sub> that was applied at crush was bound to phenolics, enzymes, and carbonyl compounds. The relatively little SO<sub>2</sub> remaining could explain why the sulfated control wines were not exceptionally better in lightness and stability than the other wines. Industry adds sulfites to juice for winemaking at crush and at bottling, and this method has been shown to be a more effective use of sulfiting agents in wine (26).

**Color stability:** After 40 days of exposure to oxygen at room temperature, the absorbance values for each wine within each cultivar – SO<sub>2</sub> control, unfiltered control, wines receiving UF prior to fermentation, and those receiving UF after fermentation - were plotted versus the number of days exposed (Fig. 5).

It was observed that in the Seyval wines, the pre-fermentation UF wines were the lightest and most stable of all the wines from that cultivar. The nontreated wine was the least stable and browned dramatically upon exposure to oxygen, and at the fortieth day its absorbance decreased, probably due to the polymerized phenol precipitation (7,15). The wine that was ultrafiltered after fermentation appeared to be the least stable after 40 days and oxidized more readily than the other treated wines, and the sulfite-treated wine was only slightly better (Fig. 5).

In the Vidal wines, the post-fermentation filtered wines started out as the lightest wines with the lowest optical density, but by the twentieth day of exposure to air they began to oxidize dramatically until they were the most oxidized group in the cultivar. The most stable treatment after 40 days was the sulfite-treated wine, which maintained its absorbance of around 0.14 throughout the forty-day exposure to air. The pre-fermentation ultrafiltered wines, which showed little browning in the Seyval cultivar, were unstable and oxidized rapidly after 20 days of exposure. The unfiltered wine, although not as stable as the sulfited control, was more stable than either of the ultrafiltered groups.

## Conclusions

With the Seyval cultivar, the best treatment to prevent oxidation and yield a visually acceptable product appeared to be the ultrafiltration of juice prior to fermentation at the 50,000 d MWCO. This size filter would probably be better than the 30,000 d MWCO as evidenced by comments from the panelists about 'watery' appearance occurring in the 30,000 d MWCO ultrafiltered wines. The loss of buffering capacity of the wines and possible acidity would suggest that ultrafiltration after fermentation would not yield a marketable wine product unless it was used to blend with another wine with heavier flavors and little astringency. A good product with little oxidation may also be produced by combining sulfite treatment at crush and then ultrafiltration of the juice. While this method would not eliminate SO<sub>2</sub> entirely, it would remain only in small amounts - possibly below the limit requiring labeling.

With the Vidal cultivar, ultrafiltration seemed to encourage rather than reduce oxidation. Sulfites at crush and not at bottling appeared to yield a good product, as did the wine that received no treatment at all; however, untreated wine would have a much greater chance of oxidation during storage - especially if stored for longer than a year. If the public opinion concerning sulfiting is not improved, the consumer may be willing to purchase and drink young white wine products to avoid ingesting

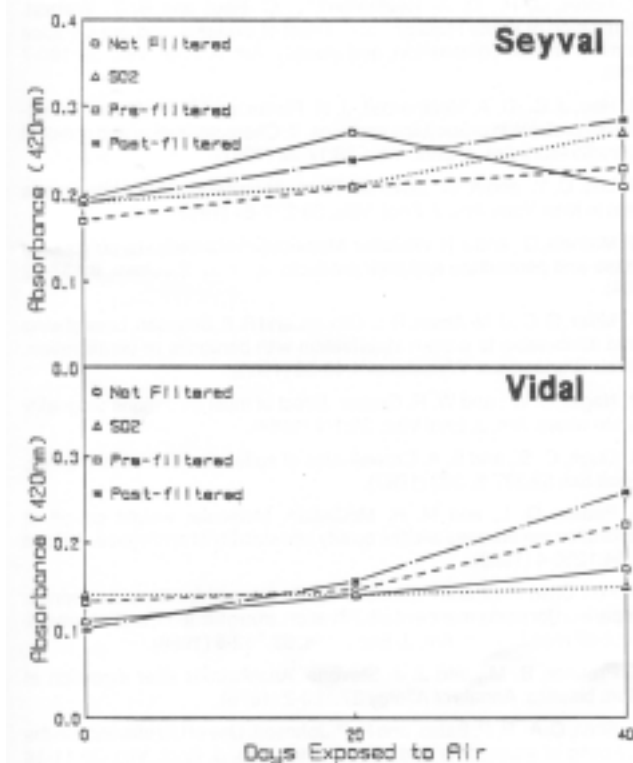


Fig. 5. The progression of browning of Seyval and Vidal wine samples after exposure to air at room temperature.

sulfiting agents, even though there is an increased chance of product degradation.

Reduced headspace would also contribute to the maintenance of good quality wines. The introduction of inert gas headspace may reduce oxidation in nonsulfited wine products.

Although this study did not endorse the replacement of sulfur dioxide in wines with ultrafiltration, the possibilities of ultrafiltration and its contributions to the wine industry are many. Further research is needed to determine the place of ultrafiltration, and with modified winemaking techniques, it is possible that ultrafiltration can replace sulfiting in the future.

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