Research Note
The Influence of Micro-oxygenation on the Long-term Ageing Ability of Pinot noir Wine

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In this study, Pinot noir wines were bottle aged for 12 and 18 months after micro-oxygenation (MOX) applied before or after malolactic fermentation (MLF) at two doses (10.8 and 52.4 mg/L/month). After ageing, a greater decrease in the total SO_2 concentration was found in wines with the higher MOX dosage, demonstrating a long-term impact of higher oxygen exposure on wines' SO_2 requirement. Meanwhile, a negative impact of MOX on wine colour development occurred over time, resulting in a large loss of colour measures (i.e., 420 nm for brown hues, 520 nm for red colour, SO_2 resistant pigments, and colour intensity), which was greater with the early oxygen exposure. This was linked to a significantly lower content of large polymeric pigments in MOX treatments. Tannin concentration was, in the end, not affected by the MOX treatments. However, regarding tannin composition, considerably higher (-)-epicatechin extension units but much lower (-)-epicatechin terminal units were found with MOX treatments. In addition, a significant reduction of tannin trihydroxylation (%Tri-OH) but a higher galloylation (%Galloyl) and mean degree of tannin polymerisation (mDP) remained in wines with MOX, indicating a long-term negative influence on astringency intensity.

INTRODUCTION

Over the years, it has been observed that Pinot noir wines are lighter in colour than other red table wines (Burns and Osborne 2013, Casassa *et al*., 2015) and usually have poor colour development, low pigment stability, and limited ageing potential (Sacchi *et al*., 2005, Sparrow *et al*., 2016). These issues have been associated with Pinot noir grape phenolic composition: the lower phenolic extractability and skin to seed tannin ratio, the lack of acylated anthocyanins in the skin, as well as fewer cofactors for copigmentation (Boulton *et al*., 2001, Casassa *et al* 2015, Sparrow *et al*., 2016).

Previous studies have shown that micro-oxygenation (MOX) can be a useful tool for promoting colour development and stability in Pinot noir wines (Durner *et al*., 2010, Yang *et al*., 2022 a). It involves redox cycling of Fe (II) and Fe (III) that affects phenolic oxidation (Danilewicz 2016), during which acetaldehyde may form and induce tannin and anthocyanin modifications, yielding more stable polymeric pigments (Anli and Cavuldak 2012, Peterson and Waterhouse 2016). However, in Yang *et al*., (2022 b), tannin concentration increased after MOX with a higher degree of galloylation and a large decrease of (-)-epigallocatechin extension units, both of which have been associated with an increase of astringency intensity in the literature (Huang and Xu 2020). This could be a problem for the overall wine quality (McRae and Kennedy 2011). However, wine ageing in the bottle is also critical for final wine quality, given that oxygen ingress through the bottle closure can further alter colour and phenolic composition (Avizcuri *et al*., 2016).

To our knowledge, the long-term impact of MOX on Pinot noir wines after bottle ageing has not been reported before. In the present study, Pinot noir wines after MOX, as described by Yang *et al*., (2022 a) and Yang *et al*., (2022 b), were analysed after 12 and 18 months of bottle ageing. The results on wine colour parameters, polymeric pigment content, and tannin composition are reported.

MATERIALS AND METHODS

Pinot noir wine and micro-oxygenation

Vitis vinifera L. cv. Pinot Noir wine (100%) produced from the 2019 vintage from Marlborough, New Zealand was treated with MOX before or after malolactic fermentation (MLF) at two oxygen doses (10.8 and 52.4 mg/L/month) for 30 days. Winemaking protocols and experimental design are

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described in Yang *et al*., (2022 a). Treatments are labeled as follows: T1 (MOX applied before MLF at 10.8 mg/L/month), T2 (MOX applied before MLF at 52.4 mg/L/month), and C1 (the pre-MLF MOX control treatment received no oxygen); T3 (MOX applied after MLF at 10.8 mg/L/month), T4 (MOX applied after MLF at 52.4 mg/L/month), and C2 (the post-MLF MOX control treatment received no oxygen).

SO_2 additions, wine bottling and storage conditions

Before bottling, the free SO_2 concentration was adjusted to 42.5 ± 2.3 mg/L in each treatment. The total SO_2 concentration in each treatment was: C1, 85.6 ± 5.2 mg/L; T1, 78.7 ± 1.0 mg/L; T2, 85.2 ± 2.9 mg/L; C2, 91.2 ± 1.6 mg/L; T3, 77.6 ± 3.4 mg/L; T4, 81.6 ± 4.1 mg/L.

The wine from each control and MOX treatment was sterile filtered at 0.2 µm pore size (Sartopore 2 MaxiCaps, Göttingen, Germany) and bottled under the same conditions in 750 mL olive green Bordeaux-style glass bottles and sealed with Oenoseal (Guala Closures Group, Auckland, New Zealand) screwcap enclosures (oxygen transmission rate <0.008 cc/day/bottle). Upon bottling, N_2 was first purged into the bottles and was maintained over the headspace of each MOX and control vessel to avoid oxygen exposure. The dissolved oxygen (DO) in wine after bottling was measured using the NomaSense™ O_2 Trace Oxygen PSt3 dipping probe (LOD, 15 µg/L). For bottled wines in the ageing trial, DO accumulation was not detected. Afterwards, the wines were boxed and stored at 9°C in the upright position and analysed after 12 and 18 months of bottle ageing.

Basic wine composition

Wine pH and TA were measured using an automatic wine titrator (Hanna Instruments, Woonsocket, RI, USA) equipped with a pH meter that was calibrated at pH 4 and 7. Free and total SO_2 [limit of detection (LOD): 2 and 5.28 mg/L, respectively] were determined using Megazyme enzymatic test kits (Megazyme, Bray, Ireland).

Spectrophotometric analyses

Colour absorbances (Iland *et al*., 2013), the Harbertson-Adams assay (Heredia *et al*., 2006) and tannin reactive with methylcellulose (Mercurio *et al*., 2007) were determined accordingly and measured by a PharmaSpec UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan). The concentration of anthocyanins and tannins was determined as malvidin-3-glucoside equivalents (mg/L) and (-)-epicatechin equivalents (g/L), respectively.

Tannin extraction and high-performance liquid chromatography analyses of tannins

The method for tannin extraction was as detailed by Yang *et al*., (2022 b). The extracts were reconstituted to 10 g/L using pure methanol, according to the tannin concentration of the wine sample. The methanolic extracts were then kept in a -80°C freezer until analysis. Tannin composition was analysed on a reversed-phased C18 column (Kinetex C18, 2.6 µm, 100 Å, 100 mm x 4.6 mm) using an Agilent Technologies 1200 series HPLC system (Santa Clara, CA, USA) and equipped with a G1315D diode array detector. The main detection wavelength was 280 nm and tannin subunits (i.e., flavan-3-ol monomers as terminal units and phloroglucinol adducts as extension units) were calculated according to their concentrations expressed as (−)-epicatechin equivalents (in moles). The mean degree of polymerisation was calculated using the sum of all tannin subunits divided by the sum of all terminal units. The tannin mass conversion (yield %) was determined by using the total mass of all tannin subunits (in grams), which excluded the phloroglucinol portion of the adducts, divided by the concentration of tannins (i.e., 10 g/L) used for the analysis.

Sampling

Three bottles from each treatment were selected and the bottles opened. The analyses of free and total SO_2 concentrations, colour absorbances, the Harbertson-Adams assay and tannin concentration were carried out in a day. Afterwards the headspace of each bottle was evacuated with N_2 and bottles closed and stored at -3°C until phenolic analyses. Tannin extraction was conducted on the second day after bottles were opened. Later, the analyses of pH and TA were completed within 72 h. All measurements were carried out in duplicates (i.e., six replications for each treatment).

Chemicals and reagents

All calibration standards were of HPLC grade quality and chemical reagents were of the highest available analytical grade quality. Milli-Q water was obtained from the Thermo Scientific Barnstead Nanopure water purification system.

Statistical analyses

The statistical analyses for the ANOVA analyses were performed in R Studio version 1.2.5033 (R version 4.0.3, Boston, MA, USA). Correlation matrices of the colour parameters were also produced in R Studio, where the correlation coefficient (r) was calculated using the Pearson's method and organised in the hierarchical clustering order. The levels of correlations with raw p value <0.05 were considered significant.

RESULTS AND DISCUSSION

A comparison of basic composition of wines with and without MOX are shown in Table 1. The pH and TA of wines were not affected by MOX treatments. Both free and total SO_2 concentrations decreased in all wines over the 18 months. However, the total SO_2 concentration was considerably lower in T2 and T4, whereas the free SO_2 concentration did not significantly differ among treatments. It has been shown that release of bound SO_2 occurs in wines during bottle ageing, which compensates for the loss of free SO_2 due to oxygen ingress (Waterhouse *et al*., 2016) and other reactions of tannins with SO_2 (Ma *et al*., 2018). In wines of T2 and T4, the higher oxygen dosage applied during MOX could have resulted in oxidation reactions where quinones, acetaldehyde and other electrophiles react with nucleophiles such as flavanols. As a result, free SO_2 could be the only antioxidant protecting the wines from the oxygen ingress, thus the faster depletion during ageing (Gambuti *et al*., 2019) and more bound SO_2 dissociated to maintain a minimal amount of free SO_2 (Waterhouse *et al*., 2016). Similar results were found in Waterhouse *et al*., (2016) where Chardonnay wines exposed

TABLE 1
Wine pH, TA, free and total SO_2 of the control and the MOX treatments after 12 and 18 months bottle ageing (mean ± standard error, n = 6).

Timeline & Treatments	Parameters			
	pH	TA (g/L)	Free SO_2 (mg/L)	Total SO_2 (mg/L)
After 12 months ageing				
C1	3.5 ± 0.1 a	6.2 ± 0.1 a	24.7 ± 4.4 a	70.6 ± 4.0 a
T1 (low oxygen dose)	3.5 ± 0.0 a	6.2 ± 0.0 a	30.4 ± 3.5 a	63.3 ± 3.7 ab
T2 (high oxygen dose)	3.5 ± 0.0 a	6.0 ± 0.1 a	29.3 ± 4.6 a	48.1 ± 2.4 bc
C2	3.5 ± 0.0 a	6.2 ± 0.1 a	26.4 ± 3.8 a	72.7 ± 2.7 a
T3 (low oxygen dose)	3.5 ± 0.0 a	6.2 ± 0.1 a	26.9 ± 4.2 a	50.9 ± 3.1 bc
T4 (high oxygen dose)	3.5 ± 0.0 a	6.2 ± 0.2 a	25.9 ± 3.5 a	44.3 ± 6.2 c
After 18 months ageing				
C1	3.6 ± 0.0 a	6.1 ± 0.1 a	14.1 ± 4.5 a	70.8 ± 2.1 a
T1 (low oxygen dose)	3.6 ± 0.0 a	6.0 ± 0.0 a	22.2 ± 5.1 a	69.3 ± 4.3 a
T2 (high oxygen dose)	3.6 ± 0.0 a	6.0 ± 0.1 a	22.3 ± 1.6 a	54.6 ± 6.2 ab
C2	3.5 ± 0.0 a	6.2 ± 0.0 a	21.7 ± 1.6 a	73.9 ± 1.8 a
T3 (low oxygen dose)	3.6 ± 0.1 a	6.0 ± 0.1 a	22.6 ± 2.1 a	57.4 ± 5.1 ab
T4 (high oxygen dose)	3.5 ± 0.0 a	6.2 ± 0.0 a	17.1 ± 3.3 a	46.8 ± 6.4 b

to 4% oxygen during bottle ageing for 11 months had a higher loss of total than free SO_2 concentration.

Concerning wine colour, although MOX had promoted colour development at the time of bottling, during bottle ageing, wines with MOX, regardless of dosage and timing, had a large decline in the absorbance at 420 nm (Fig. 1a) and 520 nm (Fig. 1b), and thus overall colour intensity measurement (Fig. 1c). Wines of the control treatments, on the contrary, had only a slight decrease in these colour measures. In addition, the SO_2 resistant pigments (Fig. 1d) increased in the control treatments and became notably higher than the pre-MLF MOX treatments (T1 and T2). Among wines with MOX, post-MLF MOX treatments (T3 and T4) had increased absorbance at 520 nm and higher colour intensity than T1 and T2. After 18 months, the colour readings were no longer significantly different between the post-MLF MOX treatments (T3 and T4) and the control treatments, however, the absorbances were much lower in the pre-MLF MOX treatments (T1 and T2).

Therefore, although MOX promoted the colour development of Pinot noir wines after the treatment (Yang *et al*., 2022 a), it had negatively affected the long-term colour development of the wines, especially with the early oxygen exposure. The results are contrary to previous reports, where MOX was shown to stabilise colour intensity and maturity of Cabernet Sauvignon wines (Gambuti *et al*., 2019) and Cabernet Sauvignon blends (Oberholster *et al*., 2015) during bottle ageing. Besides the variation on MOX application, this might also be due to the significance of grape variety, Cabernet Sauvignon versus Pinot noir, as well as the regional and winemaking variations. For Pinot noir wines, an issue could be the lower phenolic content and the lack of acetylated anthocyanins, resulting in the low pigment stability and limited ageing potential.

Within the first 12 months of bottle ageing, the total anthocyanin concentration decreased by 17 to 18% in the control treatments and up to 10 % in T3 and T4 but remained relatively unchanged in T1 and T2 (Fig. 2). Later at 18 months, a further decline was found in all wines (i.e., between 9 and 24 %) and the final concentration of total anthocyanins did not differ significantly among treatments. The concentration of small polymeric pigments (SPP) increased in all wines during bottle ageing (Fig. 2) and was not affected by MOX treatments. For large polymeric pigments (LPP), however, the concentration decreased in all wines (Fig. 2) and was significantly lower in wines with MOX than the control treatments. In addition, the content of LPP was positively correlated with the 520 nm absorbance, SO_2 resistant pigments, and colour intensity at both 12 (Fig. 3a) and 18 months (Fig. 3b) and to the 420 nm absorbance at 18 months bottle ageing. Therefore, the induced colour loss in wines with MOX appears to be associated with the loss of LPP that would be due to further cleavage and addition reactions, leading to structural diversity among the polyphenols (Cheynier *et al*., 2006). In a study conducted by Teng *et al*., (2021), higher concentrations of ethyl-linked anthocyanins were found in tannin precipitates, indicating the incorporation of polymeric pigments into tannins.

Regarding the tannin profile, tannin concentration was not significantly different among treatments (Table 2). The mean degree of polymerisation (mDP), however, was higher in wines with MOX (by 0.3 to 0.5 units). Tannin molecules with higher mDP values have been positively linked to the astringency perception, by having more binding sites for interactions with salivary proteins (Huang and Xu 2020). A lower tannin mass conversion (yield%), indicating a

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higher proportion of tannin macromolecules that could not be easily depolymerised, can also be seen in wines with MOX, although it did not always significantly differ from the control treatments.

Compared to the control treatments, MOX treatments had a much higher proportion of (-)-epicatechin extension units, which was seen together with a much lower percentage of (-)-epicatechin terminal units. It is known that (-)-epicatechin in extension positions positively add to the perceived astringency intensity (Huang and Xu 2020). For grape seed derived tannins, a decrease of %Galloyl was found in all wines with the extended bottle aging, which

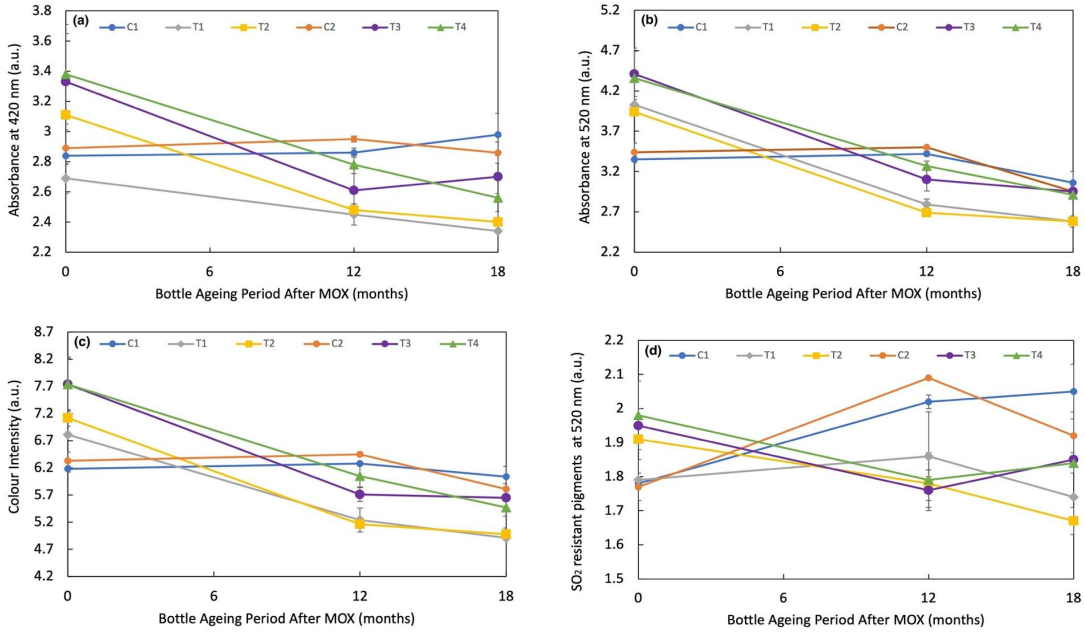

FIGURE 1

Evolution of colour absorbances during bottle ageing in wines with micro-oxygenation (MOX) at 10.8 mg O_2/(L · month) (◆,●) and 52.4 mg O_2/(L · month) (■, ▲) before (◆,■) and after MLF (●, ▲) compared to the control treatments (●, ●) by spectrophotometric measures at (a) 420 nm, (b) 520 nm, (c) colour intensity (420 + 520 nm), and (d) resistant pigments at 520 nm (mean ± SE, n = 6).

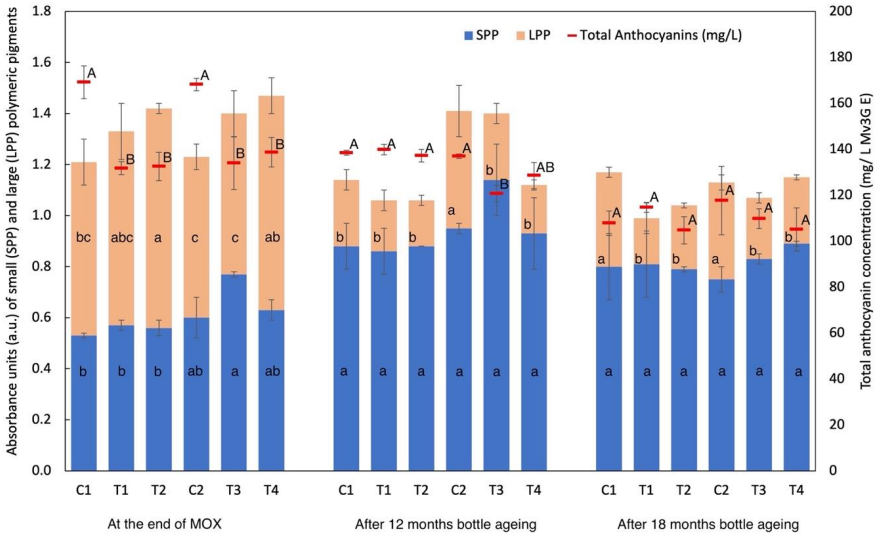

FIGURE 2

The changes of small (SPP) and large (LPP) polymeric pigments and total anthocyanins (mg/L, malvidin-3-glucoside equivalent) in wines treated with and without MOX after 12 and 18 months of bottle ageing. C1: pre-MLF MOX control wines; T1: pre-MLF MOX treatment at 10.8 mg/L/month; T2: pre-MLF MOX treatment at 55.2 mg/L/month; C2: post-MLF MOX control wines: T3: post-MLF MOX treatment at 10.8 mg/L/month; T4: post-MLF MOX treatment at 55.2 mg/L/month. Different lower-case letters indicate statistical differences in LPP and SPP among treatments, while different capital letters differentiate statistical differences in total anthocyanins among treatments ($p < 0.05$).

TABLE 2
Tannin composition of control and MOX treated Pinot Noir wines after 12 and 18 months bottle ageing (mean ± standard error, $n = 6$).

Timeline & Treatments	Parameters											
	MCP Tannin	mDP	%Tri-OH	%Galloyl	Extension Unit (mole %)			Terminal Unit (mole %)				Yield%
					EGC	CAT	EPI	ECG	CAT	EPI	ECG	
After 12 months ageing												
C1	0.75 ± 0.07 a	2.5 ± 0.0 c	12.4 ± 0.1 a	3.6 ± 0.3 a	12.4 ± 0.1 a	7.9 ± 0.0 a	47.1 ± 0.1 bc	1.8 ± 0.4 a	15.9 ± 0.0 b	13.1 ± 0.3 a	1.8 ± 0.0 c	60.1 ± 0.2 a
T1 (low oxygen dose)	0.82 ± 0.04 a	2.9 ± 0.0 a	11.6 ± 0.3 ab	3.7 ± 0.1 a	11.6 ± 0.3 ab	8.2 ± 0.0 a	51.9 ± 0.4 a	1.5 ± 0.0 a	16.6 ± 0.2 ab	8.1 ± 0.3 c	2.2 ± 0.1 bc	51.3 ± 1.6 ab
T2 (high oxygen dose)	0.81 ± 0.04 a	2.7 ± 0.0 b	9.7 ± 1.0 b	4.1 ± 0.1 a	9.7 ± 1.0 b	7.7 ± 0.2 a	52.2 ± 1.0 a	1.6 ± 0.0 a	17.0 ± 0.2 a	9.4 ± 0.3 bc	2.4 ± 0.1 a	49.1 ± 4.6 bc
C2	0.73 ± 0.02 a	2.5 ± 0.0 c	12.5 ± 0.1 a	4.0 ± 0.0 a	12.5 ± 0.1 a	7.7 ± 0.5 a	46.8 ± 0.7 c	1.5 ± 0.0 a	16.9 ± 0.1 a	12.1 ± 0.0 a	2.5 ± 0.0 ab	51.3 ± 0.5 abc
T3 (low oxygen dose)	0.84 ± 0.06 a	2.7 ± 0.0 b	11.4 ± 0.7 ab	4.3± 0.1 a	11.4 ± 0.7 ab	7.9 ± 0.3 a	49.6 ± 0.6 ab	1.6 ± 0.0 a	17.2 ± 0.4 a	9.7 ± 0.4 b	2.6 ± 0.1 a	45.0 ± 0.3 bc
T4 (high oxygen dose)	0.86 ± 0.06 a	2.7 ± 0.1 b	10.3 ± 0.2 ab	4.0 ± 0.1 a	10.3 ± 0.2 ab	8.2 ± 0.1 a	51.4 ± 0.5 a	1.6 ± 0.0 a	17.0 ± 0.1 a	9.4 ± 0.4 b	2.4 ± 0.1 bc	42.1 ± 1.1 c
After 18 months ageing												
C1	0.83 ± 0.01 a	2.6 ± 0.0 b	7.0 ± 0.8 ab	1.4 ± 0.1 b	7.0 ± 0.8 ab	9.3 ± 0.2 a	53.3 ± 0.3 bc	1.1 ± 0.1 b	18.2 ± 0.2 a	10.9 ± 0.2 a	0.4 ± 0.1 a	57.3 ± 1.8 a
T1 (low oxygen dose)	0.96 ± 0.04 a	3.1 ± 0.1 a	5.9 ± 0.2 bc	2.4 ± 0.2 a	5.9 ± 0.2 bc	8.7 ± 0.1 a	59.1 ± 0.8 a	2.0 ± 0.2 a	15.3 ± 0.4 bc	8.7 ± 0.3 b	0.3 ± 0.1 a	55.3 ± 1.9 ab
T2 (high oxygen dose)	1.02 ± 0.04 a	3.2 ± 0.1 a	5.6 ± 0.3 c	2.3 ± 0.1 a	5.6 ± 0.3 c	8.7 ± 0.1 a	59.6 ± 1.1 a	2.1 ± 0.1 a	15.0 ± 0.5 c	8.8 ± 0.4 b	0.3 ± 0.0 a	52.0 ± 2.0 abc
C2	0.89 ± 0.01 a	2.6 ± 0.0 b	7.5 ± 0.3 a	1.4 ± 0.0 b	7.5 ± 0.3 a	8.9 ± 0.4 a	52.2 ± 0.4 c	1.0 ± 0.1 b	18.5 ± 0.0 a	11.4 ± 0.5 a	0.4 ± 0.0 a	56.1 ± 0.5 ab
T3 (low oxygen dose)	0.93 ± 0.10 a	2.8 ± 0.1 ab	5.9 ± 0.2 bc	2.5 ± 0.1 a	5.9 ± 0.2 bc	8.6 ± 0.2 a	56.5 ± 1.4 ab	2.0 ± 0.2 a	17.1 ± 0.8 ab	9.4 ± 0.4 b	0.6 ± 0.1 a	46.4 ± 2.8 c
T4 (high oxygen dose)	1.01 ± 0.04 a	3.0 ± 0.1 a	6.2 ± 0.2 bc	2.3 ± 0.1 a	6.2 ± 0.2 bc	8.7 ± 0.1 a	58.2 ± 0.6 a	1.8 ± 0.0 a	16.0 ± 0.3 bc	8.7 ± 0.3 b	0.5 ± 0.1 a	48.2 ± 1.3 bc

Values with different letters are significantly different ($P < 0.05$). MCP Tannin (g/L), tannin concentration determined by the methylcellulose tannin precipitation assay; mDP, the mean degree of polymerization; %Tri-OH, the percentage of trihydroxylation; %Galloyl, the percentage of galloylation; Extension and terminal Unit (mole%): EGC, (-)-epigallocatechin, CAT, (+)-catechin, EPI, Epicatechin and ECG, (-)-epicatechin gallate; yield%, the percentage of tannin mass conversion.

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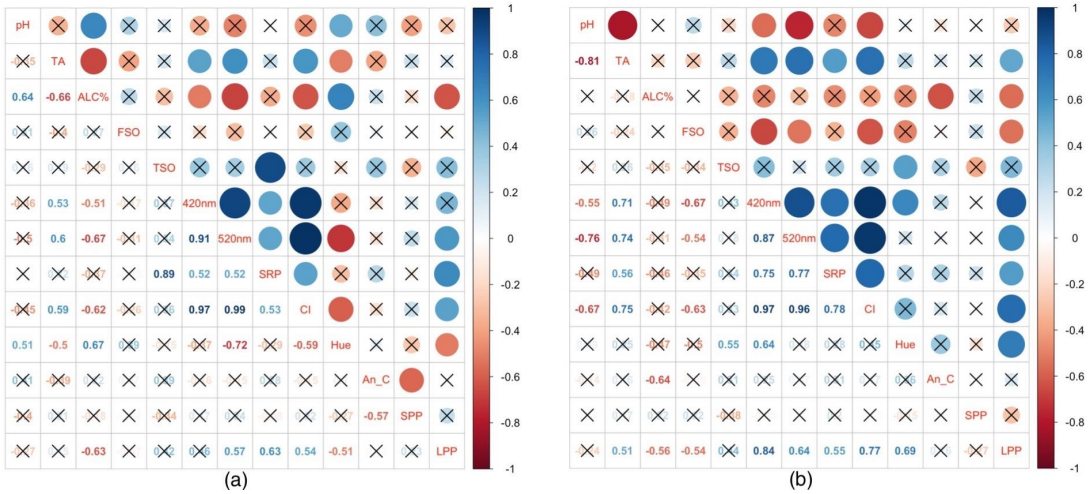

FIGURE 3

Correlation at 12 (a) and 18 months (b) bottle ageing between wine basic composition (pH, TA and alcohol content, ALC%), free and total SO_2 concentration (FSO and TSO), colour absorbance at 420 nm and 520 nm, SO_2 resistant pigments (SRP), colour intensity (CI), wine hue (Hue), total anthocyanin concentration (An_C), small and large polymeric pigments (SPP and LPP). Non-significant correlation ($P > 0.05$) is crossed off.

was mainly due to the decline of (-)-epicatechin-O-gallate terminal units. However, the overall %−Galloyl remained higher in the MOX treatments. For grape skin derived tannins, a decrease of %−Tri-OH was also found in all wines at the end, but the concentration remained much lower in the MOX treatments. In the literature, (−)-epigallocatechin has been shown to minimise or mask the "coarse" sensations from tannins (Vidal *et al*., 2003) and induce a softer taste in skin tannins (Ma *et al*., 2014). The results of this study have indicated that MOX has an impact on the Pinot noir wines' tannin composition, potentially leading to an increase of wine astringency perception even after bottle ageing for 18 months.

CONCLUSIONS

Overall, our results suggest that SO_2 concentration at bottling should be a critical factor determining the long-term ageing ability of wines treated with MOX. For wines with higher oxygen exposure, a higher SO_2 addition rate at bottling would be required to protect from oxygen ingress. Colour development of the studied Pinot noir wines was associated with the higher loss of large polymeric pigments. Meanwhile, the early oxygen exposure (T1 and T2) considerably reduced the wines' long-term ageing ability, resulting in a greater decline in the 520 nm absorbance, SO_2 resistant pigments, and colour intensity. Finally, the content of %− Tri-OH remained significantly lower in wines with MOX and together with other MOX induced changes in tannin composition, favouring the increase of astringency intensity. More research is required to investigate the long-term impact of MOX on light-colour Pinot noir wines, especially for the sensory impacts.

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