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Characterisation and extractability of tannins in Pinot noir grape skin, seed, and stem: Impact of leaf removal, clone, and rootstock

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ABSTRACT

This study examined the impact of leaf removal timing, rootstock, and clone selection on Pinot noir grape composition and subsequent impact on tannin extractability. Results showed that leaf removal increased anthocyanin levels in berries and tannins in stems and seeds, while also raising the mean degree of polymerisation (mDP) of skin tannins. Schwarzman rootstock had lower seed tannins but higher mDP in skin and seed tannins compared to own roots. UCD5 clone exhibited higher mDP in skin tannins than AM10/5 clones in acetone extraction. Tannin extractability was determined by the ratio of model wine-extracted tannins and acetone-extracted tannins. For the first time, this study reported that stems had the highest tannin extractability (64%-78%) compared to skins (37–52%) and seeds (26–34%). Compositional differences in grape tissues from different treatments had no impact on tannin extractability except for grape skins from two treatments. This suggests that tannin extractability is mainly influenced by grape ripeness which largely determines the composition of cell wall materials that can interact with tannins and thus influence tannin extractability. The differences in grape skin tannin extractability observed in this study between treatments were likely due to the significantly different galloylation (G%) levels in the extracted tannins.

1. Introduction

Tannins play a crucial role in wine quality, directly influencing taste and mouthfeel, while also indirectly affecting its colour through interactions with anthocyanins to form pigmented tannins (Cheynier et al., 2006; Hanlin et al., 2010). The knowledge about grape tannin concentration, composition, and extractability can provide insightful information for effective tannin management, enabling the production of high-quality wine. Pinot noir winemaking can be challenging due to its thin skin and lower skin-to-seed tannin ratio, leading to lower tannin and anthocyanin levels being extracted during winemaking (Carew et al., 2013; Harrison, 2018). Many previous studies have reported that Pinot noir wines tend to have much lighter colour and lower tannin concentration compared to other red wines (Dimitrovska et al., 2011; Harbertson et al., 2008; Kennedy, 2008). Hence, different vineyard management strategies and winemaking interventions are employed in Pinot noir winemaking to optimize tannin extraction and stabilise colour (Kemp et al., 2011; Wimalasiri et al., 2023; Wimalasiri et al., 2021).

The flavonoid concentration and composition of wine are largely determined by the composition of the grapes at harvest (Downey et al., 2006) as grape skins, seeds, and stems are the main sources of tannins in wine (Kennedy, 2008; Wimalasiri et al., 2022). Many researches studied the tannin development in grape tissues, and factors influencing their concentration and composition in grapes. In general, the studies on Pinot noir berry development have observed that both skin and seed tannins reach maximal concentration near véraison, followed by a decreasing trend towards harvest (del Rio and Kennedy, 2006; Harbertson et al., 2002). At harvest, the highest tannin concentration in Pinot noir berries was found in the seeds which was 3–5 times higher than per berry skin tannin (Cortell and Kennedy, 2006; Sparrow et al., 2015).

Factors influencing the concentration and composition of tannins in grape tissues include cultivar and rootstock (Berdeja et al., 2014; Blank et al., 2022; Casassa et al., 2021), viticultural practices (e.g. leaf removal, irrigation, and nitrogen application) (Berdeja et al., 2014; Cortell and Kennedy, 2006; Kemp et al., 2011), and environmental factors (e.g. location, vintage) (Chira et al., 2011). A recent study on Pinot noir in Edna Valley observed that wines made from 777 and 2A

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clones were higher in phenolics than those of clone 115, confirming the influence of cultivar and clone on the flavonoid biosynthesis in grapes (Casassa et al., 2021). Concerning the influence of rootstock, many previous studies have reported that rootstock had a small or no influence on the tannin concentration in Chardonnay, Merlot, Cabernet Sauvignon, and Syrah varieties (Harbertson and Keller, 2012; Koundouras et al., 2008). However, a study on Pinot noir conducted in Rheingau of Germany demonstrated that scions grafted onto SO4 (high vigour rootstock) were characterised by a 15% higher tannin concentration in seed and skin tissues compared to those grafted onto the low vigour Riparia rootstock (Blank et al., 2022), although, the wines produced from the two rootstocks did not exhibit a significant difference in tannin concentration. The influence of mechanical leaf removal on Pinot noir berry and resulting wine composition has been investigated in previous studies (Cortell and Kennedy, 2006; Kemp et al., 2011). The leaf removal study conducted by Kemp et al. (2011) in Waipara, New Zealand found no significant effect on grape tannin concentration but had higher tannin concentrations in wines made from earlier leaf removal treatments and had no influence on wine tannin composition. In Oregon, fruit exposure increased tannin concentration in berries, and altered subunit proportions in skins and seeds (Cortell and Kennedy, 2006). More noticeably, exposed berries exhibited higher B ring tri-hydroxylation and resulted in a higher proportion of epigallocatechin extension subunits in skins. These discrepancies could be attributed to the differences in geographical location, vintage, and the tannin extraction and analysis protocols used in these studies.

Grape skins are an important source of tannins and anthocyanins to be extracted in winemaking. Grape skin tannins are easier to extract compared to seed tannins (Canals et al., 2005; Rousserie et al., 2019). According to previous studies on Pinot noir and Tempranillo (Canals et al., 2005; Sparrow et al., 2015), concentration of skin tannins plateaued after 4–6 days of fermentation, whereas extraction of seed tannins continued progressively, possibly due to the physical structure of the seeds (Canals et al., 2005; Cerpa-Calderón and Kennedy, 2008; González-Manzano et al., 2004; Rousserie et al., 2020). Adams (2006) proposed that the extraction of tannins from seeds is influenced by the inherent leakiness or lack thereof of the thin-walled cells surrounding the inner lignified layers and the diffusion barrier posed by the surrounding cuticle of the seed coat. In addition, berry ripeness also plays a major role in tannin extractability. Bautista-Ortín et al. (2012) observed a decrease in seed tannin extractability in three different grape varieties (Cabernet Sauvignon, Shiraz and Monastrell) during ripening and suggested this could be due to the histochemical changes occurring in seeds during maturation. Another study on Cabernet Sauvignon skins conducted by Bindon et al. (2014) also observed a decreasing skin tannin extractability and suggested this may be due to the adsorption of tannin into highly porous cell walls in ripened grapes. Moreover, the tannin and cell wall material (CWM) interactions also play an important role in tannin extractability because tannin binding to CWM such as proteins, and polysaccharides through hydrogen bonds and hydrophobic interactions could limit tannin extraction from grape tissues during berry ripening (Bindon et al., 2012; González-Centeno et al., 2010; Hanlin et al., 2010).

The existing literature has well-documented how various viticultural practices, e.g. clone selection, rootstock selection, and grape ripening, influence Pinot noir grape tannin concentration and composition (Blank et al., 2022; Casassa et al., 2021; Cortell and Kennedy, 2006; Kemp et al., 2011), but few studies investigated the influence of these common practices on the extractability of tannins from grape tissues. In the present study, a comprehensive analysis was conducted to elucidate the influence of initial tannin concentration and composition within different grape tissues on their subsequent extractability. To our knowledge, this represents a novel approach for winemakers to manage tannin extraction, as prior studies have provided limited insights into this specific aspect of tannin dynamics during harvest.

2. Materials and methods

2.1. Experimental design and grape sampling

Whole clusters of Pinot noir grape samples were collected at harvest in 2021 from the Pegasus Bay vineyard in Waipara, New Zealand. The treatments used in this study are outlined in Table 1. Mechanical leaf removal was applied on both sides of the rows using a Collard E2200 pulsed-air leaf removal machine (Wilyabrup Western Australia, Australia) to obtain 50% cluster exposure in the fruiting zone by visual assessment and maintained until harvest. In this study, the impact of leaf removal timing on berry composition was assessed by comparison among AM0, AM7, AM30 and AM60 treatments, the impact of rootstock was assessed by comparison between AM30 and AMS30 treatments, and the impact of clone selection was assessed by comparison between AMS30 and UCDS30 treatments.

The vines, 25 years old and spaced 1.3 m apart with a 2.7-meter gap between rows, had approximately 200 vines per row. The vines were spur pruned on each side with 8 buds, drip-irrigated, and trained on a vertical shoot position (VSP) trellis system. To obtain a representative vineyard sample, 30 whole bunches were collected randomly at the harvest with no more than two bunches taken per vine. The whole bunches were transported to the laboratory on ice, and the weight of each whole bunch was recorded. Grape berries were then sampled from different positions of each bunch. In total, six lots of 30 berries (three lots for acetone extraction and three lots for model wine extraction) were randomly selected and weighed, and then stored at −20 °C for tannin extraction. Samples were stored at −20 °C until further processing. The stems were separated and measured for the stem weight and stored at −20 °C until further processing. The frozen berries were sliced transversely without cutting through the seeds using a scalpel. Skins and seeds were then rinsed with deionised water and gently dried with a paper towel. Recovered skins and seeds were weighed and the number of seeds was counted. For grape stem samples, the frozen stems from at least 10 bunches were cut into small pieces (<20 mm) and used for the extraction. Fresh tissues were used for the model wine extraction, while freeze-dried tissues were used for the acetone extraction.

2.2. Optimisation of the tannin extraction process

The effect of the extraction time and the optimum solid-to-liquid ratio were determined when extracting tannin using 2:1 acetone-water. Four solid-to-liquid ratios (g sample/mL solvent: 1/10, 1/25, 1/50, 1/75) were used to extract tannin from the freeze-dried tissues using acetone for 48 hr on a tumbler at 60 rpm speed. The headspace in the tubes was sparged with nitrogen to avoid oxidation. During the extraction, a 1 mL sample was taken at 6, 12, 24, 36, and 48 hr intervals and centrifuged at 4750 g for 10 minutes using a Heraeus Multifuge X1R centrifuge (Thermo Fisher Scientific, Lower Saxony, Germany) to remove solid material. The acetone extracts were vacuum evaporated to remove all acetone and adjusted back to 1 mL using deionised water to avoid interference with the absorbance reading at 280 nm in the MCP tannin assay (Corona et al., 2015). After that, the aqueous extracts were analysed for tannin concentration as described below. Optimised time and the solid-to-liquid ratios were used to extract tannin from the grape

Table 1
Summary of the treatments.

Treatment	Clone	Rootstock	50% Leaf removal (Days after flowering)
AM0	AM10/5	Own	No leaf removal
AM7	AM10/5	Own	7
AM30	AM10/5	Own	30
AM60	AM10/5	Own	60
AMS30	AM10/5	Swartzman	30
UCDS30	UCD5	Swartzman	30

tissues to avoid saturation of the extraction medium.

The effect of extraction time was evaluated when extracting tannin using the model wine. The model wine was prepared according to the method described in Mattivi et al. (2002), containing 12% (v/v) ethanol, 5 g/L tartaric acid, and 100 ppm SO_2, adjusted the pH to 3.2 using 1 M sodium hydroxide and followed the same solid-to-liquid ratio mentioned in the original paper (1.25 mL per 1 g berry weight) to mimic wine-making conditions. The headspace in the tubes was sparged with nitrogen to avoid oxidation. The extraction was carried out at room temperature for 96 hr on a tumbler at 60 rpm speed. During the extraction, a 1 mL sample was taken at 12, 24, 48, 72, and 96 hr intervals and centrifuged at 4750 g for 10 minutes before analysing for tannin concentration. The time required to reach plateau concentration was used to extract tannin from grape tissues.

2.3. Tannin extraction with the optimised conditions

Freeze-dried tissues were extracted with a 2:1 v/v acetone-water mixture (50 mL per 1 g freeze-dried tissue weight) at room temperature for 24 hr on a tumbler at 60 rpm speed. The headspace in the tubes was sparged with nitrogen to avoid oxidation during the extraction. Extracts were then centrifuged at 4750 g for 10 minutes to remove solids and vacuum evaporated at 40°C to remove acetone. The remaining solution was adjusted to a volume of 20 mL with deionised water and kept at −20°C until further analysis.

Fresh tissues were extracted into the model wine (1.25 mL per 1 g berry weight) at room temperature for 72 hr on a tumbler at 60 rpm speed. The headspace in the tubes was sparged with nitrogen to avoid oxidation during the extraction. Extracts were then centrifuged at 4750 g for 10 minutes to remove solids and clear extracts were stored at −20°C until analysis.

2.4. Methyl cellulose precipitable (MCP) tannin

Tannin in extracts was determined using the 1 mL methyl cellulose precipitation method (Mercurio et al., 2007). The absorbance of the sample and the respective control at 280 nm was measured using a Shimadzu 1800 UV-Visible spectrophotometer (Shimadzu, Tokyo, Japan). The absorbance difference between treatment and control was used to calculate the concentration of MCP tannins against an epicatechin calibration curve. Appropriate volume correction was done when calculating tannin concentration in the acetone extracts (based on the recovered volume after the extraction). Model wine extracts were analysed directly for MCP tannin concentration.

2.4.1. Phloroglucinolysis

Extracted tannin was characterised by acid catalysis in the presence of excess phloroglucinol followed by reversed-phase HPLC. Phloroglucinolysis was carried out using the method described by Kennedy and Jones (2001) with modifications. Briefly, the volume of the sample loaded into the cartridge was determined based on the MCP tannin concentration, with less than 20 mg of tannin to obtain maximum recovery and avoid breakthrough. Model wine extracts were vacuum evaporated under reduced pressure at 40°C to remove ethanol in the media and reconstituted with deionised water before loading into the cartridge. Acetone extracts were loaded directly into the cartridge as acetone was vacuum evaporated previously. A 500 mg C18 SEP-PAK cartridge (WAT043395, Global Science, Auckland, New Zealand) was conditioned with 7.5 mL of methanol, 7.5 mL of ethyl acetate, and 7.5 mL of deionised water. The sample was loaded onto the C18 cartridge and the cartridge was washed with 5 mL of deionised water and allowed to dry with nitrogen gas at a flow rate of 1 L/min for 60 minutes. After that, 5 mL of ethyl acetate was passed through the cartridge to wash off monomeric phenols and the required polymeric fraction was eluted with 5 mL of methanol. This methanolic solution was rotary evaporated at 40°C and reconstituted with methanol to a final volume of 1 mL. Then 0.5 mL of this prepared methanolic solution was reacted with an equal volume of phloroglucinol reagent (double strength) (100 g/L phloroglucinol in methanol, containing 0.2 M hydrochloric acid, and 20 g/L ascorbic acid) in a screw cap tube in a water bath at 50°C for 20 minutes. To stop the reaction, 5 mL of 40 mM sodium acetate solution was added into the screw cap tube and vortexed the mixture for dissolving. This solution was filtered through a 0.45 μm pore size 13 mm diameter PTFE filter into an HPLC vial (the first few drops discarded). Reversed-phase HPLC analysis and calculations were carried out according to the method described in Wimalasiri et al. (2022) using an Agilent 1100 series HPLC (Waldbronn, Germany) machine equipped with a quaternary pump, diode array detector (DAD), and fluorescence detector (FLD) to identify and quantify proanthocyanidin cleavage products.

2.5. Statistical analysis

Data were presented as mean ± standard deviation of three replicates. Viticulture data obtained at the harvesting stage, MCP tannin concentration and composition, and anthocyanin concentration were subjected to one-way analysis of variance (ANOVA) at a 0.05 level of significance, followed by a post-hoc analysis using Tukey's HSD test using Minitab 18 (Minitab Inc., State College, PA, USA, version 18.1). The structural characteristics of the tannins extracted into the two solvents were subjected to a two-sample t-test at a 0.05 level of significance using Minitab 18.

3. Results and discussion

3.1. Optimisation of tannin extraction conditions

In Acetone extraction, the optimum solid-to-liquid ratio and tannin extraction progress with time were assessed to ensure the extraction efficiency was not influenced by the saturation of the solvent. Fig. 1 shows tannin extraction curves over time when using different solid-to-liquid ratios. More than 85% of the skin tannin, 60% of seed tannin, and 75% of stem tannin were extracted at 12 hr and all tissues reached a plateau by 24 hr. A previous study on Cabernet Sauvignon has demonstrated that skin tannin comes to the peak concentration at 42 hr when using 70% (v/v) acetone (Bindon et al., 2014). The shorter extraction period in this study could be due to the differences in the extraction process and the grape variety. The use of freeze-dried tissues in this study may have facilitated more efficient tannin extraction from grape tissues due to the creation of a highly porous structure through sublimation (Felsot and Rosen, 2004). A decreased solid-to-liquid ratio in the extraction gave rise to an increase in tannin concentration, agreeing with previous findings (Pinelo et al., 2005; Rajha et al., 2014). However, there was no significant difference observed in tannin concentration among the ratios of 1:25, 1:50, and 1:75 after 24 hr of extraction. Therefore, a 1:50 solid-to-liquid ratio and 24 hr extraction time were chosen as the optimum conditions to extract tannin from all three tissues when using acetone and freeze-dried tissues in the extraction process. In this study, the extracted tannin using acetone with these optimised conditions was referred to as 'total tannin', although it has been shown that there is a small amount of non-extractable high molecular weight tannins remaining in grape tissues after acetone extraction (Bindon et al., 2014; Bindon et al., 2010).

Tannin extraction progress with time was assessed when using the model wine to extract tannin from skin, seed and stem tissues (Fig. 2). The model wine extracted fewer tannins from grape tissues compared to the acetone extraction, supporting previous studies (Bindon et al., 2014; Downey and Hanlin, 2010). Extraction of tannins from skin and stem tissues reached a plateau at 48 hr and seeds reached a plateau at 72 hr, and after that, the tannin concentration remained constant in all three tissues. Hence, the extraction time of 72 hr was selected to extract tannin from all three tissues using model wine. When comparing the

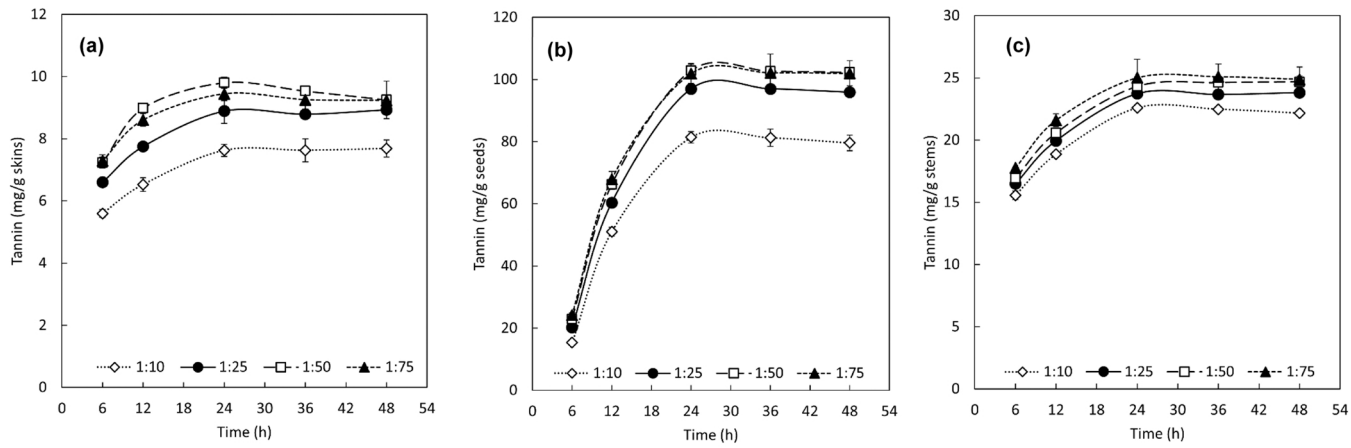

Fig. 1. Tannin extraction progress with time when using different solid-to-liquid ratios in 2:1 acetone-water extraction. (a) skins; (b) seeds; (c) stems.

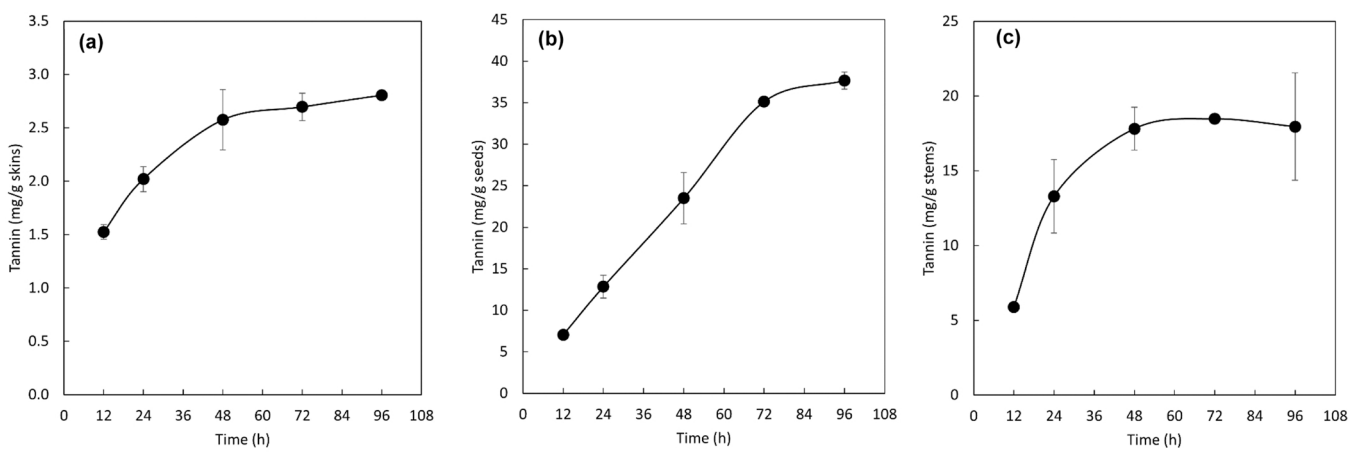

Fig. 2. Tannin extraction progress with time when using model wine. (a) skins; (b) seeds; (c) stems.

extraction efficiency among the three grape tissues, the extraction of grape skin and stem tannins was much faster than that of grape seeds, as observed in acetone extraction.

3.2. Physical and chemical properties of grapes at harvest

Leaf removal timing and clone selection significantly influenced the physical and chemical parameters of grapes at harvest (Table 2). In leaf removal treatments, earliest leaf removal (AM7) showed comparatively lower berry weight compared to other leaf removal treatments, with statistically lower weight compared to AM60. This could be due to initial carbohydrate limitations during berry development and lower average seed count, leading to insufficient gibberellins, which are important in pericarp cell enlargement (Mejia et al., 2007). AM7, AM30, and AM60 showed a significantly higher anthocyanin concentration compared to the no-leaf removal treatment (AM0), with the earliest leaf removal

Table 2
General berry parameters at harvest.

Treatment	Bunch weight (g)	Berry weight (g)	Stem weight per bunch (g)	Skin weight per berry (g)	Seed weight per berry (g)	Seeds per berry	TSS (°Brix)	pH	TA (g/L)	Anthocyanin concentration (mg per g skins)
AM0	67.5 ± 27.2a	1.13 ± 0.07ab	2.54 ± 0.70a	0.108 ± 0.007a	0.040 ± 0.008abc	1.22 ± 0.03a	22.5 ± 0.3a	3.13 ± 0.05b	10.53 ± 0.31a	5.48 ± 0.18d
AM7	58.9 ± 23.5ab	1.03 ± 0.04b	2.27 ± 0.56a	0.103 ± 0.004a	0.044 ± 0.006ab	1.14 ± 0.11a	22.2 ± 0.4a	3.05 ± 0.08b	10.67 ± 0.29a	8.64 ± 0.17a
AM30	61.3 ± 18.9ab	1.12 ± 0.05ab	2.43 ± 0.69a	0.115 ± 0.004a	0.045 ± 0.002ab	1.40 ± 0.18a	22.6 ± 0.1a	3.08 ± 0.03b	10.57 ± 0.95a	7.03 ± 0.38b
AM60	74.4 ± 20.2a	1.21 ± 0.04a	2.75 ± 0.51a	0.118 ± 0.002a	0.050 ± 0.002a	1.28 ± 0.06a	22.8 ± 0.6a	3.10 ± 0.04b	10.70 ± 0.20a	7.58 ± 0.11b
AMS30	55.1 ± 16.0ab	1.09 ± 0.09ab	2.41 ± 0.53a	0.117 ± 0.011a	0.037 ± 0.003bc	1.20 ± 0.10a	22.0 ± 0.2a	3.14 ± 0.01b	9.51 ± 0.37a	7.08 ± 0.31b
UCDS30	43.0 ± 14.1b	1.03 ± 0.11b	2.44 ± 0.47a	0.111 ± 0.009a	0.034 ± 0.004c	1.22 ± 0.15a	22.4 ± 0.2a	3.26 ± 0.02a	8.18 ± 0.24b	6.29 ± 0.16c

Values are the means of triplicate determinations ± standard deviation
Abbreviations: TA-Titratable acidity (tartaric acid equivalent), TSS-Total soluble solids.
Lowercase letters within columns indicate significant differences between the treatments (Tukey's HSD, post-hoc testing, $p < 0.05$)

treatment displaying the highest anthocyanin concentration among all treatments. This may be associated with the increased synthesis of tri-hydroxylated anthocyanins in exposed berries compared to shaded ones (Downey et al., 2004; Feng et al., 2015; Kemp et al., 2011; Lee and Skinkis, 2013; Ristic et al., 2007). These results suggest that AM7 may potentially enhance the skin tannin proportion and anthocyanin concentration in wines due to the increased skin-to-pulp ratio of AM7 berries. When comparing AM30 and AMS30 treatments, rootstock selection had no impact on the measured physical and chemical properties of grapes at harvest. Furthermore, no difference in pH and TA between AM30 and AMS30 treatments indicates no difference in the potassium uptake between Schwartzman rootstock and own-rooted Pinot noir. A previous study on Pinot noir grafted onto 125AA and 110 R rootstocks also displayed rootstock selection had no impact on berry weight, TSS, and anthocyanin concentration in berries (Berdeja et al., 2014). However, clone selection significantly influenced pH, TA and anthocyanin concentration in berries, with AMS30 showing higher TA and anthocyanin levels (approximately 13% higher), and lower pH compared to UCDS30. This may potentially contribute to a higher concentration of anthocyanin, ionized anthocyanin proportion, and tannin solubility and retention via polymeric pigment formation in AM10/5 wines (He et al., 2012; Kumar et al., 2022; Ristic et al., 2010). As expected, when comparing all six treatments together, all parameters except stem weight, skin weight, number of seeds per berry and TSS showed significant differences between treatments, mainly due to the greater variability of experimental parameters.

3.3. Tannin concentration

Fig. 3 shows the tannin concentration of different grape tissues extracted using acetone and model wine under different treatments at harvest. The similar TSS among treatments minimized the potential impact of berry ripeness on tannin extractability, enabling the study of the influence of different treatments on tannin concentration and composition of grape tissues. Tannin concentration was expressed as per kilogram of grapes, in order to assess the extractable tannins for a given weight of grapes.

In acetone extraction, skin, seed, and stem tannins ranged from 0.65 to 1.00 g/kg, 2.75–3.56 g/kg, and 0.72–1.16 g/kg grapes at harvest, respectively. For a given weight of grapes, stems showed a much lower weight proportion than skins (Stems: 3.69–5.68%, skins: 9.24–10.27% w/w of the grapes) but a similar amount of tannins, suggesting that stems are a good source of tannin. Leaf removal timing, rootstock, and clone selection had no impact on skin tannin concentrations. However, when comparing all treatments together, UCDS30 tends to show the highest skin tannin concentration extracted by acetone. In grape seeds, leaf removal treatments increased seed tannin concentrations, partially due to the reduced seed weight per berry. Schwartzman rootstock showed a lower seed tannin concentration than the own roots, possibly attributed to low vine vigour. Cortell et al. (2005) also observed that low vine vigour was associated with lower seed tannin concentration in Pinot noir berries. No difference in seed tannin concentration was observed between the two clones. When comparing all treatments together, AM7 and AM30 treatments showed significantly higher seed tannin concentrations compared to AM0, AMS30, and UCDS30 treatments. In grape stems, leaf removal tends to increase tannin concentration, possibly due to the lower water content in stems (AM0: 71%, AM7: 60%, AM30: 66%, and AM60: 70%). Rootstock and clone showed no influence on stem tannin concentration.

In model wine extraction, skin, seed, and stem tannins ranged from 0.26 to 0.45 g/kg, 0.73–1.19 g/kg, and 0.56–0.80 g/kg grapes at harvest, respectively. In general, model wine-extracted tannins in three tissues showed a similar trend as acetone-extracted tannins, although they were not statistically significant always. When comparing all the treatments, AMS30 showed a significantly higher skin tannin concentration than AM60, which was not observed in the acetone extraction.

As expected, the extraction solvent had a significant impact on the extracted tannin concentration from grape tissues due to the differences in their hydrophobicity, with acetone extracting significantly higher tannin levels from skins, seeds, and most stems than the model wine. The presence of hydrophobic interactions between tannins and CWM such as protein and polysaccharides limit the extractability of tannin from grape tissues. However, when the extraction solvent becomes more hydrophobic (acetone is more hydrophobic than model wine), it can disrupt these hydrophobic interactions, enabling greater extraction of tannin (Bindon et al., 2014; Hanlin et al., 2010).

3.4. Tannin composition and mean degree of polymerisation (mDP)

The relative proportion of terminal and extension subunits varied across tissues (Fig. 4). Catechin was the primary terminal subunit, and epicatechin was the major extension subunit in all three tissues. In grape skins and stems, epigallocatechin extension subunit was observed in addition to the catechin, epicatechin and epicatechin gallate subunits detected in the seeds, which is in agreement with previous findings (Downey et al., 2003; Kyraleou et al., 2017; Souquet et al., 2000). In addition, the epicatechin terminal subunit was not detected in grape skins. This is also consistent with similar observations in grape skins of Cabernet sauvignon (Busse-Valverde et al., 2010; Kennedy et al., 2002), Shiraz and Chardonnay (Kennedy and Jones, 2001). Tannin composition analysis revealed that stem tannins had intermediate galloylation levels (4–7%) between seed tannins (13–15%) and skin tannins (1–2%), lower prodelphinidin content (4–10%) than skin tannins (29–37%), and a similar mDP (10−11) to seed tannins (8−9) but lower compared to skin tannins (20−27) at harvest (Table 3). Seed tannins exhibited the lowest conversion yield, suggesting their higher resistance to acid catalysis,

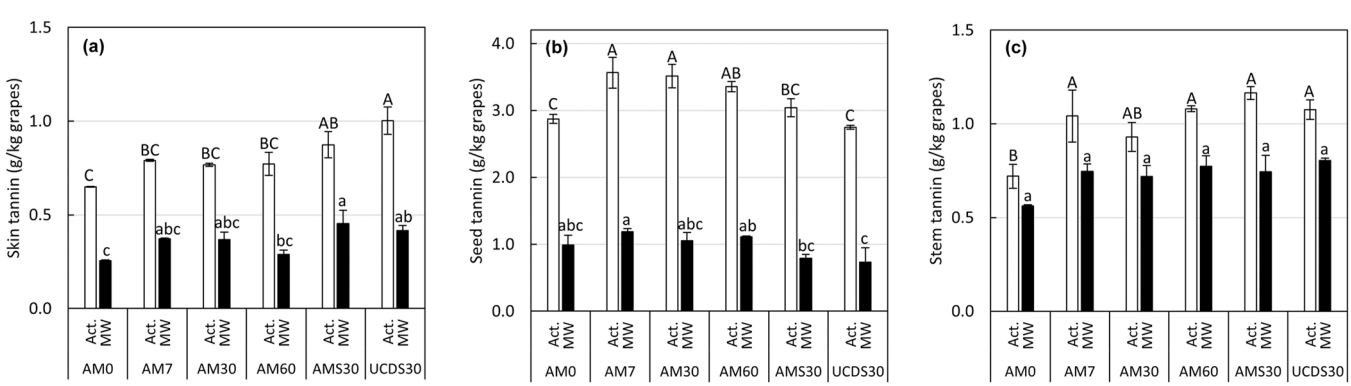

Fig. 3. Acetone (Act.) and model wine (MW) extracted tannin concentrations of skins (a), seeds (b), and stems (c) from different treatments. Different uppercase letters denote significant differences between acetone-extracted tannin, and lowercase letters denote significant differences between model wine-extracted tannin (evaluated by Tukey's HSD, post-hoc testing at p-values < 0.05).

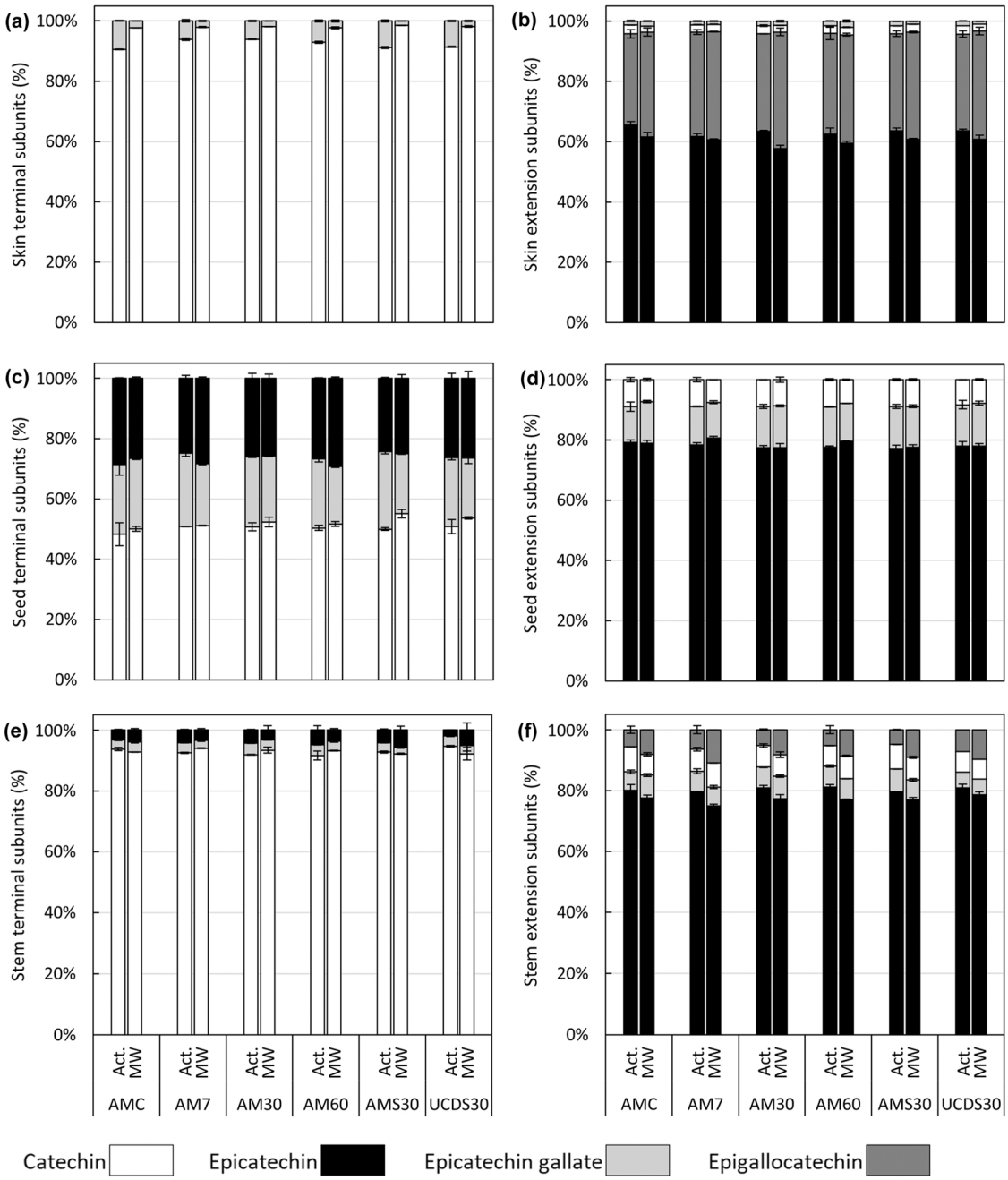

Fig. 4. Acetone- (Act.) and model wine- (MW) extracted tannin composition of skin, seed, and stems from different treatments.

consistent with previous findings in Pinot noir (Lee, 2010), Chardonnay (Kennedy and Jones, 2001), and Merlot grape varieties (Yu et al., 2016). The overall variation in tannin concentrations between treatments measured by phloroglucinolysis and MCP tannin methods showed similar trends in both acetone and model wine extractions. Although, major differences were observed between different grape tissues and extractions solvents due to the significant variability in conversion yield between tannins extracted from different grape tissues and solvents.

In the acetone extract, leaf removal increased skin tannin mDP (Table 3), as suggested by the slightly higher extension subunit concentrations observed in exposed treatments. A previous study on Shiraz has also observed that increased sunlight exposure could lead to an increased skin tannin mDP, extracted with acetone (Downey et al., 2004). The higher mDP of exposed berry skins may be due to the production of more secondary metabolites as a defensive mechanism against UV stress (Downey et al., 2004; Feng et al., 2015), thereby contributing to increasing mDP by forming new interflavan bonds. Rootstock and clone selection influenced skin tannin mDP, with AMS30 showing a higher mDP compared to AM30 and a lower mDP compared to UCDS30, respectively. These results indicate a possible influence of rootstock on skin tannin composition. The influence of clone selection on skin tannin mDP could be associated with genetic influence on flavonoid biosynthesis (Casassa et al., 2021; Pantelić et al., 2016). When comparing all treatments together, UCDS30 had a notably higher mDP than all other treatments. Meanwhile, AMS30 exhibited a significantly higher G% compared to AM7, primarily attributed to the higher epicatechin gallate terminal subunits proportion in AMS30 (8.8%) compared to AM7 (6.1%) (Fig. 4a). In grape seeds, leaf removal and clone selection did not influence tannin composition, as suggested by the similar terminal and extension subunit proportions (Fig. 4c and Fig. 4d). These results agree with those of previous studies on Cabernet sauvignon, and Shiraz varieties, showing sunlight exposure had no impact

Table 3
Tannin concentration and mean degree of polymerisation (mDP) by Phloroglucinolysis.

	mDP		Yield (w/w%)[1]		Galloylation (G%)		Prodelphinidins (P%)		Total Terminal (μmol per g tissue)		Total Extension (μmol per g tissue)	
	Acetone	Model wine	Acetone	Model wine	Acetone	Model wine	Acetone	Model wine	Acetone	Model wine	Acetone	Model wine
Skin												
AM0	19.9 ± 0.3E	20.6 ± 0.1bc	47.4 ± 1.1 A	64.5 ± 2.8b*	1.6 ± 0.2AB	1.6 ± 0.1ab	28.6 ± 1.3 A	33.0 ± 1.3b	0.58 ± 0.00 A	0.30 ± 0.02b*	10.92 ± 0.23 A	5.87 ± 0.31b*
AM7	23.0 ± 0.3CD	20.5 ± 0.5bc*	55.6 ± 8.7 A	73.1 ± 0.3ab*	1.4 ± 0.1B	1.1 ± 0.1b	33.1 ± 0.8 A	34.0 ± 0.1ab	0.68 ± 0.10 A	0.48 ± 0.01ab*	15.05 ± 2.47 A	9.28 ± 0.04ab
AM30	22.2 ± 0.2D	21.9 ± 0.7abc	42.8 ± 1.9 A	77.3 ± 5.6ab*	1.7 ± 0.0 AB	1.4 ± 0.2b	30.9 ± 0.0A	36.9 ± 1.3a*	0.52 ± 0.01 A	0.45 ± 0.00ab*	10.95 ± 0.37 A	9.48 ± 0.32ab
AM60	23.8 ± 0.7BC	20.1 ± 1.4c	49.3 ± 1.1 A	63.8 ± 4.5b*	1.8 ± 0.1 AB	2.1 ± 0.3a	32.0 ± 2.1A	34.1 ± 0.6ab	0.58 ± 0.02 A	0.34 ± 0.08b*	13.32 ± 0.79 A	6.43 ± 0.97b*
AMS30	24.9 ± 0.1B	23.5 ± 0.8ab	52.1 ± 1.5 A	83.4 ± 2.8a*	1.8 ± 0.0 A	1.0 ± 0.0b*	30.9 ± 0.9 A	34.0 ± 0.3ab*	0.58 ± 0.00 A	0.54 ± 0.08a	14.00 ± 0.15 A	12.20 ± 2.32a
UCDS30	27.3 ± 0.3 A	24.6 ± 0.7a*	43.3 ± 1.3 A	73.7 ± 2.3ab*	1.7 ± 0.0 AB	1.0 ± 0.0b*	31.0 ± 1.0A	34.5 ± 1.1ab	0.56 ± 0.01 A	0.43 ± 0.03ab*	14.81 ± 0.03 A	10.04 ± 0.31ab*
Seed												
AM0	7.9 ± 0.0B	8.4 ± 0.1a*	32.8 ± 0.3 BC	44.6 ± 0.8a*	13.3 ± 1.8 A	14.9 ± 0.3a	ND	ND	11.96 ± 0.18BC	5.26 ± 0.78a*	82.57 ± 1.12B	39.04 ± 6.50a*
AM7	8.3 ± 0.3AB	8.5 ± 0.4a	36.8 ± 0.4 AB	46.0 ± 1.7a*	14.1 ± 0.0 A	12.8 ± 0.4b	ND	ND	13.23 ± 0.55AB	5.42 ± 0.70a*	97.22 ± 7.74 A	40.54 ± 2.90a*
AM30	7.9 ± 0.2B	8.9 ± 0.1a*	34.9 ± 1.5 BC	46.0 ± 2.8a*	14.9 ± 0.6 A	14.7 ± 0.3a	ND	ND	13.74 ± 0.30 A	4.84 ± 0.81a*	95.00 ± 0.87AB	38.32 ± 6.81a*
AM60	8.0 ± 0.2B	8.1 ± 0.3a	33.6 ± 0.4 BC	48.2 ± 3.4a*	14.6 ± 0.1 A	13.3 ± 0.1ab*	ND	ND	12.24 ± 0.46BC	5.76 ± 0.70a*	85.34 ± 0.65AB	40.71 ± 2.93a*
AMS30	9.0 ± 0.1 A	9.2 ± 0.7a	31.2 ± 0.4 C	49.7 ± 0.2a*	15.2 ± 0.7 A	14.3 ± 0.3ab	ND	ND	11.05 ± 0.32 C	4.58 ± 0.65a*	87.99 ± 3.58AB	37.41 ± 2.30a*
UCDS30	8.5 ± 0.1AB	9.3 ± 0.4a	31.1 ± 0.6 C	43.4 ± 4.0a*	14.7 ± 1.4 A	14.9 ± 0.8a	ND	ND	11.23 ± 0.02 C	3.87 ± 0.95a*	84.62 ± 1.35AB	31.73 ± 6.20a*
Stem												
AM0	9.3 ± 0.6 A	9.8 ± 0.5b	38.9 ± 1.9 AB	71.7 ± 2.9a*	5.7 ± 0.6AB	7.0 ± 1.1a	5.0 ± 1.1 A	7.2 ± 1.4a	2.78 ± 0.29AB	3.76 ± 1.01a	22.89 ± 0.75B	33.42 ± 10.74a
AM7	9.9 ± 0.2 A	10.1 ± 0.2ab	40.3 ± 0.4 A	76.5 ± 0.9a*	6.4 ± 0.7AB	5.8 ± 0.3a	5.7 ± 1.2 A	9.7 ± 0.4a*	3.79 ± 0.54 A	5.00 ± 0.91a	33.62 ± 4.07 A	45.76 ± 9.52a
AM30	10.1 ± 0.1 A	10.3 ± 0.1ab	39.6 ± 0.6 AB	67.5 ± 0.0a*	6.6 ± 0.0AB	7.0 ± 0.8a	4.7 ± 0.2 A	7.4 ± 1.6a	3.20 ± 0.34AB	4.09 ± 0.86a	28.92 ± 2.79AB	38.15 ± 7.77a
AM60	9.3 ± 0.2 A	10.4 ± 0.1ab*	38.0 ± 0.2 AB	68.6 ± 2.1a*	6.5 ± 0.3AB	6.6 ± 0.2a	4.7 ± 1.2 A	7.8 ± 0.8a	2.55 ± 0.42AB	4.79 ± 0.54a*	21.18 ± 3.06B	44.84 ± 4.55a*
AMS30	11.2 ± 0.0 A	10.1 ± 0.1ab*	36.1 ± 0.3 B	72.2 ± 5.0a*	4.3 ± 0.1 A	6.2 ± 0.8a	4.3 ± 0.1 A	8.1 ± 0.7a*	2.95 ± 0.11 AB	4.20 ± 0.91a	30.11 ± 1.12AB	38.29 ± 7.94a
UCDS30	10.7 ± 1.1 A	10.9 ± 0.1a	37.5 ± 0.9 AB	75.8 ± 2.0a*	5.1 ± 0.4B	5.0 ± 0.5a	6.5 ± 1.1A	8.8 ± 0.7a	2.23 ± 0.25B	3.38 ± 0.01a*	21.56 ± 0.02B	33.61 ± 0.36a*

Values are the means of triplicate determinations ± standard deviation
Abbreviations: ND-not detected
Different letters in the same column indicate significant differences between treatments in each grape tissue extracted with acetone (upper case) and model wine (lower case) using Tukey's HSD, post-hoc testing ($p < 0.05$); * indicates significant difference between the two solvent extractions by Student's t-test at $p < 0.05$; 1 conversion yield was calculated using epicatechin equivalent mass of total subunits divided by epicatechin equivalent MCP tannin

on seed tannin composition and mDP (Blancquaert et al., 2019; Downey et al., 2004). However, Schwartzman rootstock showed a higher mDP than the own roots, suggesting a possible influence of rootstock on tannin composition as observed for skin tannin. When comparing all treatments together, AMS30 had a significantly higher mDP compared to AM60, and AM0 treatments, while AM7 showed a significantly higher conversion yield compared to AMS30 and UCDS30 treatments. In grape stems, leaf removal and rootstock did not influence tannin composition. However, clone selection influenced G%, with AMS30 showing a higher G% than UCDS30. This was mainly due to the significantly higher epicatechin gallate extension subunit proportion observed in AMS30 (7.6%) compared to UCDS30 (5.2%) (Fig. 4f). When comparing all treatments together, AM7 showed a significantly higher conversion yield compared to AMS30, possibly attributed to the relatively lower stem tannin mDP.

In model wine extraction, leaf removal affected G% and P% of skin tannin, with AM60 showing a higher G% compared to AM7 and AM30 treatments, while AM30 had a significantly higher P% compared to AM0 (Table 3). The higher G% of AM60 skin tannin was mainly due to the higher epicatechin gallate extension subunits proportion (2.1%) than AM7 (1.1%) (Fig. 4b). While the higher P% of AM30 skin tannin was mainly due to the higher epigallocatechin extension subunits proportion (38.7%) than AM0 (34.7%) (Fig. 4b). These results support prior research, indicating light exposure can increase B ring tri-hydroxylation of epicatechin in grape skins, leading to a higher epigallocatechin proportion and a lower epicatechin proportion in grape skin tannin (Downey et al., 2004; Yu et al., 2016). Rootstock and clone selection did not influence skin tannin composition, as suggested by the similar concentrations and proportions of terminal and extension subunits. However, when comparing all treatments together, UCDS30 showed a higher mDP than AM0, AM7, and AM60 treatments, AMS30 showed a higher conversion yield than AM0 and AM60 treatments, and AM60 showed a higher G% than all other treatments. In grape seeds, leaf removal had a significant impact on G% of seed tannin, with AM7 showing a lower G% compared to AM0 and AM30 treatments. This was mainly due to the lower epicatechin gallate extension subunit proportion of AM7 (11.8%) seed tannin compared to AM0 (13.8%) and AM30 (13.8%) treatments (Fig. 4d). As expected, rootstock and clone selection did not affect tannin composition, due to the similar terminal and extension subunit proportions (Fig. 4c and Fig. 4d). When comparing all treatments together, only G% showed differences, with AM7 showing a significantly lower G% compared to AM0, AM30, and AMS30

treatments. In grape stems, leaf removal timing, rootstock, and clone selection did not affect stem tannin composition, as suggested by the similar concentrations and proportions of terminal and extension subunits between individual trials (Fig. 4e and Fig. 4f). However, when comparing all treatments together, UCDS30 showed a significantly higher mDP compared to AM0.

Overall, the two solvents showed different trends in tannin composition between treatments. These discrepancies are possibly due to the compositional differences between extractable and unextractable tannin fractions as observed in previous studies (Bindon et al., 2014; Fournand et al., 2006). When comparing all treatments together, most of the parameters showed significant differences between treatments, mainly due to the greater variability of experimental parameters. In addition, model wine-extracted skin, seed, and stem tannins had a lower proportion of epicatechin gallate terminal subunits and a higher proportion of catechin terminal subunits than acetone-extracted tannins (Fig. 4a). In grape stems and skins, model wine-extracted tannins had a comparatively higher proportion of epigallocatechin extension subunits and a lower proportion of catechin extension subunits compared to the acetone-extracted tannins (Fig. 4b and Fig. 4f). Hence, comparatively higher P% was observed in model wine-extracted skin and stem tannins, even though all of them were not statistically significant. Similar to these observations, prior research on Pinot noir skin tannin and Monastrell seed tannin found that the extraction solvent had no impact on extension subunit composition and a minor influence on terminal subunit composition of tannins extracted with model wine and acetone (Bautista-Ortín et al., 2012; Peyrot des Gachons and Kennedy, 2003). However, extraction solvent had a major effect on conversion yield, with tannin extracted with model wine showing a significantly higher conversion yield than acetone-extracted tannin in all three tissues. This indicates the presence of tannin molecules that are resistant to acid catalysis, possibly due to the larger molecular size of acetone-extracted tannin than model wine-extracted tannin. However, the mDP of the tannins extracted with the two solvents did not show a difference in this study, potentially due to extracting both extractable and non-extractable fractions in the acetone extraction. The gel permeation chromatography (GPC) results, as reported by Bindon et al. (2014), provide evidence that high molecular mass skin tannins are increasingly extracted by the less polar 70% acetone compared to the polar aqueous ethanol solutions. Tannins with larger molecular weight can often be more resistant to acid catalysis particularly due to the presence of more active sites to form intramolecular bonds (such as hydrogen bonds), which causes incomplete depolymerisation of tannin into flavan-3-ol subunits (Du Preez et al., 2022; Poncet-Legrand et al., 2010).

3.5. Tannin extractability

The tannin extractability of different tissues from different treatments is shown in Fig. 5. Stems showed the highest tannin extractability ranging from 64% to 78% compared to skins (37–52%) and seeds (26–34%). Tannin extractability largely depends on the adsorption of tannin onto CWM, and the existence of diffusive barriers (Harrison, 2018). For example, a recent study on Pinot noir skin CWM found that lignin and protein content in CWM correlate negatively with the phenolics and polymeric phenolics extracted into a hydroalcoholic solution, possibly due to the enhanced tannin-CWM interactions (Medina-Plaza et al., 2021). Moreover, skin tannin extractability also appeared to be dependent on mDP and G% of tannin, with higher mDP and G% of tannins showing reduced tannin extractability due to their greater affinity for CWM (Bindon et al., 2014; Fournand et al., 2006; Le Bourvellec et al., 2004). In grape seeds, the lignification of the inner layers and outer integument of grape seeds have been observed to act as a diffusion barrier during tannin extraction (Adams, 2006; Rousserie et al., 2020). Hence, the higher stem tannin extractability suggests a weaker binding of stem tannins to CWM materials, possibly due to the lower mDP compared to skin tannin, lower G% compared to seed tannin, lower

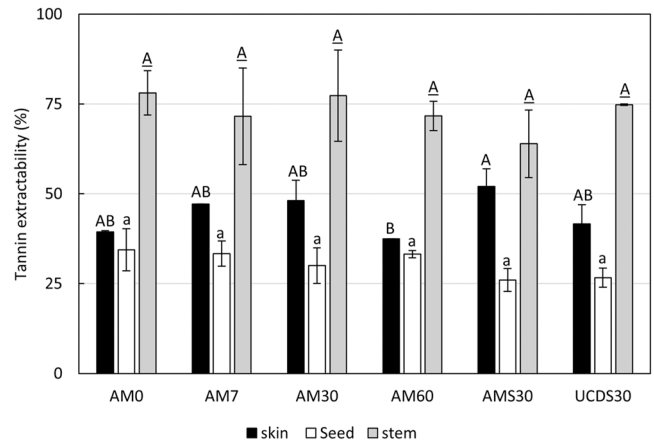

Fig. 5. Tannin extractability of skins, seeds, and stems when using acetone and model wine for tannin extraction. Different uppercase letters denote significant differences between skins, lowercase letters denote significant differences between seeds, and uppercase letters with underscore denote significant differences between stems (evaluated by Tukey's HSD, post-hoc testing at p-values < 0.05.

lignin, protein, and polysaccharide levels in CWM composition, and/or the presence of a weak diffusion barrier than in grape skins and seeds. Thereby, a faster tannin extraction is expected in stem inclusion fermentation.

Despite the influence of treatments on berry composition, results showed that the tannin extractability of different grape tissues is largely influenced by grape ripeness which largely determines CWM composition. Many previous studies have observed that grape ripeness decreased skin and seed tannin extractability mainly due to the adsorption of tannins onto cell walls, due to the increase in porosity during ripening (Bindon et al., 2014), the interaction with other cell wall components such as proteins and polysaccharides (del Rio and Kennedy, 2006), and lignification of the inner-most cell layers of the outer integument of seeds (Cadot et al., 2006; Rousserie et al., 2020). In this work, treatments had no impact on seed and stem tannin extractability, while grape skins differed significantly, with AMS30 skins showing a higher skin tannin extractability than AM60. Skin tannin extractability also appeared to be dependent on mDP and G% of tannin, as described earlier. However, the model wine-extracted AM60 skin tannins showed a higher G% in agreement with previous findings but also had a higher mDP in contrast (Bindon et al., 2014; Fournand et al., 2006; Freitas and Mateus, 2001). This indicates that G% of skin tannin plays a major role in skin tannin extractability. Previous studies have also observed that G % increased the binding capacity of tannins with protein (Freitas and Mateus, 2002; Ricardo-da-Silva et al., 1991), because epicatechin gallates possess well-exposed galloyl functions, which enable binding to several sites via hydrophobic effects and hydrogen bonding (Freitas and Mateus, 2001).

4. Conclusions

This study investigated the effect of initial berry composition on the tannin extractability of skin, seed, and stem tissues of Pinot noir grapes at harvest. Leaf removal, rootstock and clone all showed significant impact on grape tissue composition. Increased anthocyanin, stem and seed tannin concentrations, and the mDP of skin tannins were observed in the leaf removal trial. Compared to own roots, Schwarzman rootstock had lower seed tannins but higher mDP of skin and seed tannins. The UCD5 clone exhibited higher mDP of skin tannins than the AM10/5 clone. For the first time, this study revealed that stems have the highest tannin extractability, followed by grape skins and seeds. This provides valuable information for winemakers to manage tannin extraction,

especially when grape stems are included in the fermentation. Various grape tissue compositions obtained from different treatments showed no impact on the tannin extractability of individual grape tissue, except for grape skins from AMS30 and AM60. This indicates that tannin extractability is mainly influenced by grape ripeness. However, the galloylation (G%) of tannins also influences tannin extractability, with higher G% (observed in AM60) showing a lower extractability. Future studies on cell wall materials could further improve the understanding of how grape tissue composition influences tannin extractability.

Statement of the significance of the study

1) Statement of the problem addressed and originality of the approach. Very few studies have investigated on how the initial grape berry composition influences the tannin extractability, which is very important for quality red wine production. Thus, this study used Pinot noir grapes sourced from different clones, rootstocks, and leaf removal trial to understand how initial tannin concentration and composition in grape berries effects on the subsequent tannin extractability. Furthermore, a special attention has been given to the investigation of tannin extractability from Pinot noir grape stems in considering the increased popularity of whole bunch fermentation in Pinot noir production.
2) Contribution of the work to create new knowledge in the field.

The major findings of the study include:

- Leaf removal increased anthocyanin concentration, and tannins in stems and seeds, and mean degree of polymerisation (mDP) of skin tannin.
- Schwarzman rootstock exhibited lower seed tannins but higher mDP in skin and seed tannins compared to own roots.
- The UCD5 clone showed higher mDP in skin tannins compared to AM10/5 clones in acetone extraction.
- Grape stems showed the highest tannin extractability (64%-78%) compared to skins (37–52%) and seeds (26–34%).
- Compositional differences in grape tissues generally showed no impact on tannin extractability but the tannin extractability of grape skins seems being correlated with the level of galloylation.

3) Relevance of the work to advance research and impact to the field of agricultural and food chemistry, including the specific role of molecular research in the study.

Managing tannin extraction is critical in red wine production as tannins are important for wine quality by contributing to the mouthfeel and taste. Findings of this study help winemakers better manage tannin extraction during Pinot noir production especially when using grape stems in the production. For the first time, this study reported that grape stems had the highest tannin extractability compared to skins and seeds. In addition, this study reported the initial differences in tannin concentration and composition of the grape tissues had limited impact on tannin extractability, but the different galloylation (G%) levels in grape skin tannins could significantly influence the tannin extractability.

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CRediT authorship contribution statement

Belinda Kemp: Writing – review & editing, Supervision. **Bin Tian:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Roland Harrison:** Writing – review & editing, Supervision, Conceptualization. **Ivan Donaldson:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Pradeep M. Wimalasiri:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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