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Effect of Pre-Fermentative Bentonite Addition on Pinot Noir Wine Colour, Tannin, and Aroma Profile

Pradeep M. Wimalasiri [1], Tanya Rutan [2] and Bin Tian [1,*]

[1] Faculty of Agriculture and Life Sciences, Lincoln University, Lincoln 7647, New Zealand
[2] Bragato Research Institute, Blenheim 7201, New Zealand
* Correspondence: bin.tian@lincoln.ac.nz

Abstract: Pinot noir is a grape variety with thin grape skin, which means the extraction of colour and polyphenols is more challenging than other red grape varieties. The aim of this study was to investigate the impact of protein removal by adding bentonite prior to fermentation on Pinot noir wine composition. Four treatments were conducted, including the control without bentonite addition and Pinot noir wines produced with the addition of three different types of bentonites before cold soaking. The juice and wine samples were analysed for pathogenesis-related proteins, tannin, wine colour parameters, and aroma composition. The results showed that bentonite addition at 0.5 g/L had little impact on tannin and aroma compounds but more impact on wine colour, especially significantly higher level of SO_2 resistant pigments observed in Na bentonite addition treatment. This study indicates the potential use of bentonite to modulate the Pinot noir juice composition that may facilitate the extraction of colour components from grape into juice, which plays an important role in colour stabilization in finished wine.

Keywords: anthocyanins; bentonite; cold soaking; colour; pathogenesis-related proteins; Pinot noir; tannin; wine aroma

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1. Introduction

The extraction of anthocyanins and tannins is critical for red wine production as they are associated with important sensory attributes, e.g., wine colour, taste, and mouthfeel [1]. Pinot noir is a grape variety with thin grape skin, so the winemaking techniques that can enhance the extraction of colours and polyphenols are useful tools to produce quality Pinot noir wine.

Previous studies [2,3] have reported that tannins in wine can interact with proteins, which influences the perception of astringency [4]. Tannins and proteins can bind with each other through the hydrogen bonding and hydrophobic interactions [5]. From a winemaking point of view, the tannin–protein interaction may also influence the extraction of those components from grape into juice and wine. As the major soluble proteins in white grape juice and wine, pathogenesis-related (PR) proteins have been well studied as they could cause protein haze formation in white wine [6–8]. PR proteins have been reported to be present in both the grape skin and pulp of but not in the seed in Sauvignon blanc [9] and their concentrations were gradually increased in grape skin and pulp during ripening [10], but the diversity of PR proteins decreased during grape maturation [11]. The level of PR proteins in grapes can also be influenced by UV radiation and fungal infection [12]. To remove PR proteins in white wine in order to prevent protein haze formation [13], bentonite is commonly used as a fining agent. Bentonite is a negatively charged clay belonging to the group of montmorillonites (hydrated aluminium silicates), which is able to swell when combined with water and produce a gel-type suspension [14,15]. Bentonite interacts with positively charged wine proteins to form flocculation and precipitate [16]. The exchangeable cations mainly Na^+, Ca^{2+}, and Mg^{2+} localized on the external surface of clay particles and in-between the layers are important for balancing the net negative

charge of the clay [17]. The three major types of bentonite used in the wine industry are sodium (Na) bentonite, calcium (Ca) bentonite, and sodium and calcium combined (NaCa) bentonite, with Na bentonite having the greatest swelling capacity and Ca bentonite having the most compact lees [18]. In red wine production, fining is also commonly carried out but for different purposes, e.g., adjusting wine colour using charcoal and lowering tannins using egg albumin. Bentonite has been used in finished red wines to achieve colloidal stability and reduce astringency [14,19,20].

In red wine, proteins have also been reported and characterized in Cabernet Sauvignon [21] and a recent study suggested the PR proteins can limit the retention of added condensed tannins during red wine fermentation [22]. Thus, the removal of or reduction in proteins in red grapes may facilitate the extraction of phenolic substances from grape into juice and wine during fermentation. The typical bentonite addition rates for protein stabilization in white wines are between 0 and 1 g/L and the protein content in red grape juice is normally lower than in white grape juice, so in this study three common types of bentonites are added at 0.5 g/L, with the aim to investigate the impact of early protein removal by adding bentonite on Pinot noir wine composition.

2. Materials and Methods

2.1. Grapes and Wine Samples

Pinot noir grapes (clone/rootstock: AM 10-5/Swartzman) were harvested in 2021 from the Pegasus Bay vineyard located in north Canterbury. Four treatments, including control, were carried out in this study. In each treatment, the wines were produced in triplicate and, in each replicate, 700 g of destemmed and crushed grapes were placed in a 1 L plastic bucket. Three types of bentonite (Enartis Pacific, Napier, New Zealand), including sodium (Na), calcium (Ca), and sodium and calcium combined (NaCa) bentonites, were added at a rate of 0.5 g/L into grape must prior to the cold maceration (5 days). After five days cold maceration, the grape must was warmed up to room temperature at 20 °C and then inoculated with EC1118 yeast. Alcoholic fermentation was carried out in a temperature-controlled room at 28 °C and cap management was conducted once a day. The fermentation was considered finished when the residual sugar is less than 2 g/L and all the ferments have gone through three days of post-fermentation maceration. The free-run wines from different treatments were collected at the end of alcoholic fermentation for chemical analysis. No malolactic fermentation was carried out in this study as the aim of this study was to investigate the immediate effect of bentonite addition on Pinot noir wine composition at the end of alcoholic fermentation.

2.2. Oenological Parameters

The free-run wines collected at the end of alcoholic fermentation were analysed for pH, titratable acidity (TA), and alcohol content according to the methods described previously [23].

2.3. Methylcellulose Precipitable Tannins

The total tannins were measured in the free-run wines collected at the end of the alcoholic fermentation using the methylcellulose precipitation method [24].

2.4. Analysis of Pathogenesis-Related Proteins by HPLC

Two major pathogenesis-related (PR) proteins, thaumatin-like proteins (TLPs) and chitinases, were measured in grape juice at crushing, after 5 days' cold maceration, and in resultant wines according to the method published previously [25]. In brief, the juice/wine samples (50 µL) were loaded at 1 mL/min and the elution of proteins was monitored by absorbance at 210, 220, 260, 280, and 320 nm. The identification of thaumatin-like proteins (TLPs) and chitinases was assigned from the 210 nm chromatogram by comparison of the peak retention times to the purified TLPs and chitinases. The protein was quantified

through a comparison with the peak area of thaumatin from *Thaumatococcus daniellii* (Sigma–Aldrich) and thus the protein concentration was expressed as thaumatin equivalents.

2.5. Colour Parameters in Resultant Wines

The wine samples were analysed for colour parameters using the modified Somer's assay [24]. All wine samples were diluted by 10 times in the model wine (0.5% *w*/*v* tartaric acid in 12% *v*/*v* ethanol adjusted to pH 3.4 with 5 M NaOH) and treated with 0.375% *w*/*v* sodium metabisulphite, 0.1% *v*/*v* acetaldehyde, and 1 M HCl, respectively. The absorbance of the mixtures was measured at 280 nm, 420 nm, and 520 nm, respectively, after incubation using UV-Visible spectrophotometer (Model UV-1800, Shimadzu Corporation, Kyoto, Japan) and colour parameters were calculated using the formulas described in the method.

2.6. Aroma Profiling by SPME GC-MS

The volatile aroma compounds in the wine samples were analysed according to the previously published method [26]. Two different methods were used to quantify two groups of aroma compounds: esters and alcohols and low concentration compounds. In brief, the esters and alcohols were analysed using a headspace solid-phase micro-extraction (HS-SPME)-GC/MS method. The wine samples (0.9 mL) were pipetted together with 8.06 mL of pH 3.5 acidified water, followed by 40 µL of deuterated internal standard solution, and 4.5 g of sodium chloride into a 20 mL SPME vial. The samples were incubated and agitated for 10 min at 60 °C. The SPME fibre was conditioned for 60 min at 60 °C before being desorbed in the injection port at 270 °C for 5 min. The GC/MS was equipped with dual columns. The MS source was operated in the electron impact (EI) mode with an ionization energy of 70 eV. The analysis of the chromatograms was performed on GC/MS Solution software, version 2.5 (Shimadzu, Auckland, NZ). The compounds of low concentration were analysed by changing the acquisition mode to the selected ion monitoring (SIM) to increase the sensitivity.

2.7. Statistical Analysis

All data were presented as means and standard deviations of three replicates, which were analysed using one-way analysis of variance (ANOVA), followed by a post hoc analysis (Tukey's test $p \leq 0.05$) using Minitab 18 (Minitab Inc., State College, PA, USA).

3. Results

3.1. Fermentation Kinetics and Oenological Parameters

The grapes were harvested with total soluble solids (TSS) at 22°Brix and the titratable acidity (TA) and pH at harvest were measured at 9.51 g/L and 3.14, respectively. The addition of bentonite prior to the cold soaking did not show a significant impact on fermentation dynamics. The fermentation progress was measured as ferment cumulative weight loss and all the fermentations in different treatments were finished after eight days (Figure 1). The pH, TA, and alcohol content in resultant wines were determined at the ranges of 3.62–3.67, 6.71–6.88 g/L, and 12.09–12.31% abv, respectively (Table 1). No significant difference in pH, TA, and alcohol content was observed in the resultant wines between treatments. There was also no significant difference in the tannin concentration between treatments, with the concentration ranging from 890 mg/L to 929 mg/L.

Table 1. Oenological parameters and tannins measured in resultant wines.

	Control	Na	Ca	NaCa
pH	3.65 ± 0.11 a	3.63 ± 0.03 a	3.62 ± 0.02 a	3.67 ± 0.01 a
TA (g/L)	6.88 ± 0.08 a	6.73 ± 0.15 a	6.88 ± 0.04 a	6.71 ± 0.04 a
Ethanol (%)	12.11 ± 0.22 a	12.14 ± 0.26 a	12.09 ± 0.32 a	12.31 ± 0.22 a
Tannin (mg/L)	890 ± 98 a	929 ± 61 a	929 ± 95 a	918 ± 17 a

Different letters in the same row indicate significant differences statistically between treatments.

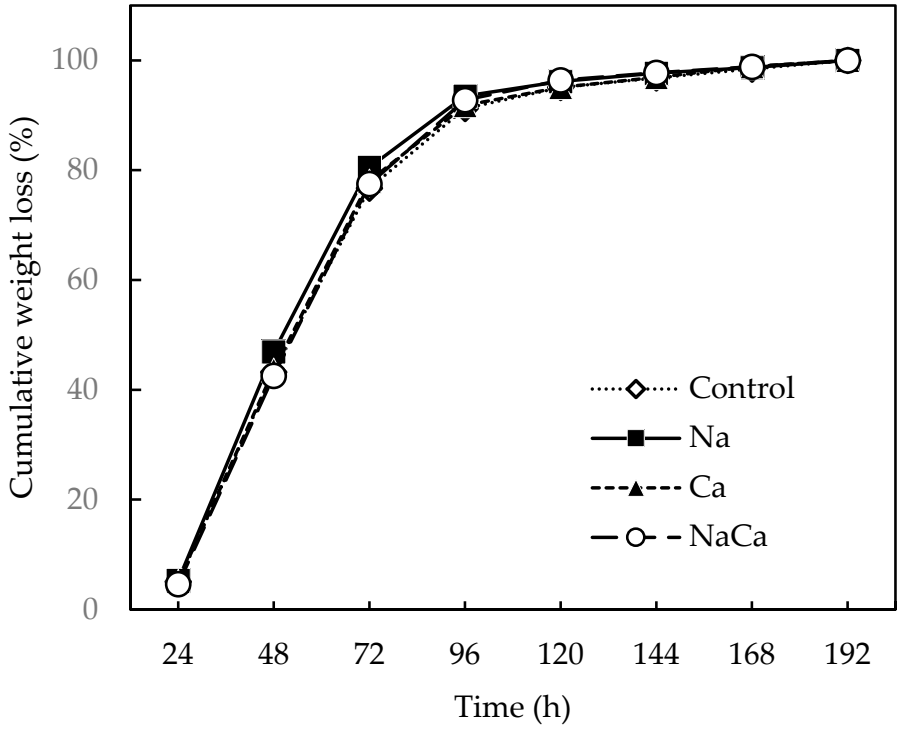

Figure 1. Fermentation progress of Pinot noir wine with or without bentonite addition, measured as ferment cumulative weight loss.

3.2. Pathogenesis-Related Proteins in Juice

The free run Pinot noir grape juice was analysed for both TLPs and chitinases, with concentrations determined at 70.4 mg/L and 58.7 mg/L, respectively (Table 2). After five days cold soaking, the concentrations of both TLPs and chitinases decreased to 37.5 mg/L and 38.7 mg/L, respectively.

Table 2. Analysis of pathogenesis-related proteins in juice with and without bentonite addition.

PR Proteins (mg/L)	Juice at Crushing	Juice after Cold Soaking			
		Control	Na	Ca	NaCa
TLPs	70.4 ± 0.6 a	37.5 ± 4.0 b	16.3 ± 6.1 c	25.5 ± 4.5 bc	28.2 ± 5.9 bc
Chitinases	58.7 ± 2.7 a	38.7 ± 4.8 b	28.1 ± 10.0 b	33.1 ± 5.6 b	33.9 ± 6.8 b

Different letters in the same row indicate significant differences statistically between treatments.

In the treatments with a bentonite addition, the concentration of TLPs and chitinases was decreased even further, with the Na bentonite-added treatment showing the lowest level of TLPs and chitinases at 16.3 mg/L and 28.1 mg/L, respectively. Compared to the control, the concentrations of TLPs and chitinases were not significantly different in treatments added with a Ca bentonite or NaCa bentonite. After fermentation, no PR proteins were observed in wines from any treatments (data not shown).

3.3. Wine Colour and Phenolics

The colour parameters measured by the modified Somer's assay are shown in Table 3. At the end of fermentation, all colour parameters except chemical age I, hue, and total phenolics showed significant differences between the treatments. However, Na bentonite addition seems to have a greater effect on wine colour compared to the other types of bentonites used in this study. The Na bentonite addition showed a significantly higher chemical age II, colour density, SO_2-corrected colour density, and SO_2 resistant pigments, compared to the control treatment. In contrast, the addition of Ca or NaCa bentonite

showed no significant difference in chemical age I, colour density, SO_2 corrected colour density, hue, and SO_2 resistant pigments in wines compared to the control treatment.

Table 3. Colour parameters of resultant wines measured by modified Somer's assay.

Parameters	Control	Na	Ca	NaCa
Chemical age I	0.44 ± 0.02 a	0.47 ± 0.01 a	0.47 ± 0.02 a	0.45 ± 0.01 a
Chemical age II	0.13 ± 0.01 a	0.16 ± 0.01 b	0.17 ± 0.01 b	0.15 ± 0.01 ab
Degree of ionization of anthocyanins (%)	21.11 ± 1.38 a	25.56 ± 1.92 b	26.83 ± 1.90 b	23.18 ± 0.97 ab
Total anthocyanin (mg/L)	170.89 ± 8.06 a	150.89 ± 7.82 b	139.44 ± 7.00 b	153.89 ± 2.84 ab
Colour density	6.37 ± 0.21 a	7.32 ± 0.28 b	7.02 ± 0.50 ab	6.48 ± 0.35 ab
SO_2 corrected colour density	6.45 ± 0.17 a	7.26 ± 0.30 b	6.99 ± 0.36 ab	6.52 ± 0.34 ab
Hue	0.96 ± 0.03 a	1.04 ± 0.03 a	1.01 ± 0.06 a	0.98 ± 0.03 a
SO_2 resistant pigments	1.45 ± 0.05 a	1.67 ± 0.09 b	1.62 ± 0.10 ab	1.48 ± 0.10 ab
Total phenolics	32.88 ± 0.68 a	33.50 ± 0.75 a	30.00 ± 3.00 a	31.42 ± 0.40 a

Different letters in the same row indicate significant differences statistically between treatments.

The total anthocyanin concentration was reduced by 10–18% in all the bentonite-treated wines compared to the control treatment, although the reduction degree varied in each wine. For example, the NaCa bentonite addition did not show a significant difference in the total anthocyanin concentration, while the Na and Ca bentonite addition significantly reduced the total anthocyanin level in wines compared to the control treatment. The degree of ionization of anthocyanin (DIA) in wines ranged from 21.11% to 25.74%, with a significantly higher DIA observed in the Na and Ca bentonite treatments compared to the control treatment. A significantly higher colour density and SO_2 corrected colour density values were observed in Na-bentonite-added treatment. Furthermore, the Na bentonite addition also showed a significantly higher SO_2 resistant pigments compared to the control treatment. However, the Na bentonite-added treatment did not show significantly higher total phenolic concentration but still showed averagely higher total phenolic results compared to the control treatment.

3.4. Wine Aroma Composition Affected by Bentonite Addition

There were 36 aroma compounds, including esters, higher alcohols, terpenes, and volatile phenols, analysed in the resultant Pinot noir wines, and the concentrations of these aroma compounds are shown in Table 4.

There was no significant difference in the concentrations of aroma compounds between treatments except for three aroma compounds, ethyl cinnamate, hexyl acetate, and *cis*-3-hexenol, which showed significantly lower concentrations in the treatments added with bentonite compared to the control. The significantly higher concentrations of ethyl cinnamate, hexyl acetate and *cis*-3-hexenol in the control Pinot noir wines were determined at 1.15 μg/L, 4.3 μg/L, and 51.08 μg/L, respectively. The wines from Na bentonite treatment showed significantly lower concentrations of ethyl cinnamate and hexyl acetate at 0.61 μg/L and 3.48 μg/L, respectively. The wines from the Ca bentonite treatment showed significantly lower concentrations of ethyl cinnamate and *cis*-3-hexenol at 0.55 μg/L and 36.54 μg/L, respectively. The wines from the NaCa bentonite treatment showed significantly lower concentrations of ethyl cinnamate, hexyl acetate, and *cis*-3-hexenol at 0.71 μg/L, 3.54 μg/L, and 38.33 μg/L, respectively.

In general, the addition of bentonite at the level of 0.5 g/L prior to the cold soaking seems to have no impact on the aroma profile in resultant Pinot noir wines except for the aforementioned three aroma compounds.

Table 4. Quantification of aroma compounds in resultant Pinot noir wines.

Aroma Compounds *	Control	Na	Ca	NaCa
Esters				
Ethyl 2-Methyl Butyrate	5.75 ± 0.41 a	5.41 ± 0.57 a	5.07 ± 0.49 a	5.46 ± 0.58 a
Ethyl Hydrocinnamate	0.78 ± 0.17 a	0.59 ± 0.03 a	0.57 ± 0.05 a	0.60 ± 0.06 a
Ethyl Cinnamate	1.15 ± 0.30 a	0.61 ± 0.06 b	0.55 ± 0.08 b	0.71 ± 0.03 b
Ethyl Acetate (mg/L)	58.03 ± 5.80 a	53.46 ± 7.39 a	50.59 ± 2.88 a	57.39 ± 4.93 a
Ethyl Isobutyrate	29.86 ± 2.60 a	26.62 ± 0.74 a	26.08 ± 1.87 a	26.78 ± 2.25 a
Ethyl Butanoate	292.78 ± 18.94 a	285.52 ± 28.12 a	255.54 ± 13.50 a	290.97 ± 29.70 a
Ethyl Isovalerate	5.92 ± 1.27 a	5.48 ± 0.18 a	5.29 ± 0.70 a	6.12 ± 0.79 a
Ethyl Pentanoate	1.77 ± 0.14 a	1.67 ± 0.10 a	1.64 ± 0.10 a	1.76 ± 0.13 a
Ethyl Hexanoate	1020.45 ± 16.90 a	1057.21 ± 66.13 a	1004.36 ± 51.83 a	998.97 ± 60.26 a
Ethyl Lactate	3413.55 ± 161.66 a	3304.15 ± 233.72 a	3258.34 ± 44.85 a	3432.74 ± 123.63 a
Ethyl Heptanoate	8.18 ± 0.29 a	7.66 ± 1.11 a	8.19 ± 0.35 a	7.70 ± 0.86 a
Ethyl Octanoate	2369.14 ± 34.52 a	2659.39 ± 285.58 a	2446.15 ± 131.14 a	2298.64 ± 175.76 a
Isoamyl Acetate	188.70 ± 7.77 a	167.30 ± 7.93 a	156.20 ± 22.90 a	172.45 ± 3.08 a
Isobutyl Acetate	49.99 ± 2.58 a	45.93 ± 2.52 a	37.65 ± 9.11 a	46.00 ± 2.77 a
Octyl Acetate	10.00 ± 0.56 a	10.73 ± 0.45 a	9.15 ± 2.31 a	9.35 ± 0.91 a
2-Phenylethyl Acetate	17.88 ± 1.03 a	16.63 ± 1.50 a	15.21 ± 1.84 a	16.12 ± 0.71 a
Hexyl Acetate	4.30 ± 0.23 a	3.48 ± 0.07 b	4.31 ± 0.49 a	3.54 ± 0.20 b
Higher alcohols				
Trans-2-Hexenol	4.19 ± 0.47 a	4.63 ± 0.48 a	4.07 ± 0.24 a	3.94 ± 0.79 a
1-Octanol	42.49 ± 3.03 a	38.87 ± 5.98 a	34.35 ± 8.90 a	40.42 ± 6.70 a
Isoamyl Alcohol (mg/L)	201.74 ± 6.79 a	199.58 ± 6.91 a	194.38 ± 10.16 a	201.97 ± 2.98 a
Hexanol	2670.13 ± 124.75 a	2445.67 ± 124.61 a	2440.31 ± 77.77 a	2429.43 ± 121.52 a
Trans-3-Hexenol	51.54 ± 3.63 a	49.32 ± 0.71 a	47.94 ± 2.59 a	49.45 ± 2.46 a
Cis-3-Hexenol	51.08 ± 6.76 a	39.56 ± 6.15 ab	36.54 ± 1.98 b	38.33 ± 2.48 b
1-Heptanol	80.03 ± 4.61 a	72.40 ± 4.13 a	72.21 ± 3.32 a	76.55 ± 3.01 a
Phenyethyl Alcohol (mg/L)	59.41 ± 3.70 a	57.66 ± 4.00 a	57.28 ± 1.07 a	58.40 ± 1.65 a
Terpenes				
Linalool	36.65 ± 2.31 a	36.21 ± 1.21 a	35.20 ± 3.22 a	37.91 ± 0.97 a
Citronellol	24.72 ± 0.83 a	24.06 ± 0.18 a	23.89 ± 0.96 a	24.93 ± 0.94 a
Nerol	8.58 ± 0.54 a	8.61 ± 0.60 a	8.13 ± 0.69 a	9.11 ± 0.39 a
β-Damascenone	12.44 ± 0.45 a	11.82 ± 0.46 a	12.30 ± 1.12 a	12.50 ± 0.25 a
Geraniol	9.75 ± 0.39 a	9.73 ± 0.20 a	9.38 ± 0.40 a	10.06 ± 0.47 a
α-Ionone	ND	ND	ND	ND
β-Ionone	0.63 ± 0.06 a	0.64 ± 0.02 a	0.65 ± 0.03 a	0.61 ± 0.00 a
Volatile phenols				
Guaiacol	5.45 ± 0.61 a	4.02 ± 0.68 a	3.81 ± 1.27 a	5.40 ± 1.18 a
Phenol	3.80 ± 0.27 a	3.65 ± 0.12 a	3.98 ± 0.26 a	4.21 ± 0.37 a
4-Ethyl Guaiacol	0.10 ± 0.02 a	0.09 ± 0.01 a	0.10 ± 0.02 a	0.10 ± 0.00 a
Eugenol	3.79 ± 0.16 a	4.06 ± 0.49 a	4.49 ± 0.23 a	4.34 ± 0.22 a

* concentration of aroma compounds is expressed as μg/L, except of three compounds specified in the table that are expressed as mg/L; ND: not detected; different letters in the same row indicate significant differences statistically between treatments.

4. Discussion

As expected, the addition of bentonite prior to the cold soaking had no impact on general oenological parameters. After cold soaking, the concentration of the PR proteins was significantly reduced in all treatments, including the control. The reduction in PR proteins during cold soaking might be due to their interactions with phenolic compounds [27] and a consequent complex may precipitate during the cold soaking process. Treatments added with bentonite showed a further reduction in PR proteins, especially a significant reduction in TLPs observed in Na bentonite treatment. Bentonite can bind PR proteins and remove them, which has been well studied in white wine [28]. The greater reduction in PR proteins by adding Na bentonite could be associated with its higher swelling ability and cation exchange capacity compared to Ca and NaCa bentonites [29].

The reduction in anthocyanins due to the bentonite addition observed in this study is in agreement with previous studies [14,20,30]. The decrease in total anthocyanins could be

associated with the binding ability of bentonite with anthocyanins. The bentonite indirectly binds phenolic compounds that have complexed with proteins and they can also bind anthocyanins, with a resulting loss in wine colour [31]. However, a previous study [32] reported that the addition of Na bentonite at rate of 15 g/100 g of grapes during pre-fermentation maceration increased the malvidin concentration in wines due to the removal of suspended solids and yeast lees in the fermenting juice, which consequently prevents the adsorption of anthocyanins into solids. The contradictory results might be due to the much higher dosage rate of bentonite.

The degree of ionization of anthocyanin (DIA) in wines ranged from 21.11% to 25.74%, which is in the range previously reported in young red wines [33]. A significantly higher DIA were observed in Na and Ca treatments compared to the control treatment. However, bentonite has proven to be an important agent for the removal of colouring matters and is constituted of ionized anthocyanins (flavylium cations), tannins, polysaccharides, and proteins when added in a 0.2–0.5 g/L dose rate in wines [19]. The increased degree of ionization of anthocyanin in bentonite-treated wines may be due to the lower anthocyanin levels observed in Na and Ca treatments, which has been observed in our previous study that wines with lower anthocyanin (e.g., older wines) showed a higher degree of ionization of anthocyanins (data not shown).

The higher colour density and SO_2-corrected colour density values were observed in Na bentonite treatments, which agrees with a previous study on Monastrell wines [31]. The increase in colour density may be attributed to the self-association and co-pigmentation of anthocyanins and the higher concentration of ionized anthocyanins found in wine. Interestingly, some studies [14,30,34] suggested that the addition of bentonite (0.5 g/L) at the end of fermentation could reduce the colour density in wines by 1–7%. This suggests that the wine colour could be influenced by the timing of bentonite addition and vary between grape varieties.

The Na bentonite addition also showed significantly higher SO_2 resistant pigments compared to the control treatment. This might associate with the reduced concentrations of PR proteins observed in Na bentonite treatment. A previous study [22] reported that PR proteins could limit the retention of added tannins and, thus, reducing the PR proteins is likely to aid tannin retention, which may favour the formation of polymeric pigments by the interaction with anthocyanins and increase the colour density in wine.

Although 36 aroma compounds have been analysed in resultant Pinot noir wines, only three aroma compounds—ethyl cinnamate, hexyl acetate, and *cis*-3-hexenol—showed significant differences between the treatments. Ethyl cinnamate could contribute to sweet floral notes to the wine, but it has a relatively short life in wine as it can slowly hydrolyse to form alcohol [35], so its reduction in bentonite-added treatments may have little impact on Pinot noir in long term. The concentration of hexyl acetate, contributing red berry aromas to the wine, was significantly reduced in the Na and NaCa bentonite treatments, but its concentrations in all the treatments were almost 100 times lower than its perception threshold at 400 µg/L [36]. The *cis*-3-hexenol has been reported to be associated with grassy herbaceous notes in wine [37]. The lower level of *cis*-3-hexenol in Pinot noir wines produced from bentonite treatments may reduce the green notes that are normally negatively associated with Pinot noir wine quality [38].

5. Conclusions

This study reveals that the bentonite addition prior to fermentation had a significant impact on the Pinot noir wine colour but little impact on the aroma profile at the end of fermentation. One aroma compound, *cis*-3-hexenol, showed significantly lower levels in bentonite-added treatments, which may positively contribute to Pinot noir wine quality by reducing the negative green notes. The significantly higher level of colour density and SO_2 resistant pigments observed in Na bentonite treatment suggests more colour stability in resultant wine, which is beneficial for varieties similar to Pinot noir that are challenging in colour extraction. In this study, PR proteins still remained after cold soaking, indicating the

dosage rate at 0.5 g/L is not sufficient to remove all the PR proteins in the must. Further studies could investigate if a higher dosage rate of bentonite addition could further increase the formation of un-bleachable pigments and even increase the extraction of tannins in Pinot noir wine, without having a negative impact on the aroma profile.

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