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Phenolic Compositions of 50 and 30 Year Sequences of Australian Red Wines: The Impact of Wine Age

Jacqui M. McRae,[†] Robert G. Dambergs,[†,‡] Stella Kassara,[†] Mango Parker,[†] David W. Jeffery,[§] Markus J. Herderich,[†] and Paul A. Smith*[,†]

[†]The Australian Wine Research Institute, P.O. Box 197, Glen Osmond, SA 5064, Australia

[‡]The Tasmanian Institute of Agriculture, Private Bag 98, Hobart, TAS 7001, Australia

[§]The University of Adelaide, School of Agriculture, Food and Wine, PMB1, Waite Campus, Glen Osmond, SA 5064, Australia

ABSTRACT: The phenolic composition of red wine impacts upon the color and mouthfeel and thus quality of the wine. Both of these characteristics differ depending on the age of a wine, with the purple of young wines changing to brick red and the puckering or aggressive astringency softening in older wines. This study investigated the color parameters, tannin concentrations and tannin composition of a 50 year series of Cabernet Sauvignon wines from a commercial label as well as 30 year series of Cabernet Sauvignon and Shiraz wines from a separate commercial label to assess the impact of wine age on phenolic composition and concentration. The wine color density in wines of 40 to 50 years old was around 5 AU compared with 16 AU of wine less than 12 months old, which correlated well with the concentration of non-bleachable pigments and pigmented polymers. Conversely, the anthocyanin concentrations in 10 year old wines were substantially lower than that of recently bottled wines (around 100 mg/L compared with 627 mg/L, respectively), adding further evidence that non-bleachable pigments including pigmented polymers play a much larger role in long-term wine color than anthocyanins. No age-related trend was observed for tannin concentration, indicating that the widely noted softer astringency of older red wines cannot necessarily be directly related to lower concentrations of soluble wine tannin and is potentially a consequence of changes in tannin structure. Wine tannins from older wines were generally larger than tannins from younger wines and showed structural changes consistent with oxidation.

KEYWORDS: *tannin, wine color, wine age, phenolics, anthocyanins*

INTRODUCTION

Phenolics, particularly anthocyanins and modified flavan-3-ol polymers (wine tannins), are responsible for the color and mouthfeel of red wine, which is directly related to wine quality.[1−3] The concentration of monomeric anthocyanins in wine decreases rapidly after fermentation[4] due to degradation[5] and the formation of pigmented polymers via condensation reactions with flavan-3-ols,[6] as well as anthocyanin-derived pigments including pyranoanthocyanins, which form via cycloaddition reactions with pyruvic acid or acetaldehyde.[1,7] This decrease in anthocyanin concentration has been shown to contribute to the change in wine color from bright purple to orange-red.[8]

The astringency and mouthfeel of red wine is generally attributed to the interaction of wine tannins with oral proteins,[9,10] which is influenced by a range of factors including tannin concentration and structure. Wine tannins originate from grape-derived condensed tannins that are modified during winemaking due to condensation, rearrangement and oxidation reactions.[11−13] The concentration of tannins in young wine depends on many factors, including the tannin concentration in the grapes as well as the winemaking techniques used.[14,15] The intensity of the astringent sensation increases with higher tannin concentrations[16] and differences in the subqualities of astringency, such as 'puckering' or 'fine grain', have been associated with particular tannin structural characteristics including larger molecular size and more flavan-3-ol subunits with gallic acid groups.[17] Red wine generally loses astringency intensity with aging[1] and it is unclear as to whether this is related to a decrease in tannin concentration over time or structural changes induced by progressive depolymerization reactions.[18] A study of wine age correlations with sensory analysis have demonstrated that aged wines are generally perceived as more 'mellow' than younger wines, regardless of tannin concentrations in the wine.[19]

Experiments investigating the effect of wine age have examined the change in wine color and phenolic composition over only a few years[6,8,20] or have focused on the formation of smaller pigments and classes of pigments.[4,21] There is little information on the tannin concentration and structure in decades old wines. The objective of this study was to investigate the differences and trends in wine color parameters as well as tannin concentrations and composition of two major varieties grown in Australia, using a 50 year vertical series of Cabernet Sauvignon (50CAS) wines from a commercial Australian label ranging from 1954 to 2004 and two 30 year vertical red wine series of CAS and Shiraz (SHZ) from a separate commercial Australian label spanning 1977 to 2006 (30CAS and 30SHZ).

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MATERIALS AND METHODS

Chemicals. All solvents used were high-performance liquid chromatography (HPLC) grade, all chemicals were analytical reagent grade, and water was obtained from a Milli-Q purification system. Acetic acid (100%), acetone, acetonitrile, ethanol, formic acid (98−100%), HCl (32%), octan-1-ol, orthophosphoric acid (85%) and Tris were all purchased from Merck Australia (Kilsyth, VIC, Australia). Ammonium sulfate, ascorbic acid, LiCl, methyl cellulose, *N*,*N*-dimethylformamide, phloroglucinol, sodium acetate and NaCl were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia).

Wine Samples. Cabernet Sauvignon (CAS) wines for the 50 year vertical series (50CAS) were sourced from Wynns Coonawarra Estate in South Australia, Australia, and the CAS and SHZ 30 year vertical series (30CAS and 30SHZ) were obtained from Taltarni in the Australian Pyrenees region of Victoria, Australia. Reserve edition CAS wines (R30CAS) were also periodically released from Taltarni. These wines were made from the same fruit as the standard release wines using different winemaking techniques and sold at a higher price bracket. Five R30CAS wines, from the 1992, 1988, 1984, 1979, and 1977 vintages, were analyzed for comparison with the standard release wines from the corresponding vintage.

Wine Characterization. The basic wine chemistry for each sample was determined using a *FOSS* WineScan (FT-120) rapid-scanning infrared Fourier-transform spectrometer with *FOSS* WineScan software Version 2.2.1 (P/N 1010968), analyzing for alcohol concentration, pH, specific gravity, titratable acidity at pH 8.2 and 7.0, glucose and fructose concentration and volatile acidity as acetic acid.

Tannin Concentration and Color Measures. Methyl cellulose precipitable (MCP) tannin assay and modified Somers color measurements were performed as reported by Mercurio et al.[22] For the MCP tannin assay, wine (25 μL) was reacted with 300 μL polymer solution (0.04% MCP in H_2O) or H_2O for a control, in a 96 well plate for a total of 3 min (shaken for 1 min and allowed to rest for 2 min) prior to the addition of saturated ammonium sulfate solution (200 μL). Each reaction mixture (samples and controls) were made up to 1 mL in H_2O, shaken for 1 min and allowed to stand for 10 min prior to centrifuging at 2000 rpm for 5 min. The supernatant (300 μL) was transferred to a UV plate and absorbance was measured at 280 nm (A280) with a Spectral M2 max UV−vis Microplate reader (Molecular Devices). Tannin concentration was calculated as the difference between the A280 of the control and treatment wells compared with a standard curve of epicatechin concentrations and hence reported in epicatechin equivalents (mg/L).

The modified Somers method provided wine color density (WCD), hue, SO_2 non-bleachable pigment, and total anthocyanin. For the WCD and hue, wine was diluted 1:9 with a buffer solution (0.5% tartaric acid in 12% EtOH) and the absorbance measured at 420 and 520 nm (A420 and A520, respectively). The combined absorbance units of both wavelengths gave the WCD and the hue was given by A420/A520. The SO_2 non-bleachable pigment measurements were the absorbances at 520 nm of the wine samples after reaction (1:9) with a buffer solution (0.375% sodium metabisulfite, 0.5% tartaric acid in 12% EtOH) for 1 h (A520/sulfite). The total anthocyanin concentration of the wine samples was calculated based on the absorbance at 520 nm after reaction (1:49) with 1 M HCl solution for 3 h (A520/HCl) and the A520/sulfite and compared with a standard curve of malvidin 3 glucoside (M3G). Hence, the total anthocyanin concentration was reported in mg/L M3G equivalents.

Anthocyanin Concentration by HPLC. The concentrations of particular anthocyanins were determined using the method of Mercurio et al.[22] and Cozzolino et al.[23] using an Agilent 1100 HPLC (Agilent, Australia) with a Phenomenex Synergi Hydro-RP C18 column (4 μm particle size, 80 Å pore size, 150 × 2 mm) at 25 °C. Solvent A consisted of 1% (v/v) acetonitrile and 1.5% (v/v) orthophosphoric acid in water and solvent B was 80:20 acetonitrile/solvent A, using a gradient as previously described.[23] The identity of individual anthocyanins including M3G and pinotin A was achieved by comparing the retention times and UV spectra with reference standards.

Tannin Isolation and Fractionation. Tannin was isolated from wine as previously reported.[24] Briefly, 1 mL of wine was loaded under gravity onto an OASIS HLB SPE cartridge (Waters) that had been previously activated with 2 mL of MeOH and equilibrated with 2 mL of H_2O. The cartridge was dried with nitrogen and washed with 40 mL of 95% acetonitrile/5% 0.01 M HCl (v/v) under vacuum (F1). For tannin fractionation, the first tannin fraction (F2) was eluted with 5 mL of MeOH/0.1% (v/v) formic acid, and the second tannin fraction (F3) with 300 μL of formic acid followed by 2.7 mL of 95% (v/v) MeOH/H_2O. Total tannin was also isolated from some selected vintages for characterization by phloroglucinolysis and gel permeation chromatography (GPC). After F1 was removed from the cartridge, the total tannin was eluted with 300 μL of formic acid followed by 3 mL of 95% (v/v) MeOH/H_2O. The fractions and total tannin were concentrated under nitrogen at 30 °C and dissolved in MeOH for analysis.

Tannin Characterization by Phloroglucinolysis. The composition of the total tannin and tannin fractions isolated from selected wine samples was characterized using acid-catalysis in the presence of excess phloroglucinol (phloroglucinolysis) as previously described.[10,25] Tannin fractions and total tannin were dissolved in MeOH to 10 g/L and 25 μL was reacted with 25 μL of phloroglucinol solution (phloroglucinol (100 g/L) in MeOH with 20 g/L ascorbic acid and 0.2 N HCl) at 50 °C for 25 min. Sodium acetate solution (70 mM, 150 μL) was added and the reaction products were analyzed using HPLC with two Chromolith RP-18e columns (100 × 4.6 mm) connected in series at 30 °C. The mobile phase was 1% (v/v) acetic acid/H_2O for solvent A and 1% (v/v) acetic acid/acetonitrile for solvent B with a gradient as follows: 0−4 min (3% B), 14 min (18% B), 14.01−20 min (80% B). The flow rate was 3 mL/min and elution of peaks monitored at 280 nm.[26] The chromatogram peaks were integrated and the proportions of flavan-3-ol monomers were calculated as previously reported.[25] Percent mass conversions were calculated based on the total peak areas and respective molar extinction coefficients of each flavan-3-ol subunit (i.e., the mass converted by the depolymerization reaction) relative to the mass of tannin used in the reaction.[25,26]

Tannin Molecular Size by Gel Permeation Chromatography. The molecular sizes of the total tannin samples and tannin fractions isolated from selected wine samples were estimated using gel permeation chromatography (GPC) as previously described.[27] Briefly, 80 μL of *N*,*N*-dimethylformamide (DMF) was added to 20 μL of tannin sample in MeOH to a final tannin concentration of 2 g/L. Samples were analyzed using two PLgel columns (300 × 7.5 mm, 5 μm, 500 Å followed by 10^3 Å) connected in series and protected by a guard column containing the same material (50 × 7.5 mm, 5 μm), all purchased from Polymer Laboratories (Amherst, MA), and a mobile phase of DMF with 0.15 M LiCl and 10% (v/v) acetic acid. Chromatograms were analyzed using Agilent ChemStation software and the retention time range was measured against a standard curve of fractionated preveraison (PV) grape skin tannins, which had been previously isolated and characterized.[28,29] The molecular sizes of the fractionated PV grape skin tannins were estimated using phloroglucinolysis as described above, and ranged from 1277 to 8996 (mDp 4.3−30.2) with the retention time of the monomer, epicatechin, incorporated into the calibration curve as the smallest subunit.

Octanol-Buffer Partition Coefficients. The relative hydrophobicity of the isolated tannin fractions was determined by measuring the octanol-buffer partition coefficients as previously reported.[29] Briefly, 50 μL of tannin solution (1 mg/mL in 10% (v/v) ethanol/H_2O containing 0.1% (v/v) formic acid) was added to 450 μL of buffer solution (20 mM Tris-HCl, pH 7.4) and 500 μL of octanol, mixed with a vortex mixer for 1 min and then shaken gently at room temperature using a rotating wheel for 30 min. The mixture was then centrifuged at 4000 rpm for 5 min and the UV absorbance of each phase (300 μL) was measured using a Spectral M2 max UV−vis Microplate reader (Molecular Devices) at 280 nm. The partition coefficients (LogP) were calculated as $\text{LogP} = \log_{10} [\text{ABS}_{\text{OCT}}/\text{ABS}_{\text{BUFFER}}]$.

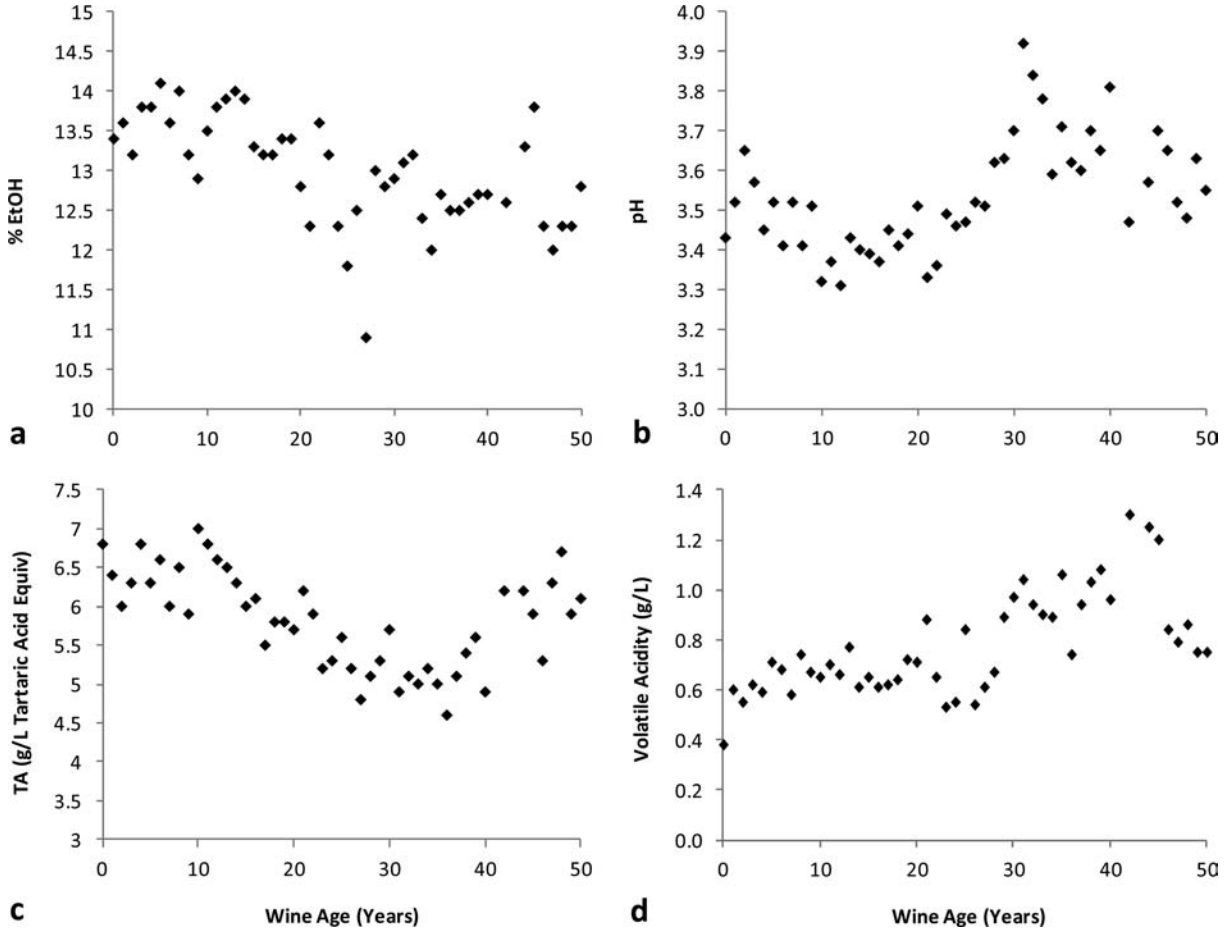

Figure 1. Wine matrix parameters for the 50 year vertical series (50CAS) as measured by WineScan: (a) alcohol concentration as % EtOH; (b) pH; (c) titratable acidity as g/L tartaric acid equivalents; (d) volatile acidity (VA) in g/L.

RESULTS AND DISCUSSION

Trends in Wine Parameters over 50 Years. A series of CAS wines spanning 1954 to 2004 from a single commercial Australian producer in the Coonawarra region (referred to as 50CAS) were analyzed using *FOSS* WineScan for alcohol, pH, and titratable and volatile acidities (Figure 1). The pH showed no age-related trend; however, it did vary across the vertical series, with younger wines (<25 years old) being lower in pH (pH 3.44 ± 0.08) than older wines (pH 3.64 ± 0.12). This change in pH is likely to be due to the changes in wine style and winemaking that occurred in Coonawarra in the 1980s. There was no trend observed in the titratable acid concentration over time at 5.8 ± 0.52 g/L over the series, yet there was a general increase in volatile acidity with wine age. More recent vintage wines were slightly higher in EtOH concentration than older vintages, emphasizing the stylistic variation in the wine as a result of winemaking techniques. The wine parameters across the vintages from the 50 year vertical series demonstrated stylistic variations more so than age-related trends and are therefore unlikely to promote age-related trends in the formation or structural alteration of wine tannins with age.

Impact of Wine Age on Color. The color measures of a 30 year series of CAS wines (30CAS) and SHZ wines (30SHZ) spanning 1977 to 2006 (both from the same commercial producer in the Australian Pyrenees region) were investigated along with those of the 50CAS wines to compare changes in the color in wines as a function of age. Reserve CAS wines (R30CAS) were also periodically released and five such wines were analyzed. The concentration of anthocyanins as measured using the modified Somers assay (Figure 2a) was substantially lower in the five year old wines (less than 200 mg/L) compared with younger vintages (such as 627 mg/L for the youngest wine) and there was a strong correlation (R^2 = 0.97) between anthocyanin concentration and wine age over this time. Wines of 10 or more years demonstrated half the anthocyanin concentrations of the five year old wines with concentrations ranging between 60 and 150 mg/L for all vertical series. The 30SHZ wines consistently contained slightly lower anthocyanin concentrations than 30CAS wines, which may be due to differences in the amount or composition of anthocyanins extractable from the different grape varieties. These values were consistent with those of red wines reported from other regions, such as Spain[30] and France.[31]

The exponential decrease in concentration of total anthocyanins related directly to that observed for malvidin-3-glucoside (M3G) as measured by HPLC for the 50CAS wines (Figure 2a) and indicated the instability of monomeric anthocyanins as previously reported.[21,32] Conversely, the concentration of pigments that are resistant to SO_2 bleaching as measured by the modified Somers assay (Figure 2b), including pyranoanthocyanins and pigmented polymers, showed no aged-related trends in concentration over 30 vintages for the wines in all vertical series, with an average of 4.0 ± 1.9 AU. Wines older than 40 years in the 50CAS series

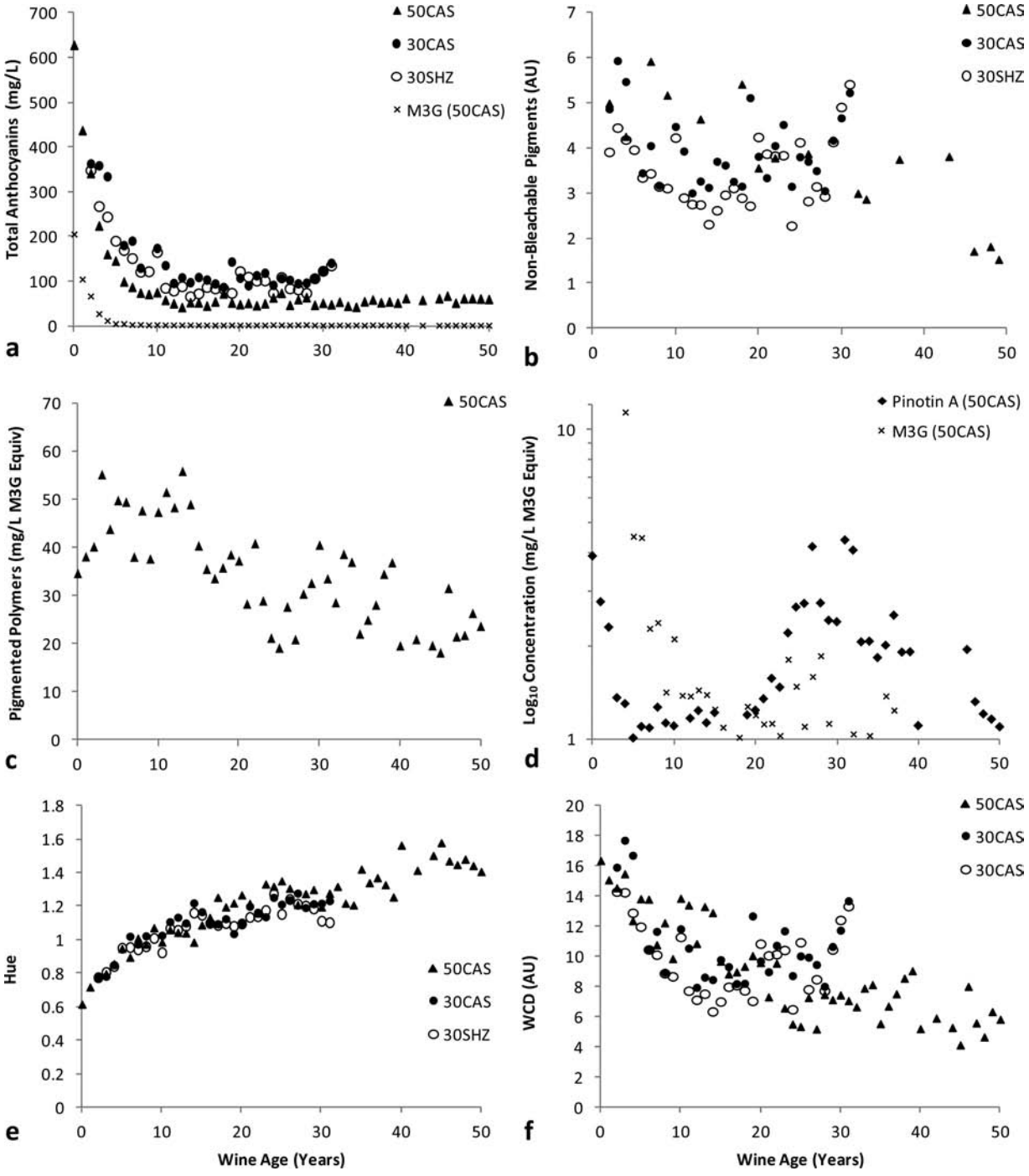

Figure 2. Wine color measures for the 50CAS wines and the 30 year vertical series of Cabernet Sauvignon (30CAS) and Shiraz (30SHZ) wines obtained using the modified Somers method: (a) total anthocyanin concentration (including the concentration of malvidin 3-glucoside (M3G) in the 50CAS wines); (b) non-bleachable pigments measured as absorbance units; (c) pigmented polymers measured by HPLC in mg/L M3G equivalents for the 50CAS wines; (d) concentration of pinotin A and M3G as measured by HPLC for the 50CAS wines; (e) hue; (f) wine color density (WCD).

showed a decline in measurable non-bleachable pigments to less than 2 AU. The concentration of pigmented polymers as measured by HPLC (Figure 2c) was slightly lower in aged wines than in younger wines, around 50 to 20 mg/L M3G equivalents, respectively. These concentrations are substantially more consistent across 50 vintages compared with the rapid decline with age of monomeric anthocyanins, and therefore, although the structure of the pigmented polymers may change over time, they remain responsible for wine color, particularly in older wines. This is further demonstrated in Figure 3, which shows the HPLC chromatograms recorded at 520 nm for the 50 year old and 2 year old wines from the 50CAS series. The solvent gradient of the HPLC method enables the separation of monomeric anthocyanins as well as the elution of all pigmented polymers, at 24 min. The only peaks remaining in the chromatogram of the 50 year old wine are those of pigmented polymers and pyranoanthocyanins, particularly pinotin A, demonstrating the absence of monomeric pigments in older

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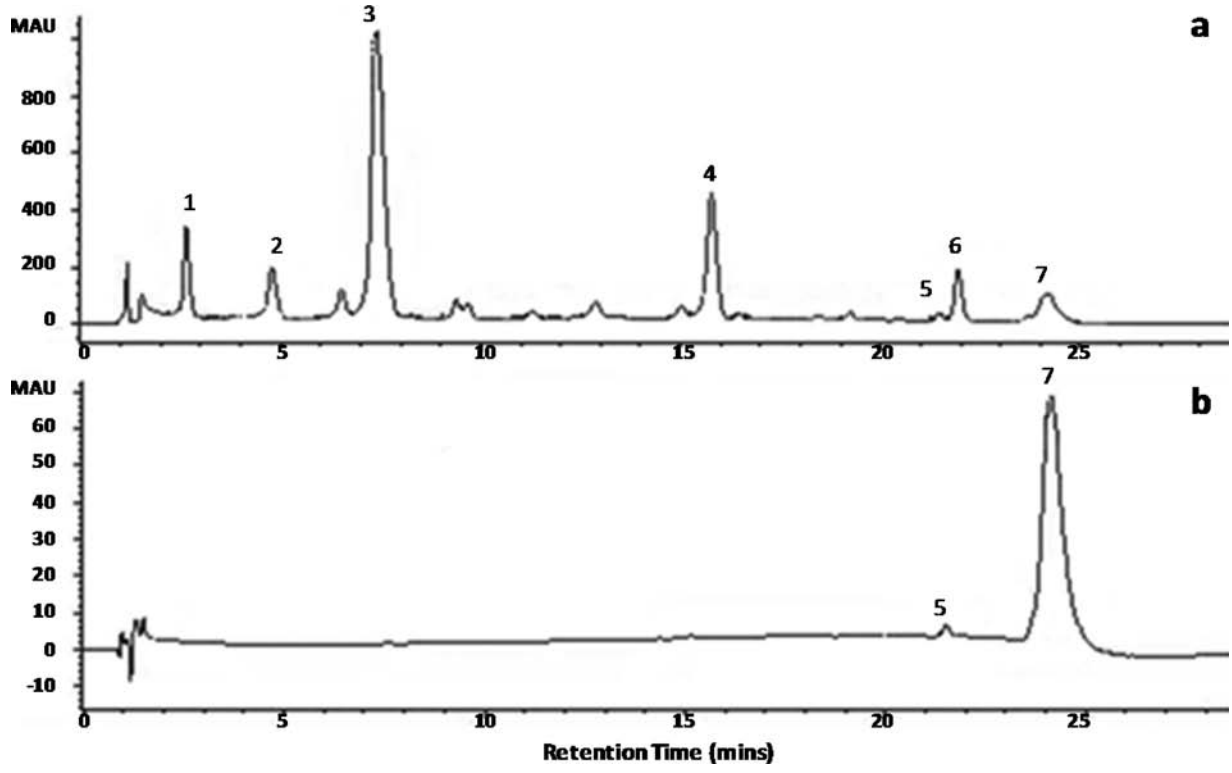

Figure 3. Chromatograms of (a) 2 year old and (b) 50 year old Cabernet Sauvignon at 520 nm. 1, delphinidin 3-glucoside; 2, petunidin 3-glucoside; 3, malvidin 3-glucoside; 4, malvidin acetyl glucoside; 5, pinotin A; 6, malvidin coumaroylglucoside; 7, pigmented polymers with small concentrations of pyranoanthocyanins.

wines. The concentration of the pyranoanthocyanin, pinotin A (Figure 2d), remained relatively low across the 50CAS series (0.8−4.4 mg/L M3G equivalents) with no age-related trends in concentration. Pyranoanthocyanins in wine have been shown to form during fermentation[33,34] and not necessarily change in concentration relative to the decrease in anthocyanins over time.[1] This suggests that the observed decrease in anthocyanin concentration with wine age may be related to the formation of pigmented polymers and the degradation of the monomeric anthocyanins, rather than the formation of pyranoanthocyanins.

The hue, or 'brownness' of each wine was measured as the relative absorbance at 420−520 nm. Wines of around 10 years old showed much greater hues than wines of only a few months old (1.1 and 0.6, respectively), yet that of wines up to 30 years old were only slightly higher at 1.2 and the hues of 40−50 year old wines were around 1.5 (Figure 2e). This suggested that chemical oxidative browning reactions occurred more rapidly in younger wines when there are more monomeric poylphenols and potentially acetaldehyde and glycoxylic acid present in the wine to promote direct and indirect condensations reactions including the formation of pigmented polymers.[13,35] This trend follows those reported for other aging trials.[19]

The wine color density (WCD) showed an expected, gradual decline over time for all three vertical series (Figure 2f) as the wine color changed from purple to red-orange with age as noted in previous studies.[6,19] This color change has been associated with a decline in anthocyanin concentration in young wines over time [6] and the formation of more stable pigments.[35] The WCD correlated strongly with total anthocyanin concentration in wines less than five years old (R^2 = 0.99, 0.93, and 0.95 for 50CAS, 30CAS, and 30SHZ, respectively), although this correlation was substantially lower across the entire series (R^2 = 0.36, 0.81, and 0.72, respectively) (Table 1). Comparatively, the correlation between WCD and the measurement of the non-bleachable pigments was higher for all three vertical series, with R^2 values of 0.94, 0.90, and 0.82 for 50CAS, 30CAS, and 30SHZ, respectively. This indicated that monomeric anthocyanins strongly impacted the WCD initially, while non-bleachable pigments were more important to the overall color stability in older wines. The non-bleachable pigments, WCD and anthocyanin concentrations were all higher in the R30CAS wines compared with the standard release, except for the 1977 Reserve, although they were made from the same fruit (Table 2). This indicated that the winemaking style was modified for the reserve releases to improve the perceived quality of the wine, highlighting the importance of winemaking and color to wine quality.

Table 1. Correlation (R^2) between the Wine Color Density (WCD) and Other Somers Color Measures over Each Vertical Series

	50CAS	30CAS	30SHZ
total anthocyanins	0.36	0.81	0.72
total anthos in wine <5 yo[a]	0.99	0.93	0.95
non-bleachable pigments	0.94	0.90	0.82

[a]Total anthocyanins for wines less than 5 years old.

Impact of Wine Age on Tannin Concentration. Red wine astringency is dependent on the concentration and composition of tannins in the wine. The concentration of tannin was measured in the 30CAS and 30SHZ wines (including the R30CAS samples) as well as 14 randomly selected 50CAS wines using the MCP tannin assay.[22] The

Table 2. Color Measures by Modified Somers Assay and Tannin Concentration by MCP Tannin Assay for the Standard and Reserve Releases of the 30CAS Wines

	1992		1988		1984		1979		1977	
	std[a]	res[b]	std	res	std	res	std	res	std	res
WCD (AU)[c]	9.3	12.7	9.7	13.0	8.8	12.4	10.7	11.2	13.7	10.4
total anthos (mg/L)[d]	103	150	106	138	91	135	105	117	142	113
NB pigments (AU)[e]	3.6	5.2	3.8	5.3	3.2	4.9	4.2	4.4	5.2	4.1
tannin (g/L)	1.43	3.52	1.67	3.53	1.85	2.91	1.96	3.01	3.42	3.11

[a]Standard release. [b]Reserve release. [c]Wine color density. [d]Total anthocyanins. [e]Non-bleachable pigments.

concentration of wine tannin in each vertical series showed a general decrease in the younger wines (Figure 4), however this

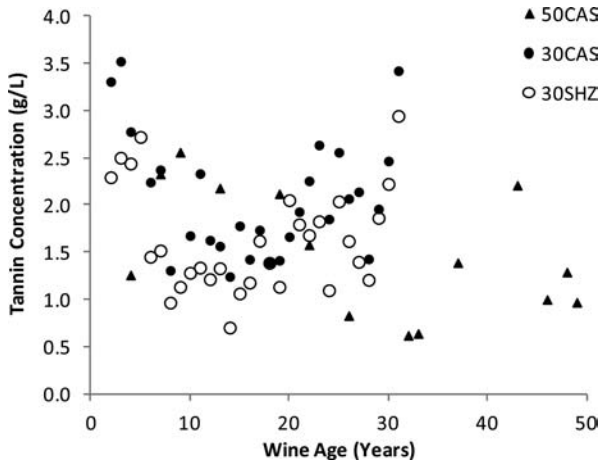

Figure 4. Tannin concentration (g/L epicatechin equivalents) by wine age for the randomly selected 50CAS wines as well as the 30CAS and 30SHZ wines obtained using the MCP tannin assay.

decline did not relate directly to the age of the wines, as some 30 year old wines were of similar concentration to wines only a few years old. This suggests that changes in wine astringency and mouthfeel in older wines is unlikely to be solely due to the change in tannin concentration. CAS wines generally showed slightly higher tannin concentrations than SHZ wines across the 30 year vertical series, potentially as a consequence of the relative concentration of tannins in the grapes of these varieties.[36] This may also have impacted upon the color measures as the 30CAS wines generally showed higher WCD and non-bleachable pigments than the 30SHZ wines, and the amount of tannins extracted from grapes during fermentation has also been shown to have a major impact on wine color.[36] The reserve release wines from the 30CAS vertical series (R30CAS) generally showed much higher tannin concentrations than the standard release wines of the same vintage (Table 2). The 1977 R30CAS wine also had high concentrations of tannin at 3.1 g/L, yet this was similar to that of the standard release for that vintage (3.4 g/L). The higher tannin concentrations of the reserve release wines correlated with the higher color measures of these wines and demonstrate the impact of both tannin and color on quality in this wine style. The high tannin concentration of the 30 year old wine for the 30CAS series from the Australian Pyrenees region is at odds with that of the 50CAS wines from the Coonawarra region of the same era (around 0.7 g/L). This may suggest that climatic differences and stylistic changes in winemaking have a greater impact on the concentrations of tannins in these vertical wine series more than any aging-related trends.

Impact of Wine Vintage on Tannin Composition. Differences in tannin structure in wines of different age were investigated by isolating tannin as described by Jeffery et al.[24] from the 30CAS and 30SHZ, wines as well as from 14 randomly selected vintages of the 50CAS wines. The structural characteristics for the isolated tannins from the 50CAS series were analyzed using phloroglucinolysis and GPC (Table 3).

Table 3. Structural Characteristics for the Total Tannin Isolated from Randomly Selected 50CAS Wines

wine age (years)	mDp[a]	% MC[b]	% GC[c]	% ECG[d]	50% GPC[e]	tannin (g/L)[f]
49	3.17	5.1	23.9	2.8	3394	0.97
48	3.42	4.9	23.6	2.9	3391	1.29
46	3.63	5.8	22.4	3.0	3196	1.00
43	3.40	4.2	20.8	4.4	2656	2.21
37	2.18	2.7	15.7	6.9	2540	1.39
33	2.47	3.3	20.8	3.0	2436	0.64
32	3.22	4.2	29.4	3.2	2168	0.62
26	2.91	3.4	20.8	4.9	2566	0.83
22	3.14	2.9	21.6	3.7	2729	1.58
19	4.71	6.1	33.2	2.0	2817	2.12
13	5.18	9.8	29.5	2.5	2530	2.18
9	5.33	17.3	24.3	2.4	2699	2.56
7	7.12	27.8	29.6	1.8	2493	2.33
4	9.22	44.8	36.1	2.4	2165	1.26

[a]Mean degree of polymerization. [b]Percent mass conversion calculated based on the wine tannin concentration as measured by the MCP tannin assay. [c]Percent epigallocatechin subunits. [d]Percent epicatechin gallate subunits. [e]Average molecular size (g/mol) estimated from GPC. [f]Calculated using the MCP tannin assay.

Phloroglucinolysis calculates the proportion of the tannin that has acid-labile interflavan bonds by measuring the mass of cleaved subunits relative to the mass of the original material.[10] Most notably, the percent mass conversion (proportion of acid-depolymerized tannin) was lower in the aged wine tannins compared with the younger wine tannins, indicating substantial changes in tannin structure due to acid-catalyzed depolymerization, condensation, rearrangement and oxidation reactions.[18,37] The most abundant subunits in all samples were epicatechin and catechin, although there were some variations in the proportion of the skin tannin-derived subunit, epigallocatechin, and the seed tannin-derived subunit, epicatechin gallate. In younger wine tannins, the percent of epigallocatechin subunits was around 30% compared with around 20% in older wine tannins, which is likely to be a consequence of gradual oxidation. The percent of epicatechin gallate subunits was slightly higher in aged wine tannins than in younger wine

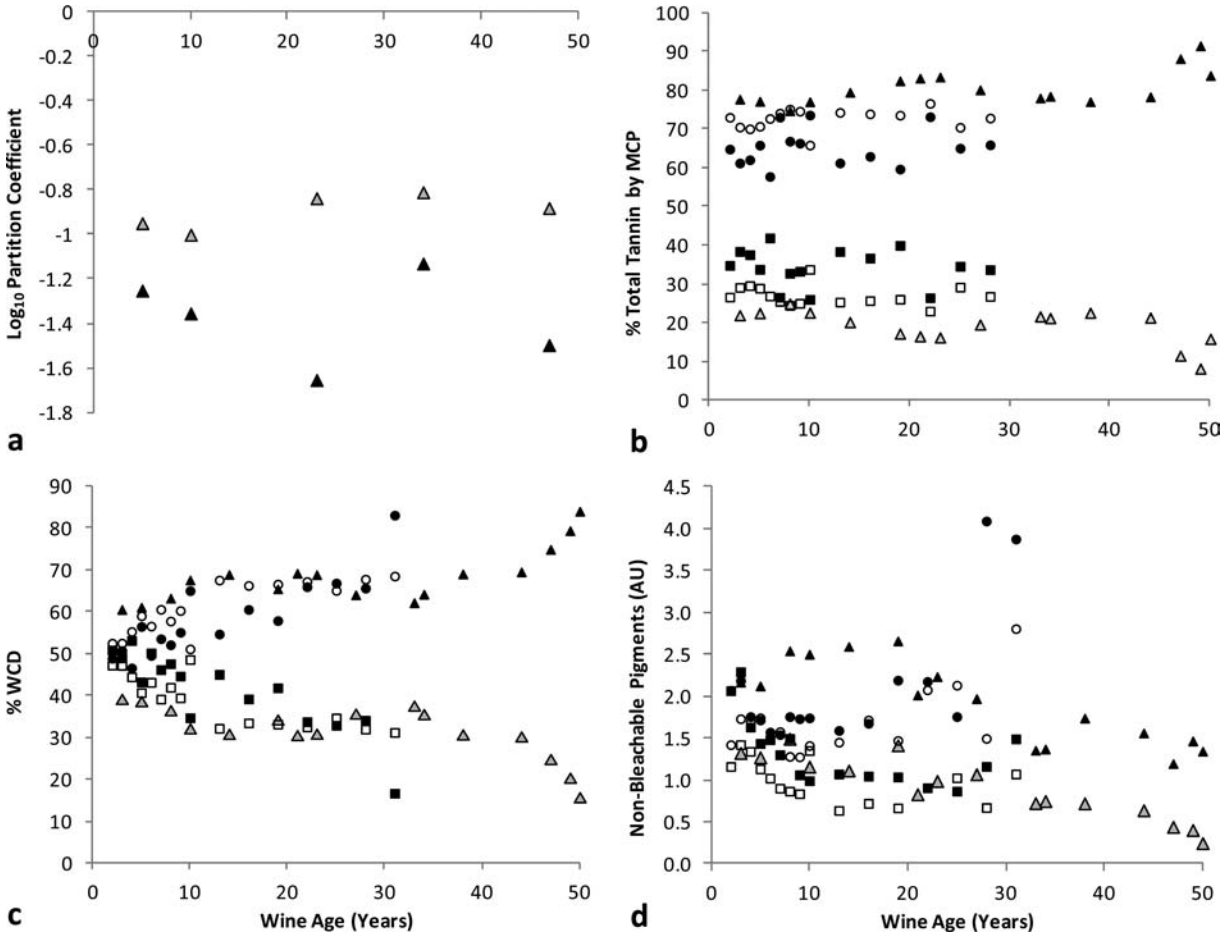

Figure 5. Characteristics of the tannin fractions over time, 50CAS F2 (gray △) and F3 (▲), 30CAS F2 (■) and F3 (●), and 30SHZ F2 (□) and F3 (○): (a) octanol/buffer partition coefficients for the selected 50CAS wines; (b) percent of tannin contributed by each tannin fraction over time; (c) percent of the tannin wine color density contributed by each fraction; (d) non-bleachable pigments for each fraction measured as absorbance units for each vertical series.

tannins, around 3 and 2%, respectively. This is in contrast to other studies in wine age [19] and what might be expected due to oxidation reactions, however this small difference may be more of a consequence of differences in the original extracted wine tannins during wine making, rather than an age-related trend. These trends are not likely to reflect the structure of the whole wine tannin given that the mass conversion of tannins from wines of 8 or more years old was less than 10%. The mDp as measured using phloroglucinolysis was lower in older wine tannins and this on first glance may imply that the tannin decreases in size with wine aging, yet the relative hydrodynamic volume as measured by GPC indicated that the aged wine tannins were actually slightly larger than the tannin isolated from younger wines. This discrepancy is likely to be due to the large proportion of wine tannin left unidentified using the phloroglucinolysis method, as has been reported with oxidized apple and grape tannins,[38] while GPC considers the whole size distribution of the wine tannin. Inferences made using the phloroglucinolysis data need to be made in context of how much material is characterized and caution needs to be applied. Furthermore, the increased oxidation of grape tannins with winemaking changes the overall tannin shape from more extended, rod-like configurations to more compact ball-like structures due to greater intramolecular bonding.[39] The fractionated preveraison grape tannin used to make the GPC standard curve is likely to be of the more rod-like configuration and would therefore potentially underestimate the determined wine tannin sizes, further exacerbating the discrepancy between the phloroglucinol mDp and the 50% GPC results. Overall, the aged wine tannins were larger than the younger wine tannins and demonstrated more characteristics that were consistent with oxidation.

Previous sensory studies on grape skin and apple tannins have demonstrated that larger tannin size as measured by mDp relates to an increase in astringency, such as the significant difference noted between mDp of 10 compared with mDp 3.[17] This grape tannin structure−function relationship may not hold for wine tannins due to the differences in structure between wine and grape tannins, particularly in older wines. For example, aged wines are often perceived as having softer astringency compared to young wines,[19] and yet aged wines have similar tannin concentrations and larger tannins (based on hydrodynamic volume) (Table 3). The reduction in astringency has been shown to correlate with mDp, with older wines showing a lower mDp.[19,40] This is more likely to be a consequence of the decreased proportion of acid-labile bonds as discussed than actual decrease in tannin size, which exemplifies the changes in wine tannin configuration with wine aging. Such structural changes may restrict the number of binding sites available for interaction with oral proteins[10] and therefore reduce the perceived astringency of the wine.

Impact of Wine Vintage on Composition of Tannin Fractions. Wine tannin was separated into two fractions, denoted F2 and F3,[24] by eluting the HLB SPE cartridge with different solvent systems, indicating that binding and solubility differences existed between wine tannin fractions. Octanol/buffer partitioning experiments confirmed that F2 was the more hydrophobic fraction across all the vertical series with consistently higher partition coefficients than F3 (Figure 5a), despite the smaller molecular size of F2 (1680 ± 280 compared with 3818 ± 596 for F3) as determined by GPC (Table 4). The

Table 4. Structural Characteristics for Tannin Fractions F2 and F3 Isolated from Selected 50CAS Wines

tannin fraction	wine age (years)	mDp[a]	% MC[b]	% GC[c]	% ECG[d]	50% GPC[e]
F2	48	1.92	9.6	12.2	6.8	1627
	37	1.88	5.2	8.4	11.1	1870
	19	2.62	8.1	20.9	6.0	1821
	4	3.64	18.8	24.6	2.2	1400
F3	48	3.21	3.7	22.5	3.1	3743
	37	2.88	15.2	16.7	5.6	3530
	19	4.59	6.2	28.5	2.4	4414
	4	9.84	39.1	36.0	2.5	3584

[a]Mean degree of polymerization. [b]Percent mass conversion calculated based on the wine tannin concentration as measured by the MCP tannin assay. [c]Percent epigallocatechin subunits. [d]Percent epicatechin gallate subunits. [e]Average molecular size (g/mol) estimated from GPC.

hydrophobicity of the wine tannin fractions varied across the 50CAS wines in patterns that were independent of wine age, which may give some indication of the differences in tannin structures. Previous work on the relative ethanol solubility of oxidized and native grape tannin fractions demonstrated that oxidized structures can be more hydrophobic,[38,39,41] and hence in this study the F2 fraction may have a more oxidized and thus modified structure compared with F3. Furthermore, the proportion of epigallocatechin subunits was greater in F3 tannins, potentially contributing to the relatively increased water-solubility of these fractions and may also indicate that F3 tannins were less oxidized than F2 tannins.

The concentrations of each fraction in the wines of each vertical series were measured using the MCP assay. The relative proportions of F2 and F3 remained consistent over the first 30 years of each vertical series (Figure 5b) indicating that the reactions over time were not favoring the formation of any particular fraction. A slight divergence was only observed in the relative proportions of F2 and F3 in the 50CAS wines after 45 years, with the concentration of F3 increasing from 80% to around 90% of the total tannin. Similar trends were observed for the WCD of wines greater than 10 years old (Figure 5c), and F3 showed a consistently greater contribution to the overall WCD than F2. The concentrations of non-bleachable pigments (Figure 5d) were consistent in each fraction across the vintages and were slightly higher in F3 than F2, which is likely to be due to the greater concentrations of F3 relative to F2.

The relative contribution of each fraction was consistent for wines of 10 to 45 years old at around 70% for F3, and diverged in wines greater than 45 years old with the contribution of F3 increasing to almost 85%. For wines less than 10 years old, the relative contribution of F2 and F3 to overall WCD in was almost 50% for each fraction, which was independent of the trends observed in relative concentration, potentially indicating that the tannin structure changes more rapidly in younger wines with the decrease in anthocyanin concentration. The strong correlation (R^2 = 0.92 and 0.83, for F2 and F3, respectively) between the WCD of the 50CAS wines and the respective concentrations of tannin fractions (Table 5) demonstrated the

Table 5. Correlation (R^2) between the Somers Color Measures of the 50CAS Wines and the Corresponding Isolated Total Tannin (TT) and Tannin Fractions F2 and F3

	TT	F2	F3
wine color density	0.92	0.92	0.83
non-bleachable pigments	0.91	0.91	0.84
hue	0.99	0.96	0.98

importance of tannin concentration to wine color. Likewise, the correlations with the non-bleachable pigments for both fractions (R^2 = 0.91 and 0.84 for F2 and F3, respectively) emphasized the contribution of pigmented polymers to tannins and wine color. The trend in wine hue over the 50CAS wines strongly correlated with that of total tannin concentration (R^2 = 0.99) as well as F2 and F3 concentrations (0.96 and 0.98, respectively) (Table 5), further suggesting that wine hue results from changes in tannin color expression with aging, rather than reflecting changes in monomeric pigment contributions.

In summary, trends in wine color were observed across the three vertical wine series as a decrease in WCD and increase in hue with wine age. There was no obvious trend observed in tannin concentration across each vertical series with wine-making style being more influential than vintage, suggesting that changes in astringency with wine age are more likely to be due to changes in tannin structure than a decrease in the concentration of tannin. The tannin structure was markedly modified in older vintage wines as demonstrated by much smaller conversion yields. There were no major trends observed in tannin size, subunit composition, or properties of the isolated tannin fractions. Long-term investigations into monitoring the change in tannin structure within individual wines over time are required for a greater understanding of how wine tannin structures relate to astringency.

AUTHOR INFORMATION

Corresponding Author

*E-mail: Paul.Smith@awri.com.au. Tel: +61 88313 6600. Fax: +61 8 8313 6601.

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Notes

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