

Color of Seyval blanc Juice and Wine as Affected by Juice Fining and Bentonite Fining During Fermentation

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Abstracts. *Two experiments were conducted to examine the effects of fining agents applied prior to and/or during fermentation on the color of Seyval blanc wine. In experiment one, kieselsol (Ke) at 719 mg/L; polyvinylpyrrolidone (PVPP) at 719 mg/L; bentonite (Bn) at 960 mg/L; combinations of these levels of Ke, PVPP, and Bn; sulfur dioxide (SO₂) at 100 mg/L; and a control were applied to the juice. The juice was analyzed and then fermented. Wine was analyzed initially and after two months of storage at 37°C. The Bn + PVPP and Bn + Ke + PVPP treatments improved juice and wine color (Color Difference Meter and absorbance at 420 nm) as compared to the control and provided results similar to those for the 100 mg SO₂/L treatment. The Ke treatment adversely affected color. In experiment two, Bn was applied to juice and/or during fermentation. All Bn applications improved juice and wine color as compared to the control. Bentonite added both to juice and during fermentation reduced browning to the same extent as did SO₂. Storage for two months at 37°C increased browning of all treatments except the SO₂ treatment in both studies, showing a need for an antioxidant at bottling.*

Health concerns, regulatory initiatives, and a desire to produce better wines have winemakers seeking to reduce the use of sulfites. A complete replacement for sulfur dioxide (SO₂) has been sought for many years without success, although several compounds can perform one or more functions of SO₂ (4,9,20). Our purpose has been to seek alternatives to SO₂ relative to color stability in Seyval blanc juice and wine.

Reducing the phenolic content of juice should reduce browning substrates. Phenolic levels can be reduced in juice and wine by compounds such as polyvinylpyrrolidone (PVPP), kieselsol (Ke), and bentonite (Bn) that are currently approved for wine use. Polyvinylpyrrolidone has an affinity for small phenolic species such as catechins and hydroxycinnamates and is often used in wine and beer to remove browning and bitter compounds (5,20). Hydrated bentonite platelets have negatively charged surfaces and positively charged edges. These platelets interact with other molecules to remove protein, reduce enzyme activity and reduce phenolic content (6,7,8,20). Kieselsol (colloidal silicon dioxide) has a negative charge and electrostatically binds and adsorbs positively charged compounds and should react similarly to Bn.

The objectives of this study were to: (a) examine the effects of Ke, PVPP, and Bn treatments applied to Seyval blanc juice, alone or in combination, on the color of juice and wine (experiment one); and (b) examine the effects of Bn additions to the juice prior to and/or during fermentation, on the color of Seyval blanc juice and wine (experiment two). These treatments were compared with a standard SO₂ juice addition.

Materials and Methods

Experiment one: Nine fining treatments were applied to Seyval blanc juice in two replications. Treatments were as follows: no additive (control), 100 mg SO₂/L, 719 mg Ke/L, 719 mg PVPP/L, 960 mg Bn/L, Ke + BVPP, Bn + Ke, Bn + PVPP, and Bn + Ke + PVPP. Materials in combination treatments were used at the same rate as in individual treatments. The juice was analyzed prior to fermentation and wines were analyzed initially and after two months of storage at an elevated temperature (37°C). This elevated temperature accelerates browning and is used in accelerated storage studies on food products; it is consistent with worse case temperature scenarios under warehouse conditions.

Phenolic analyses were made on wines held for 18 months at 2°C. Phenolic analyses were also conducted at 0 and 2 months, but the results were biased by the varying protein levels and could not be used. The analysis method was changed to eliminate protein interference and the samples retested.

Seyval blanc grapes were harvested when the fruit was 20° Brix, 3.2 pH, and 7.0 g acid/L as tartaric. The grapes were crushed and destemmed at ambient temperature (27°C); 100 g Clarex° L/1000 kg (Miles, Inc., Elkhart, IN) was added to aid juice extraction. The must was held for 45 minutes before pressing in order to allow the depectinizing enzyme to work. The must was pressed using a Willmes bladder press (type 100, Josef Willmes, Bensheim Hessen, Germany) three times at 2 bars followed by a final press at 4 bars. Juices from all pressings were held in a common vessel with continuous nitrogen sparging until 250 L had been collected. The juice was mixed and subdivided into 22-L glass carboys.

The fining and SO₂ treatments were applied immediately after the main lot of juice was subdivided. The kieselsol used was Nalco® 1072 (Nalco Chemical Co., Naperville, IL). This fining agent is supplied as a 30% dispersion of colloidal silica. Additions were made on a w/v basis as silica. The bentonite used was a sodium montmorillonite clay called Vitiben (Bentonite Corporation, Denver, CO). A 5% slurry was prepared in warm water (50°C) and used after holding for 24 hours at 2°C. The polyvinylpyrrolidone used was Polycla® VT (ISP Technologies, Inc., Wane, NJ). Potassium metabisulfite was used as the SO₂ source.

After holding at 2°C for 18 hours to facilitate settling, the juices were racked. When the juices reached ambient temperature (20°C), 0.26 g Fermaid K/L (yeast nutrient, Lallemand, Inc. Montreal, Canada) and 0.23 g *Saccharomyces cerevisiae*, Wädenswil 27/L (Lallemand, Inc.) were added. The inoculated juice was held for one day at 20°C and then fermented at 15.5°C. Lees were stirred at three- to four-day intervals until residual sugars were 0.05 g residual sugar/100 mL.

Racked wines were detartrated at 2°C for 60 days and then filtered through a 0.45- μ m filter, bottled in 375-mL bottles, and sealed with crown caps. All juice and wine transfers were made under carbon dioxide or nitrogen gas.

Juice analysis: Soluble solids were determined using a refractometer. The pH was determined with a pH meter standardized to pH 4.0 and 7.0 with standard buffer solutions. Titratable acidity was determined by diluting 5 mL of juice to 130 mL with deionized water and titrating to pH 8.2 with 0.1 N NaOH. Acidity is expressed as percent tartaric acid.

Juice phenolics were determined by the Folin-Ciocalteu assay (14) and corrected for sugar content of the juice with a 55:45 fructose: glucose syrup. Results are expressed as gallic acid equivalents (GAE).

Catecholase activity was determined by measuring the catalytic oxidation of catechol into o-benzoquinone as outlined by Traverso-Rueda and Singleton (17). These measurements were made at 25°C with a Spectronic 1201 spectrophotometer (Milton Roy Company, Rochester, NY) at 25°C. One unit of enzyme activity is the amount of enzyme that would cause an increase of 0.001 in absorbance per minute at 420 nm, pH 6.5 and 25°C.

A Gardner Color Difference Meter (CDM), model XL 10A, (BYK-Gardner, Inc., Silver Springs, MD) was used to measure tristimulus color (Hunter L, a, b) values (2). The CDM was standardized to a white plate with the following values: L = 92.4, a = -1.0, b = 1.0. The CDM's optical cup was filled with 50 mL of sample and covered with the white plate. Hue angle was calculated as the angle whose tangent is b/a (3). Browning was determined as absorbance at 420 nm.

Wine analysis: Wines were analyzed two days after bottling (initially) and following two months of storage at an elevated temperature (37°C). Titratable acidity, pH, and color were determined in the same manner as for the juice. Alcohol was determined with an ebulliometer (Dujardin-Salleron, Paris, France), and total SO₂ was determined using the aeration oxidation procedure (20). Absorbance of undiluted wine was measured at 420 and 520 nm in a cuvette with a 10-mm path length.

Flavonoid, nonflavonoid and total phenolics were determined by the method of Somers and Ziemehs (16) as modified by Tryon *et al.* (18) on wines that had been held 18 months at 2°C.

Experiment two: Five treatments were applied in two replications to juice or during fermentation. The treatments were no additive (control), 100 mg SO₂ applied to the juice, 960 mg Bn/L applied to the juice, 960 mg Bn/L applied during fermentation, 960 mg Bn/L applied both to the juice and again during fermentation. The juice was analyzed prior to fermentation. Note that only the control, SO₂ and bentonite applied to the juice treatments were available for this analysis. The remainder of the treatments are applied during fermentation. Wines were analyzed two days after bottling and after storing two months at 37°C. Wine analyses of phenolics were also made on wine held 18 months at 2°C.

Material preparation, fermentation procedures, and analyses were identical in experiments one and two. Bentonite-during-fermentation additions were made on the fifth day of fermentation when sugar concentration had decreased to 12° Balling.

Statistical analysis: Data with storage components were analyzed as a split-plot factorial. Data from the juice and data from the wine analyzed after holding for 18 months at 2°C did not have a storage component and were analyzed separately as treatments. Duncan's multiple range test at the 5% level of significance was used to separate means of main effects.

Results and Discussion

In a preliminary study (data not shown), a ten percent reduction in juice phenolics reduced browning of Seyval blanc juice. The amount of fining agent used in subsequent experiments was selected on the ability to reduce the phenolic content of Seyval blanc juice by ten percent. The levels of bentonite used in this study are higher than normal (960 mg bentonite/L versus 250 to 500 mg bentonite/L), and lees production is, therefore, also greater than normal. Due to the amount of lees production, a method of juice recovery from lees would be desirable for commercial application. The wines made from bentonite fined juices did not require further fining to be protein stable (data not shown). The use of 100 mg SO₂/L is higher than is normally used for pH 3.2 juice, but this did not inhibit fermentation and has provided some antioxidant protection through the various racking and bottling processes.

There were no significant differences in juice soluble solids, acidity, or pH in experiment one (data not shown). The SO₂ treatment had the highest phenolic level (Table 1). The Folin-Ciocalteu test used to measure total phenolics gives inflated values in the presence of SO₂ (15). Therefore, data from the SO₂ treatment were not used to calculate statistical differences for total phenolics in the juice. All treatments except Ke reduced phenolics as compared to the control. Combination treatments reduced juice phenolics the most (Table 1).

Table 1. Effects of juice treatment on total phenolics, enzyme activity, and color in Seyval blanc grape juice (Exp. 1).

Juice treatments ^a	Total phenolics GAE ^b	Catecholase activity Units/mL	CDM L	Hue angle tan ⁻¹ b/a	Absorbance at 420 nm
None	205a ^c	438b	47.9d	106d	0.185a
Kieselsof (Ke)	192ab	484a	48.7bc	107d	0.145b
PVPP	174bc	438b	48.4cd	108d	0.191a
Ke + PVPP	147d	453b	49.1b	112bc	0.141bc
Bentonite (Bn)	175bc	15c	49.8a	110cd	0.146b
Bn + Ke	150d	18c	49.9a	112bc	0.129bcd
Bn + PVPP	162cd	15c	49.9a	115ab	0.115cd
Bn + Ke + PVPP	144d	12c	50.3a	116a	0.106d
SO ₂		8c	50.2a	172a	0.143bc

^a For individual juice treatments and combinations: Ke and PVPP = 719 mg/L, Bn = 960 mg/L, SO₂ = 100 mg/L.

^b Gallic acid equivalents, mg/L.

^c Means within column having the same letter are not significantly different at the 5% level.

^d SO₂ treatment not used to determine significance of total phenols. The total phenolic level as measured by the Folin-Ciocalteu test gave the inflated value of 251 mg GAE/L.

other treatments. The hue angles for the Bn + PVPP, Bn + Ke + PVPP, and SO₂ treatments were not different from one another and had a color that was more green and less yellow than the control. The control and PVPP treatments produced juices that were the most brown, and the Bn combination treatments produced juices that were the least brown as shown by absorbance at 420 nm.

Table 2. Effect of juice treatment and storage on total phenolics and color of Seyval blanc wine (Exp. 1).

Juice treatments ^a	Total phenolics GAE ^b	CDM L	Hue angle tan ⁻¹ b/a	Abs. at 420 nm
None	199b ^c	40.0cd	89b	0.168a
Kieselsof (Ke)	187cd	39.4d	83c	0.165a
PVPP	189c	40.1cd	90b	0.163a
Ke+PVPP	184cde	40.3cd	90b	0.157a
Bentonite (Bn)	178def	41.8bc	97a	0.159a
Bn+Ke	175ef	42.0bc	97a	0.151ab
Bn+PVPP	169f	42.7ab	100a	0.130bc
Bn+Ke+PVPP	158g	43.1ab	100a	0.132bc
SO ₂	211a	44.7a	99a	0.114c
Storage at 37°C				
Initial	186	45.7a	94	0.117b
2 months	180	37.4b	93	0.180a

^a Gallic acid equivalents mg/L.

^b For individual juice treatments and combinations: Ke and PVPP = 719 mg/L, Bn = 960 mg/L, SO₂ = 100 mg/L.

^c Means within column and main effect with the same or no letter(s) are not significantly different at the 5% level.

treatments, and the Bn + Ke + PVPP treatment had the lowest phenolic level. Storage at 37°C had no effect on total phenolics.

Wine color was affected by the juice treatments (Table 2). The CDM 'L' values for the wines made from Ke-, PVPP-, and Ke + PVPP-treated juices did not differ from those of the control. The Bn + PVPP, Bn + Ke + PVPP, and SO₂ juice treatments produced the lightest wines (highest 'L' value) and were not different from one another. Hue angle was highest (less yellow, more green) for wines made from Bn- and SO₂-treated juices and lowest (most yellow) for the wines made from Ke-treated juice. Absorbance at 420 nm was lower (less brown) for the wines made from Bn + PVPP-, Bn + Ke + PVPP-, and SO₂-treated juice than for the control. Two months of storage at 37°C decreased the 'L' value (darker) and increased absorbance at 420 nm (browning).

Catecholase activity is reduced by any treatment that causes a physical change in enzyme structure or causes the enzyme to precipitate. Treatments containing Bn reduced catecholase activity to the same extent as SO₂ (Table 1). Precipitation of polyphenoloxidase through Bn fining has previously been shown to reduce enzyme activity (6,7,8). Kieselsof and PVPP may have reduced settling in the juice, thus retaining catecholase and phenolic compounds.

Juice color was affected most by SO₂ and treatments containing Bn (Table 1). The Bn-treated and the SO₂-treated juices were lighter in color (higher CDM 'L' values) than the juice with absorbance at 420 nm.

In the wine, there were no differences in pH, acidity, residual sugars, or alcohol content (data not shown). In contrast to the juice phenol analysis, the influence of SO₂ on the Folin-Ciocalteu test should be minimal in the wine due to binding of phenols with aldehydes and reduction of SO₂ during fermentation. Therefore, data from the SO₂ treatment were included in the statistical analysis for total wine phenolics. Total SO₂ was 20 mg/L in the wine made from SO₂-treated juice and averaged 4 ± 2 mg/L in all other treatments. Total phenolics in all treatments were lower than the control, with the exception of SO₂ (Table 2). The wines made from Bn-treated juices were generally lower in phenolics than other

Table 3. Spectra of Seyval blanc wine held 18 months at 2°C prior to analyses (Exp. 1).

Juice treatments	Absorbance at		Phenolics GAE* (mg/L)		
	420 nm	520 nm	Flavonoid	Non-flavonoid	Total
Bentonite additions^b					
None	0.128b ^c	0.067ab	58.2ab	17.7b	75.8ab
Kieselsof (Ke)	0.146a	0.071a	56.5abc	17.8b	74.3ab
PVPP	0.135ab	0.058bc	55.1abc	17.9b	73.0ab
Ke + PVPP	0.135ab	0.058bc	40.9cd	17.0b	57.9bc
Bentonite (Bn)	0.124bc	0.059abc	45.7bcd	19.3b	65.0bc
Bn + Ke	0.128b	0.068ab	50.9bcd	17.8b	68.8bc
Bn + PVPP	0.116c	0.047c	37.3d	17.0b	54.1c
Bn + Ke + PVPP	0.116c	0.047c	35.3d	17.3b	52.6c
SO ₂	0.102d	0.050c	68.4a	22.5a	90.9a

* GAE - Gallic acid equivalents.

^b For individual juice treatments and combinations: Ke and PVPP = 720 mg/L, Bn = 960 mg/L, SO₂ = 100 mg/L.

Table 4. Effect of bentonite and SO₂ additions on the color of Seyval blanc juice (Exp. 2).

Main effects	CDM L	Hue angle tan ⁻¹ b/a	Abs. at 420 nm	Total phenolics
Bentonite Additions^a				
None	44.1bc ^c	89b	0.157a	195a
Addition^a				
None	47.0b ^c	103c	0.25a	213b
Bentonite	49.1a	110b	0.18b	180c
SO ₂	50.2a	117a	0.14b	224a

^a 960 mg bentonite/L added to the juice and removed by racking; SO₂ = 100 mg/L.

^b Means within columns with the same letter are not significantly different.

Wine spectra were evaluated after 18 months at 2°C (Table 3). Browning (absorbance at 420 nm) was least in the wine made from SO₂-treated juice. The wines made from the Bn + PVPP- and Bn + Ke + PVPP-treated juices were less brown than those from the Ke- treated juice and the control. Absorbance at 520 nm is used as an index of anthocyanin pigments in red wines. In white wines, spectra above 500 nm are used to measure oxidative pinking. Increased absorbance at 520 nm is not a direct measurement of pinking but does suggest pinking (11). The absorbance at 520 nm was lowest in the wines made from Bn + PVPP-, Bn + Ke + PVPP-, and SO₂-treated juice as compared to the control.

Levels of flavonoid, nonflavonoid, and total phenolics are shown in Table 3. The values obtained using this test were usually lower than those of the Folin-Ciocalteu test (Table 2). This test was used to eliminate the interference that SO₂ causes in the Folin-Ciocalteu test (18). The highest levels of total phenolics were observed for the SO₂ treatment, just as they were

for the Folin-Ciocalteu test, showing that SO₂ increased the phenolic content. This increase in phenolic content may have been due to increased extraction of phenolic compounds and/or due to reduced oxidation of phenolic compounds. The nonflavonoid phenolics were highest in the SO₂ treatment, as would be expected, because SO₂ applied to the juice would prevent the oxidation of hydroxycinnamic acids (1,13). Bentonite, Ke, and PVPP would not protect the hydroxycinnamic acids from oxidation and polymerization. Polymerized compounds are often adsorbed to yeast and removed during fermentation (20). The juices treated with Bn + PVPP and Bn + Ke + PVPP produced wines with the lowest flavonoid phenolics as compared to the control (Table 3).

Bentonite and SO₂ additions to the juice did not affect soluble solids, pH, or acidity in experiment two (data not shown). Catecholase activity was reduced to the same extent by bentonite and SO₂ additions (data not shown).

Bentonite and SO₂ additions improved juice color as compared to the control (Table 4). Bentonite- and SO₂-treated juices were lighter (higher CDM 'L' value) and less yellow (higher hue angle) than the control. The SO₂-treated juice was less yellow than the Bn-treated juice. The absorbance at 420 nm showed that Bn- and SO₂-treated juices were less brown than the control. Total phenolic values were lowest in the Bn-treated juices.

The pH of the wine from SO₂-treated juice was lower than that of the Bn and control treatments (3.18 versus 3.35). A lower pH has been observed previously in wine made from SO₂-treated Seyval blanc juice as compared to nontreated juice (4). This difference should give the SO₂ treatment a small advantage, since oxidation takes place more slowly at lower pH (20). There were no differences in acidity or alcohol levels between treatments or with storage (data not shown).

Wines produced from the Bn during fermentation treatments were lighter (higher 'L' value) than those of the control or the SO₂ treatment (Table 5). All wines made from treated juice were less yellow and more green (higher hue angle) than the control. Absorbance at 420 nm was lower in the wines made from Bn- and SO₂-treated juice than in the control and lowest when Bn was added both to the juice and during fermentation. The wine became darker and more yellow

and brown with storage at 37°C. This change indicates that an antioxidant is necessary in the wine at bottling. Total phenolics were lower in wines made from Bn-treated juice than in the control or SO₂-treated juice.

Table 5. Effects of added bentonite and SO₂ and storage on color of Seyval blanc wine (Exp. 2).

Main effects	CDM L	Hue angle tan ⁻¹ b/a	Abs. at 420 nm	Total phenolics
Bentonite Additions^a				
None	44.1bc ^b	89b	0.157a	195a
Juice	46.2ab	97a	0.135b	172b
During Ferm.	46.4a	98a	0.130b	173b
Juice + Ferm.	47.2a	100a	0.105d	152c
SO ₂	42.7c	99a	0.117c	208a
Storage at 37°C				
Initial	52.6a	101a	0.104b	179
2 months	38.0b	92b	0.153a	180

^a 960 mg bentonite/L added to the juice and removed by racking or added during fermentation or added to the juice plus added during fermentation. SO₂ = 100 mg/L.

^b Means within column and main effect with the same or no letter(s) are not significantly different at the 5% level.

Table 6. Spectra and phenolic content of Seyval blanc wine held 18 months at 2°C (Exp. 2).

Juice treatments	Absorbance at		Phenolics GAE ^a (mg/L)		
	420 nm	520 nm	Flavonoid	Non-flavonoid	Total
Bentonite additions^b					
None	0.150a ^c	0.079a	67.1a	16.8b	83.8b
Juice	0.122b	0.060b	54.2b	17.3b	71.4c
During Ferm.	0.119b	0.057b	52.9b	16.6b	69.5c
Juice + Ferm.	0.099c	0.046c	41.9c	16.5b	58.4d
SO ₂	0.102c	0.050c	68.4a	22.5a	90.9a

^a GAE - Gallic acid equivalents.

^b 960 mg bentonite/L added to the juice and removed by racking or added during fermentation or added to the juice plus added during fermentation. SO₂ = 100 mg/L.

^c Means within columns having the same letter(s) are not significantly different.

Wine spectra were evaluated after 18 months at 2°C after it was held 18 months at 2°C (Table 6). Wines treated with Bn were less brown (absorbance at 420) than when bentonite was not used. Browning was least in the wines made from SO₂-treated juice or when Bn was added both to the juice and during fermentation. Lower absorbance at 520 nm for the wines made from Bn- and SO₂- treated juice than for the control suggests that Bn and SO₂ juice treatments may reduce the amount of pinking occurring in wine.

Flavonoid content in the wine was lowest when Bn was added both to juice and again during fermentation, and all wines made from Bn-treated juice had lower flavonoid phenolics than when Bn was not used (Table 6). Flavonoid phenolics are considered to contribute the most toward browning in wine (10,12,13,19). Nonflavonoid phenolics were higher in the SO₂ treatment than in all other treatments. Again, this result was expected since SO₂ added to juice will prevent oxidation of hydroxycinnamic acids. Total phenolics were reduced by all bentonite additions. The wines made with Bn added both to the juice and again during fermentation had the lowest phenolic values. Bentonite had a greater effect on reducing flavonoid phenolics than on reducing non-flavonoid phenolics.

Conclusions

The juice fining treatments used had little effect on quality attributes tested except color. All treatments except Ke reduced catecholase activity in the juice, and treatments containing Bn reduced catecholase activity to the same extent as SO₂. Juice color was maintained best by the Bn + PVPP, Bn + Ke + PVPP, and SO₂ treatments. Phenolics were reduced most by the Bn juice treatments. Browning was least in wines made from the SO₂ and Bn juice treatments. The addition of 960 mg Bn/L both to juice and again during fermentation reduced browning in Seyval blanc wine to the same extent as 100

mg SO₂/L. The addition of Ke to the juice produced wine with a color that was more yellow than the control. Counter fining the Ke treatment with PVPP or Bn did not produce a better product than using PVPP or Bn alone. Bentonite and PVPP juice treatments or Bn added both to juice and again during fermentation may be used as a substitute for SO₂ in regard to color preservation in Seyval blanc wine. However, SO₂ or another antioxidant is still required at bottling to provide long term color stability.

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