JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Sensory Properties of Wine Tannin Fractions: Implications for In-Mouth Sensory Properties

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ABSTRACT: Different molecular structures of grape tannins have been shown to influence astringency, however, the in-mouth sensory effects of different molecular structures in red wine tannins remains to be established. The objective of this research was to assess the impact of wine tannin structure on in-mouth sensory properties. Wine tannin was isolated from Cabernet Sauvignon wines of two vintages (3 and 7 years old) and separated into two structurally distinct subfractions with liquid−liquid fractionation using butanol and water. The aqueous subfractions had greater mean degree of polymerization (mDp) and contained a higher proportion of epigallocatechin subunits than the butanol-soluble subfractions, while the older wine tannin fractions showed fewer epicatechin gallate subunits than the younger tannin fractions. The red wine had approximately 3:1 mass ratio of the aqueous and butanol tannin subfractions which approximated an equimolar ratio of tannin in each subfraction. Descriptive sensory analysis of the tannin subfractions in model wine at equimolar concentrations revealed that the larger, more water-soluble wine tannin subfractions from both wines were perceived as more astringent than the smaller, more hydrophobic and more highly pigmented butanol-soluble subfractions, which were perceived as hotter and more bitter. Partial least squares analysis indicated that the greater hydrophobicity and color incorporation in the butanol fractions was negatively associated with astringency, and these characteristics are also associated with aged wine tannins. As the larger, water-soluble tannins had a greater impact on the overall wine astringency, winemaking processes that modulate concentrations of these are likely to most significantly influence astringency.

KEYWORDS: *astringency, hydrophobicity, sensory, tannin structure, wine tannin*

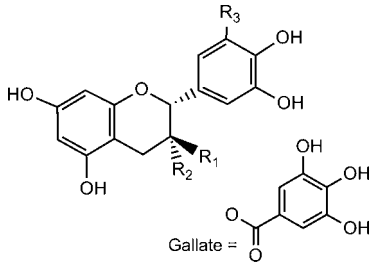
INTRODUCTION

Tannins in wine consist largely of condensed tannin polymers that are extracted from grapes and structurally altered during winemaking. Prior to veraison, when red wine grapes start to ripen with the beginning of sugar accumulation and pigmentation due to anthocyanin formation, isolated grape tannins are readily susceptible to acid-catalyzed cleavage reactions and thus can be characterized by analysis of the cleaved subunits.[1] Wine tannin is structurally quite different from preveraison grape tannin due to the incorporation of anthocyanins[2] and changes resulting from the chemical and enzymatic oxidation and rearrangement reactions that occur during the grape crushing and fermentation processes.[3] As wines age, tannin structures continue to be altered due to further oxidation and the rearrangement reactions that occur when tannins are exposed to an acidic medium.[4,5]

The tannins in red wine influence the in-mouth sensory properties including mouthfeel, particularly with respect to astringency,[6] and therefore the perceived quality of the wine. Astringency refers to a drying or puckering sensation that largely involves the interaction of wine tannins with oral proteins[7,8] and is influenced by many factors. Tannin induced astringency can be increased in matrices with lower ethanol concentrations, lower pH,[9,10] lower viscosity,[11,12] and lower concentrations of other macromolecules such as polysaccharides.[13,14] In particular, tannin concentration has been shown to correlate strongly with perceived astringency intensity.[15−17] As red wines age they are often perceived as decreasing in astringency.[5,18] Some reports suggest that this is related to a decrease in tannin concentration with wine age,[19] although other reports show that this occurs irrespective of tannin concentration,[20] suggesting that other changes, including the gradual modification of wine tannin structures, influence the perceived wine astringency.

Differences in grape and apple tannin structures have been shown to influence mouthfeel perception and protein interactions in model wine solutions. Molecular weight (degree of polymerization) and proportion of epicatechin gallate subunits (percent galloylation, Figure 1) correlated positively with both the perceived astringency [21] and protein binding in vitro,[22] while the proportion of the trihydroxylated flavan-3-ol subunit, epigallocatechin (Figure 1), reduced the coarse perception of astringency.[21] These results have been interpreted with respect to the astringency characteristics of tannins from grape seeds and skins, with the highly galloylated seed tannins being considered more astringent and less desirable than skin tannins,[5] which consist of higher concentrations of epigallocatechin subunits.[23] The incorporation of anthocyanins into the tannin structure has also been shown to reduce astringency in model systems and may relate to greater proportions of pigmented polymers, such as those found in grape skins rather than grape seeds, being present in the wine tannin.[24] Studies on wine quality gradings of young red wines have indicated that larger proportions of skin-derived

Received: October 3, 2012
Revised: December 20, 2012
Accepted: January 5, 2013
Published: January 5, 2013



Catechin: R_1=OH, R_2,R_3=H
Epicatechin: R_1,R_3=H, R_2=OH
Epicatechin gallate: R_1,R_3=H,R_2=Gallate
Epigallocatechin: R_1=H, R_2,R_3=OH

Figure 1. Structures of the catechin derivatives found as grape tannin subunits.

tannin subunits in the wine are associated with a higher quality grading.[25]

While much research has investigated the impact of grape tannin structures on astringency and mouthfeel perception, there have been far fewer studies of the relationship between wine tannin structure and in-mouth sensory properties. Some studies have investigated the astringency perception of whole wine,[26,27] however, the complex matrix of red wine with its inherent differences, such as pH and ethanol concentration, makes the direct association of these results to the structural composition of the wine tannin very difficult. Further research is required on isolated wine tannin in systems with a consistent matrix to better understand the effect of differences in wine tannin structure on mouthfeel. In this paper, we report the isolation of tannins from Cabernet Sauvignon wines of two vintages (3 and 7 year old wines) from an Australian commercial wine and the use of liquid–liquid fractionation to separate the tannin into two distinct subfractions of different molecular size and solubility. The main objective of this study was to investigate structure–functions relationships of the wine tannin subfractions in model wine and their perceived in-mouth sensory properties.

MATERIALS AND METHODS

Chemicals. Food grade ethanol (EtOH, 96%) was purchased from Tarac Technologies Pty Ltd. (Nuriootpa, SA, Australia). Formic acid (98–100%) was purchased from Rowe Scientific (Lonsdale, SA, Australia). Ethyl acetate (EtOAc, 99.8% by GC), methanol (MeOH, 99.8% by GC), acetone (99.8% by GC), 1-butanol (99.5% by GC), 1-octanol (>99% by GC), glacial acetic acid, and acetonitrile (ACN, gradient grade for liquid chromatography), all of analytical grade or higher, were obtained from Merck (Kilsyth, VIC, Australia). Potassium bitartrate, *N*,*N*-dimethyl formamide (DMF), and lithium chloride (LiCl) were sourced from Sigma-Aldrich (Castle Hill, NSW, Australia), and food grade tartaric acid was purchased from Winequip Products (Newton, SA, Australia). Milli-Q water was obtained from a water purification system (Millipore, North Ryde, NSW, Australia). Commercial oenotannin (Grape'Tan PC seed tannin) was obtained from Amtrade (Melbourne, Australia).

Basic Wine Composition. Australian Coonawarra Cabernet Sauvignon wines (Wynns Coonawarra Wine Estates, Black Label) from two vintages (3 and 7 years old) (approximately $30–40 per bottle) were studied. The older vintage wine was bottled under cork and the younger vintage under screwcap. The basic chemical compositional measures of the wines were 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 alcohol concentration, pH, specific gravity, titratable acidity at pH 8.2 and 7.0, glucose and fructose concentration, and volatile acidity as acetic acid. The concentrations of free and total sulfur dioxide were measured by flow injection analysis using a Lachat Instruments QuickChem FIA+ 8000 series with a reagent pump (RP-100 series), and data were analyzed with Omnion 3.0 software.

Tannin Isolation. Tannin was isolated from wine as reported by Kennedy and Jones[1] with some modification. Briefly, wine (1 L) was loaded on to a glass low pressure chromatography column (Aldrich 50 mm × 450 mm) packed with Toyopearl HW-40F size-exclusion medium (Optigen Scientific Pty Ltd., Port Adelaide, SA, Australia), previously equilibrated with H_2O/0.1% v/v formic acid. The column was washed with H_2O/0.1% v/v formic acid/ (2 L) to remove the residual organic acids and sugars and 1:1 MeOH/H_2O with 0.1% v/v formic acid (approximately 7.5 L) to remove smaller molecules including monomeric flavan-3-ols, anthocyanins, and small oligomers. Tannin was eluted with 2:1 acetone/H_2O/0.1% v/v formic acid (2 L).

Tannin Purification. Purification of the wine tannin was achieved using liquid–liquid fractionation to remove residual monomeric polyphenols including flavonols. Acetone was removed in vacuo using a rotary evaporator at 30 °C, and water was added to bring the total tannin solution to 650 mL. EtOAc (200 mL) was added to the aqueous tannin solution in a separation funnel, shaken vigorously, and the layers allowed to separate. The aqueous fraction was removed from the funnel and re-extracted with a further 200 mL of EtOAc. Any remaining organic solvent was removed from the tannin aqueous phase in vacuo using a rotary evaporator (30 °C). HPLC monitoring was performed to assess flavonol removal and tannin recovery using the method described in Mercurio et al.[28] Briefly, samples were analyzed using an Agilent 1100 LC (Agilent, Australia) with a Phenomenex Synergi Hydro-RP C18 column (150 mm × 2 mm, 4 μm) at 25 °C. Solvent A consisted of 1% ACN and 1.5% phosphoric acid in H_2O, and solvent B was 80:20 ACN/solvent A, using the gradient previously described.[28] A small amount (approximately 50 mL) of the total tannin solution after the removal of flavonols was retained from each vintage for chemical analysis (referred to as the total tannin fraction from the 7 year old wine (TT7) and the 3 year old wine (TT3)). The remainder of the total tannin fraction from each vintage in water was further separated into two subfractions as described below.

Tannin Fractionation and Recoveries of Subfractions. The total tannin fraction in water (600 mL) from each vintage wine were then further fractionated using liquid–liquid separation. Butanol (BuOH, 600 mL) was added to the total tannin fraction solution in a separation funnel, shaken vigorously, and the layers allowed to separate. Both phases were collected and dried in vacuo using a rotary evaporator (30 °C) followed by freeze-drying to remove any residual water. The tannin isolation and fractionation procedures were repeated to give sufficient quantities of each subfraction for sensory analysis. The total recoveries of the four tannin subfractions per liter of wine were: 7 year old wine tannin aqueous phase (Aq7), 0.82 g/L; BuOH phase (Bu7), 0.23 g/L; 3 year old wine tannin aqueous phase (Aq3), 1.11 g/L; BuOH phase (Bu3), 0.38 g/L.

Tannin Composition by Phloroglucinol. The composition of the tannin fractions and subfractions were characterized using acid catalyzed cleavage reactions in the presence of excess phloroglucinol (phloroglucinolysis) as previously described.[30] Briefly, tannin solutions (25 μL, 10 g/L MeOH) were reacted 1:1 with 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 prior to the addition of sodium acetate solution (70 mM, 150 μL). Reaction products were analyzed using HPLC with 2× chromolith RPC18 columns connected in series (100 mm × 4.6 mm each) with a mobile phase of 1% v/v acetic acid/H_2O with a gradient to 80% v/v acetic acid/ACN.[30] The chromatogram peaks at 280 nm were integrated, and the proportions of flavan-3-ol monomers were calculated as previously reported.[1]

Octanol–Water Partition Coefficients. The relative hydrophobicities of the tannin fractions and subfractions were determined by measuring the octanol–water partition coefficients. Tannin solutions (50 μL, 1 mg/mL in MeOH) were mixed with H_2O (450 μL) and octanol (500 μL) via vortex mixer for 1 min and then via carousel at room temperature for 30 min. Mixtures were centrifuged at

4000 rpm for 5 min to separate the octanol and water phases and 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 (Log P) were calculated as Log P = $\log_{10}$ (ABS_{OCT} /ABS_{WATER}), where ABS refers to the absorbance of the octanol or water phases at 280 nm.

Spectral Measures. The tannin concentrations of the tannin fractions in model wine (in mg/L epicatechin equivalents) and of the whole wines were determined using the methyl cellulose precipitable (MCP) tannin assay.[28] Briefly, wine (25 μL) was reacted with 300 μL of either polymer solution (0.04% MCP in H_2O) for the treatment mixture or H_2O for the control mixture in a 96-well plate for a total of 3 min (shaken for 1 min and allowed to rest for 2 min). Saturated ammonium sulfate solution (200 μL) was added to each well and the total volume made up to 1 mL in H_2O. Plates were shaken for 1 min and allowed to stand for 10 min prior to centrifuging at 2000 rpm for 5 min. Supernatant (300 μL) was transferred to a UV plate, and the absorbance was measured at 280 nm (A280 nm) with a Spectral M2 max UV−vis microplate reader (Molecular Devices). Tannin concentration was calculated as the difference between the A280 nm of the control and treatment wells compared with a standard curve of epicatechin concentrations and hence reported in epicatechin equivalents (mg/L).

Modified Somers color measurements were performed on tannin solutions using a high throughput method in 96-well microplates [28] and UV absorbances were measured with a Spectral M2 max UV−vis microplate reader (Molecular Devices) to give wine color density (WCD), hue, A520 nm, and A280 nm (absorbance units of the samples under acidic conditions at 520 and 280 nm, respectively) as well as SO_2 nonbleachable pigment concentrations. Briefly, the wine or tannin fractions made in model wine for the sensory analysis were diluted 1:9 with 0.5% tartaric acid in 12% EtOH and the absorbances measured at 420 and 520 nm. The combined absorbance units of both wavelengths gave the WCD, and the hue was given by absorbance units at 420 nm divided by the corresponding absorbance at 520 nm. A520 nm and A280 nm were measured after dilution (1:49) with HCl solution (1 M) for 3 h. For the SO_2 nonbleachable pigment measurements, the absorbance of the wine samples at 520 nm was measured after reaction (1:9) with a buffer solution (0.375% sodium metabisulphite, 0.5% tartaric acid in 12% EtOH) for 1 h.

Tannin Molecular Size by GPC. The average molecular size was estimated for the tannin fractions and subfractions using gel permeation chromatography (GPC) as previously described.[29] Briefly, tannin solutions (2 g/L in 1:4 MeOH: DMF) were analyzed using two PLgel columns connected in series (pore size 500 Å followed by 10^3 Å, 300 mm × 7.5 mm each, 5 μm particle size, plus a guard column, 50 mm × 7.5 mm, Polymer Laboratories, Amherst, MA, USA) and an isocratic solvent system of DMF with 0.15 M LiCl and 10% v/v acetic acid. Chromatograms were analyzed using Agilent ChemStation software against a standard curve of fractionated preveraison (PV) grape skin proanthocyanidins.[23] The average molecular weight was calculated as the mass corresponding to the elution of 50% of the tannin (50% GPC). The remaining tannin was normally distributed (bell-shaped curve) around this molecular weight, and the range of the molecular mass distribution was similar for each sample.

Tannin Fraction Solutions for Sensory Analysis. Tannin subfractions were dissolved into model wine at equimolar concentrations to determine a direct structure−function relationship with the sensory analysis. At an equimolar ratio, the gravimetric proportions of each subfraction (~3:1) were similar to the proportions of the subfraction found in the original red wine and therefore the results were relevant to the relative contribution of each fraction to the whole wine. The molar concentration of each tannin subfraction was calculated using the average molecular mass determined with GPC (as described above): Aq7 = 3272 g/mol, Aq3 = 3034 g/mol, Bu7 = 1929 g/mol, Bu3 = 1803 g/mol. A tannin concentration of 0.35 mM was considered appropriate based on informal bench trials. The final gravimetric concentrations of each subfraction to give solutions at 0.35 mM (using the GPC molar mass) were as follows: Aq7 = 1.15 g/L, Bu7 = 0.68 g/L, Aq3 = 1.06 g/L, and Bu3 = 0.63 g/L.

Tannin subfractions (0.35 mM) were dissolved in model wine (10% EtOH in H_2O, saturated with potassium bitartrate, and adjusted to pH 3.5 with tartaric acid) for sensory analysis. GCMS analysis of the isolated fractions in model wine confirmed that residual solvent levels were below food safety specifications (Australia New Zealand Food Standards Code − Standard 13.3 − Processing Aids). Given that the different gravimetric weights for the tannin subfraction solutions would make comparison of the Somers color measures on a per gram of tannin basis difficult, the results for the color measures (as described above) were normalized for gravimetric concentration by dividing the results by the tannin concentration (g/L) as determined by MCP tannin assay to give $WCD_{[NORM]}$, $hue_{[NORM]}$, degree of redness, A280 $nm_{[NORM]}$, and nonbleachable $pigments_{[NORM]}$.

Sensory Analysis. All sensory data were obtained in compliance with institutional procedures for sensory evaluation, involving risk assessment and informed consent, and all samples were expectorated. Descriptive sensory analysis was performed using a panel of 14 trained assessors (five male, nine female) who were members of the AWRI trained descriptive analysis panel. Panelists attended four training sessions to determine and refine appropriate descriptors for rating in the formal sessions using a consensus method.[31] Wines were assessed by palate only and standards were presented in the training sessions for *Alcohol* (12% (v/v) food grade EtOH in H_2O), *Acidity* (1 g/L tartaric acid in 12% aqueous EtOH solution), *Bitterness* (0.015 g/L quinine sulfate in H_2O), *Astringency* (0.7 g/L commercial oenotannin in 12% aqueous EtOH solution), and *Viscosity* (2 g/L pectin in 12% aqueous EtOH solution).

Samples (30 mL, 4× tannin fractions at 0.35 mM in model wine (12% aqueous EtOH saturated with potassium bitartrate and adjusted to pH 3.5 with tartaric acid) and 1× model wine as a control) were presented to panelists in covered ISO standard wine glasses identified with 3-digit codes, at 22−24 °C in isolated booths under sodium lighting. Before formal rating sessions, a series of practice rating sessions were conducted where panel performance was evaluated, and for these sessions the samples were presented on two trays with five samples per tray in a constant presentation order. Samples were assessed in a single formal booth session, during which assessors were presented with three trays of five samples (the four tannin fractions plus control model wine with no addition) arranged in randomized order within each tray and across judges. After each sample, the assessors rinsed their mouth with a pectin solution followed by a water rinse and then rested for 90 s. There was a 10 min rest between trays, during which time assessors were required to leave the booths. In the formal session, assessors rated the samples for the nine palate attributes that were determined as a result of the attribute generation and practice sessions (Table 1). The intensity of each attribute was rated using an unstructured 15 cm line scale, with indented anchor points of "low" and "high" placed at 10% and 90%, respectively. Data was acquired using Fizz sensory software (version 2.46, Biosystemes, Couternon, France).

Sensory Data Analysis. Panel performance was assessed using Fizz, Senstools (OP&P, The Netherlands), and PanelCheck (PC, Matforsk) software and included analysis of variance for the effect of sample, judge, and presentation replicate and their interactions, degree of agreement with the panel mean, and degree of discrimination across samples. On the basis of these results, all judges were found to be performing to an acceptable standard. Data were analyzed to determine differences between treatments using Genstat ANOVA (14th ed., VSN International, UK). Relationships between the chemical properties of the tannin subfractions and their sensory properties were determined using partial least squares (PLS2) regression calculated with The Unscrambler X software (version 10.2, Camo Software, Oslo, Norway). All variables were standardized before analysis, with all sensory data (Y-variables) modeled with the chemical measures as X-variables, so that a single model was obtained. The optimal number of components for the model was obtained from assessment of residual variance explained by each PC, following full cross-validation. The tannin color measurements used in the analysis were normalized for gravimetric concentration by dividing the Somers

Table 1. Attributes and Definitions Used in the Sensory Descriptive Study of the Wine Tannin Subfractions

attribute	definition
Alcohol	the intensity of the flavor of ethanol
Acidity	the intensity of acid taste perceived in the mouth or after expectorating
Viscosity	the perception of the body, weight or thickness of the wine in the mouth: low = watery, thin mouth feel; high = thick mouth feel
Hotness	the intensity of hotness perceived in the mouth: low = warm; high = hot
Bitterness	the intensity of bitter taste perceived in the mouth and/or after expectorating
Astringency	the intensity of drying and mouth-puckering sensations in the mouth and/or after expectorating, includes furry, chalky, drying, puckering
Hot Aftertaste	the intensity of hotness perceived after expectorating: low = warm; high = hot
Burning Aftertaste	the intensity of chemical burning, sensation associated with peppery olive oil, chilli, or black pepper burn
Tingling	the intensity of the tingling sensation on the tongue and/or lips during tasting and/or after expectorating
other	any other taste or sensation in the mouth during tasting or after expectoration.

color measures by the tannin concentration as determined by MCP assay. All analyses were conducted in January–March 2012.

RESULTS AND DISCUSSION

Wine Analysis. In this study, two premium wines were analyzed, a 2008 and a 2004 vintage (3 and 7 years of age, respectively) from the same commercial winery. The basic composition analysis (Table 2) showed that the two wines had

Table 2. Basic Chemical Composition of the Two Wines Studied

component	3 year old wine	7 year old wine
alcohol (% v/v)	14.2	13.3
specific gravity	0.9950	0.9945
pH	3.54	3.50
titratable acid pH 8.2 (g/L)	6.5	6.3
glucose + fructose (g/L)	1.0	0.6
volatile acidity as acetic acid (g/L)	0.63	0.45
sulfur dioxide (free) (mg/L)	17	<4
sulfur dioxide (total) (mg/L)	80	21

similar values generally, but the alcohol level of the 2008 wine was higher, suggesting that there were higher total soluble solids in the grapes at harvest. Somers color measures were used to assess the color of the wines and the isolated tannin fractions (Table 3). The absorbance units measured at 520 nm (A520 nm) in the 7 year old wine was almost half that of the 3 year old wine. The lower A520 nm and greater hue or "brownness" associated with older wines has been reported in several studies[32−34] and is related to a reduction in anthocyanin concentration as a consequence of pigmented polymer formation[35,36] and to some extent, malvidin 3-glucoside degradation,[37] as well as to oxidation reactions.[3,19] The tannin concentration of the 7 year old wine as measured by the MCP tannin assay was lower than the 3 year old wine. Such a distinctive difference is not always observed in red wines with aging[20,27] and may be related to differences in the ripeness of the grapes at harvest or other vintage-related effects.

Table 3. Color Analysis (by Modified Somers Method) and Tannin Concentration (by MCP tannin Assay) of the Wines, the Tannin Fractions (TT), and Subfractions (Aq and Bu for Aqueous and Butanol-Soluble Fractions, Respectively) at 0.35 mM in Model Wine

sample	WCD[a] (au)	hue	A520 nm[b] (au)	A280 nm[c] (au)	NB pigments[d] (au)	tannin[e] (g/L)
Wine04	11.8	1.03	0.179	54.1	4.26	1.72
TT7	5.2	1.07	0.057	17.1	2.07	0.79
Bu7	4.1	1.08	0.042	5.5	1.57	0.72
Aq7	5.6	0.98	0.065	11.2	2.19	1.24
Wine08	16.5	0.85	0.334	70.9	5.43	2.32
TT3	3.7	0.92	0.047	12.6	1.48	0.73
Bu3	2.5	0.99	0.040	4.3	0.98	0.61
Aq3	3.7	0.84	0.057	9.52	1.46	1.10

[a]Wine color density. [b]Absorbance at 520 nm. [c]Absorbance at 280 nm. [d]Nonbleachable pigments. [e]Tannin measured in g/L epicatechin equivalents.

Impact of Wine Age on Tannin Structure. The molecular size and subunit composition of the tannin fractions and subfractions were determined using GPC and phloroglucinolysis (Table 4). The percent mass conversion (%MC) of tannin from the phloroglucinolysis assay was substantially lower in the aged wine tannin compared to the young wine tannin, which is a consequence of the gradual decline in the proportion of acid-labile bonds, resulting from oxidation and rearrangement reactions during wine aging as previously shown.[4,38,39] As wine ages and the tannin structure changes to become less susceptible to depolymerization, the measured mean degree of polymerization (mDp) of the small proportion of acid-labile tannin has been shown to decrease, although the average molecular size of the whole tannin does not necessarily change.[38,39] This is demonstrated in our data by the similar molecular size as measured by GPC for total tannin samples from each vintage wine (2850 and 2930 g/mol for TT7 and TT3, respectively) and yet the mDp of the acid-labile portion of TT7 was smaller than that of TT3 (7.7 and 9.1, respectively) (Table 4). The percent gallocatechin ((−)-epigallocatechin subunits) was similar in both vintages, although the percent galloylation (epicatechin gallate subunits) in the aged wine tannin was almost half that of the younger tannin. TT7 had higher WCD, hue, degree of redness, and nonbleachable pigments (Table 3) demonstrating the increased color incorporation in tannins with wine aging.

Characteristics of Wine Tannin Subfractions. Fractionation of the isolated tannins by liquid–liquid separation created two tannin subfractions per vintage: an aqueous fraction (Aq3 and Aq7 from the 3 and 7 year old wines, respectively) and a butanol-soluble fraction (Bu3 and Bu7 from the 3 and 7 year old wines, respectively). The aqueous subfractions were the gravimetrically dominant subfractions from both the 3 year and 7 year old wines, with the mass of Aq3 at 2.9 times that of Bu3 and the mass of Aq7 being 3.6 times that of Bu7.

Characterization of the physical and chemical properties of each subfraction demonstrated differences between the tannin subfractions (Table 4). The measured octanol–water partition coefficients (converted to Log *P*) indicated that, as expected, the aqueous subfractions were more hydrophilic than the butanol fractions. The butanol and aqueous tannin subfractions

Table 4. Chemical Properties of Tannin Subfractions from the 3 and 7 Year Old Wines

sample	mDp[a]	% MC[b]	% GC[c]	% ECG[d]	GC:ECG[e]	MW[f]	Log P[g]
TT7	7.7	12.0	29.6	2.1	14.1	2850	−0.55
Bu7	5.7	12.4	24.2	2.0	12.1	1929	−0.72
Aq7	9.6	15.4	34.7	1.8	19.3	3272	−1.34
TT3	9.1	33.9	27.9	3.6	7.8	2930	−0.61
Bu3	5.8	26.0	21.8	3.5	6.2	1803	−0.70
Aq3	11.1	30.6	33.4	3.0	11.1	3034	−1.31

[a]Mean degree of polymerization. [b]Percent mass conversion. [c]Percent epigallocatechin subunits. [d]Percent epicatechin gallate subunits. [e]Ratio of epigallocatechin subunits to epicatechin gallate subunits. [f]Average molecular weight determined by 50% GPC elution (g/mol). [g]Log_{10} (absorbance $_{OCTANOL}$/absorbance $_{WATER}$) measured at 280 nm.

Table 5. Somers Color Measures Normalized by Tannin Concentration, Calculated as the Results Presented in Table 3 Divided by Tannin Concentration (g/L)

sample	$WCD_{[NORM]}$[a] (au/gL^{-1})	$hue_{[NORM]}$	degree of redness[b] (au/gL^{-1})	A280 $nm_{[NORM]}$[c] (au/gL^{-1})	NB $pig_{[NORM]}$[d] (au/gL^{-1})
TT7	6.6	1.4	0.072	21.6	2.6
Bu7	5.7	1.5	0.058	7.6	2.2
Aq7	4.5	0.8	0.052	9.0	1.8
TT3	5.1	1.3	0.064	17.3	2.0
Bu3	4.2	1.6	0.066	7.0	1.6
Aq3	3.3	0.8	0.052	8.7	1.3

[a]Normalized wine color density. [b]Degree of redness (absorbance at 520 nm normalized for tannin concentration). [c]Normalized absorbance at 280 nm. [d]Normalized nonbleachable pigments.

Table 6. Mean Scores for the Sensory Attributes of Each Tannin Subfraction[a]

sample	Astring[c]	Hot AT[d]	Burn AT[e]	Hotness	Bitter[f]	Alc[g]	Acid[h]	Visc[i]	Ting[j]
M Wine[b]	3.32 c	2.26 b	1.70 b	1.24 b	2.29 b	4.21	4.26	2.91	1.26
Bu7	5.15 b	3.19 a	2.84 a	2.42 a	3.45 a	4.31	3.71	2.96	1.80
Aq7	6.25 a	3.00 a	2.46 a	1.71 b	2.94 ab	4.64	3.60	3.00	1.58
Bu3	5.28 b	3.25 a	3.08 a	2.20 a	3.30 a	4.35	3.73	2.83	1.78
Aq3	6.24 a	3.05 a	2.74 a	2.25 a	3.09 a	4.70	3.71	3.02	2.01
p-value	<0.001	0.001	<0.001	0.002	0.028	ns[k]	ns	ns	ns
LSD	0.71	0.60	0.64	0.65	0.75				

[a]Means of sensory attributes with different superscript letters are significantly different from one another. Where differences between means were significant, the *p*-value and least significant difference (LSD) 5% are shown. [b]M Wine = model wine. [c]Astring = astringency. [d]Hot AT = hot aftertaste. [e]Burn AT = burning aftertaste. [f]Bitter = bitterness. [g]Alc = alcohol. [h]Acid = acidity. [i]Visc = viscosity. [j]Ting = tingling. [k]ns = not significant.

from each vintage were all more hydrophilic than the total tannin fractions. The percent gallocatechin ((−)-epigallocatechin subunits) was greater in the aqueous subfractions compared with the butanol subfractions, which may contribute to the more water-soluble nature of the aqueous subfractions. The butanol subfractions were consistently the smaller of the two fractions from both vintages with much lower molecular masses. The percent galloylation (epicatechin gallate subunits) in the aqueous and butanol subfractions of each vintage was similar, while the tannin subfractions from the younger wine were higher in percent galloylation than the older wine subfractions. Color measures of the tannin model wine solutions used in sensory analysis revealed differences between fractions and between vintages (Table 3), particularly when normalized for the different gravimetric tannin concentrations of each equimolar solution (Table 5). The butanol subfractions of both vintages demonstrated greater $hue_{[NORM]}$, $WCD_{[NORM]}$, degree of redness, and nonbleachable $pigments_{[NORM]}$ than the aqueous subfractions (Table 5), indicating greater color incorporation. As with the total tannins, the tannin subfractions of the older wine (Aq7 and Bu7) showed greater color incorporation ($WCD_{[NORM]}$ and nonbleachable $pigments_{[NORM]}$) than those of the younger wine (Aq3 and Bu3).

Sensory Properties of Wine Tannin Fractions. The primary objective of this research was to investigate within a consistent matrix the relationship between the chemical characteristics of the wine tannin subfractions and their sensory characteristics and relate this back to their relative contributions to the original red wines. At an equimolar ratio, the gravimetric proportions of each subfraction (approximately 3:1 ratio of tannins between the aqueous and butanol subfractions, irrespective of wine age) were similar to the proportions of the subfraction found in the original red wine and therefore the results were relevant to the relative contribution of each fraction to the whole wine. Hence equimolar tannin solutions were prepared. The results of the sensory investigation (Table

dx.doi.org/10.1021/jf304239n | J. Agric. Food Chem. 2013, 61, 719−727

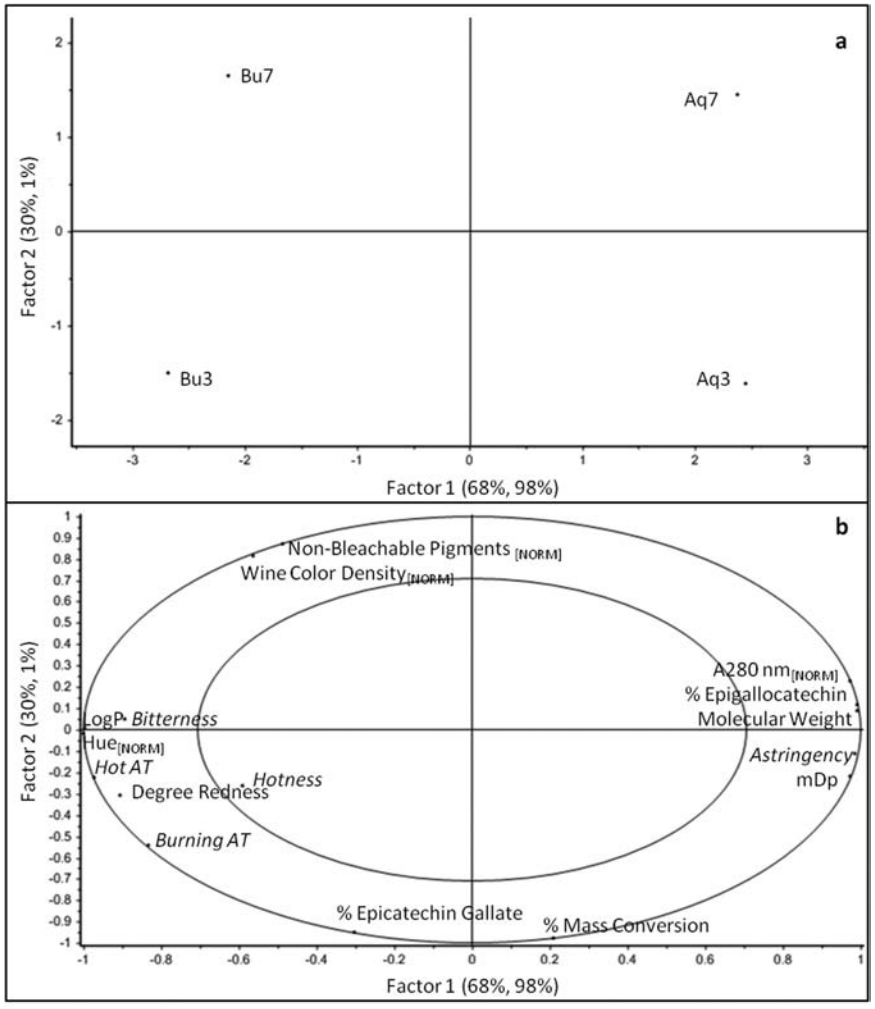


Figure 2. (a) Scores Plot for tannin samples. (b) X and Y loadings for PLS2 regression for sensory attributes (italicized) using the chemical properties from Tables 4 and 5 (Somers color measures normalized for tannin concentration). A280 $nm_{[NORM]}$ refers to the total absorbance at 280 nm normalized for tannin concentration.

6) showed that at equimolar concentrations *Astringency* was the main attribute that significantly ($p < 0.001$) differentiated the tannin subfractions from each other, with the aqueous subfractions rated higher than the butanol subfractions for both wines and the respective pairs of 3 and 7 year old wine tannin subfractions rated not significantly different from each other. There were no significant differences among the subfractions in the attributes of *Alcohol*, *Acidity*, *Viscosity*, or *Tingling*. The attributes of *Bitterness* and *Hotness*, including *Hot Aftertaste* and *Burning Aftertaste*, also significantly differentiated the tannin subfractions from the model wine, except for the Aq7 subfraction, and were rated higher in the butanol subfractions (Table 6). Partial least squares (PLS) regression analysis (Figure 2) of the four subfractions demonstrated that 98% of the variance in the sensory data was explained by the first component, indicating that those chemical measures that are most highly loaded positively or negatively on Factor 1 (A280 $nm_{[NORM]}$, molecular weight, % epigallocatechin, $hue_{[NORM]}$ and Log *P*) were most associated with the sensory attributes that differed among the four fractions. With only four samples, this analysis is only exploratory, but PLS as a soft-modeling approach is considered robust to small sample sizes and the model gives an indication of the most important measures that relate to the sensory differences.

The subfractions with the higher *Astringency* and lowest *Bitterness* and *Hotness* scores at equimolar concentrations, Aq7 and Aq3, were also highest in molecular size, A280 $nm_{[NORM]}$ and % epigallocatechin. These measures had high positive loadings on Factor 1 (Figure 2). Conversely, these subfractions were lowest in Log *P* (and hence more water-soluble), $hue_{[NORM]}$, and degree redness, which had strong negative loadings on Factor 1. The difference in *Astringency* between the tannin subfractions within a vintage was greater than the difference between subfractions of different vintages.

The aqueous tannin subfractions were characterized by larger tannins, consisted of a lower proportion of pigmented polymers, and were more water-soluble. Larger grape tannins have been previously reported as more astringent than smaller grape tannins,[21] which may relate to the greater number of tannin binding sites that are available for interaction with proteins in the oral cavity.[22] Wine tannin structures are significantly different than those of grape tannins and, as such, it was unclear at the start of this research whether the molecular mass of wine tannin and tannin subfractions would also relate to astringency or whether the structural modifications that had occurred may have offset the size-related astringency drivers. Our previous study has indicated that although wine tannins may be of similar molecular size to grape tannins, the strength

of their peptide interactions can be much weaker;[4] the impact of this observation on sensory perception remains unknown. The results of this study demonstrate for the first time that as with grape tannins, larger wine tannins were more astringent than smaller wine tannins on a molar basis despite the major structural differences between grape and wine tannins. In terms of structure−function relationships, on a molar basis the tannin subfraction with the higher average molecular mass was more astringent, suggesting that per mole of tannin, it also contained more molecular features that can interact with saliva or oral surfaces as previously suggested for grape tannins.[22]

Higher gravimetric grape and wine tannin concentrations in wine or model wine have previously shown a positive correlation with astringency intensity,[17,40] and this was also observed in this study. To maintain a final concentration of 0.35 mM for all subfractions, the larger molecular mass of the aqueous tannins required greater gravimetric amounts than for the smaller butanol subfractions. Given that the water-soluble tannin subfractions were more abundant in the original red wine and also more astringent, winemaking processes that modulate concentrations of these are likely to most significantly influence astringency.

The wine color density ($WCD_{[NORM]}$), $hue_{[NORM]}$, and degree of redness as a proportion of tannin concentration (Table 5) were much higher in the butanol subfractions than the aqueous fractions. The higher color incorporation (degree of redness) as well as the smaller molecular mass of the butanol subfractions was negatively associated with *Astringency* (Figure 2), which adds further support to a previous observation that pigmented tannin polymers are less astringent than non-pigmented tannin-like polymers.[24]

Astringency also had a negative association with Log *P* (Figure 2), leading us to propose that the greater hydrophobicity of the butanol subfractions may also contribute to the reduced perceived astringency of these tannins. Greater hydrophobicity has previously been associated with more oxidized or modified tannin structures[38,41] which have demonstrated lower percent mass conversion from depolymerization reactions than native tannins.[39] The lower mass conversion and greater hydrophobicity of the butanol subfractions compared with the aqueous subfractions of each vintage (Table 4) may mean that the butanol subfractions are more oxidized. Such oxidation may indicate a larger degree of intramolecular bonds,[39] which could reduce the number of binding sites available for interaction with oral surfaces and thus potentially contribute to the reduced astringency of these fractions.

The sensory attributes *Bitterness* and *Hotness*, including *Hot Aftertaste* and *Burning Aftertaste*, were associated with the comparatively smaller molecular mass, greater color incorporation, and relatively higher hydrophobicity of the wine tannins in the butanol subfractions (Figure 2). Smaller molecular weight flavanols such as catechin monomers or dimers are reportedly more bitter than oligomers or polymers,[42] although monomers were not present in any of the tannin samples in this study. The comparative bitterness of the butanol subfractions may have been related to the smaller molecular masses of these tannins relative to those of the aqueous subfractions. *Bitterness* has previously been shown to increase with gravimetric tannin concentration,[43] however, the opposite effect was noted in this study, suggesting that tannin structure played a greater role than gravimetric concentration for this attribute. The increased rating of *Hotness* of the tannin subfractions relative to the model wine was not due to differences in alcohol but the presence of different tannins. The reasons for this are unclear but may be similar to the sensory responses of the oral irritant, oleocanthal, from olive oil,[44] or the burning sensations reported for ginger or chili peppers.[45] Subfraction Aq7 did not differ significantly from the model wine in *Hotness* or *Bitterness*, however, the reasons for this are unclear. The ratio of epigallocatechin subunits to epicatechin gallate subunits was much higher in Aq7 than in the other subfractions (Table 4). Epigallocatechin reportedly reduces the coarseness of perceived astringency,[21] and this attribute may also have contributed to the reduced perceived *Hotness* and *Bitterness* of this tannin subfraction. Further research is required to understand how wine tannins influence hotness and how the structural characteristics of the butanol subfractions enhance this burning mouthfeel.

The sensory attributes associated with the age-related chemical properties (percent mass conversion, percent galloylation, and color incorporation) explained only 1% of the variation in the sensory data, as shown along Factor 2 of the PLS regression plot (Figure 2). Separation of the tannin subfractions based on wine vintage was mostly associated with the greater mass conversion and percent galloylation (epicatechin gallate subunits) of the 7 year old wine tannin samples and the greater WCD and nonbleachable pigments of the 3 year old wine tannin butanol subfractions. At similar concentrations (both gravimetric and molar), these structural differences did not translate into a significant discernible distinction in mouthfeel or perceived hotness between the 3 and 7 year old wine tannin subfractions. Further work comparing these tannin subfractions from young wine soon after bottling and from the same wine after aging may provide greater insight to how wine tannins change with age and how this influences astringency.

In summary, the two wine tannin subfractions from a 3 and a 7 year old wine showed distinctly different chemical characteristics and this translated into different sensory perceptions. When they were analyzed in model wine at equimolar proportions, similar to those proportions present in the original wine, the larger, more water-soluble wine tannins were perceived as more astringent than the smaller, more hydrophobic and pigmented wine tannins, which were perceived as hotter and more bitter. This suggested that the larger and more abundant water-soluble tannin subfractions in red wine have a greater contribution to wine astringency than the smaller and less abundant butanol-soluble tannin subfractions. Thus selective removal of some of the water-soluble tannins from red wine may be a tool for lessening the astringency of young red wines. Further research into the relationships between changes in wine tannin structure and the perceived sensory characteristics of tannin subfractions may provide greater insight into how tannin composition, in addition to concentration, influences red wine astringency.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to thank Sue Hodder from Wynns Coonawarra Estate for her ongoing support and discussions. Dr Leigh Francis is gratefully acknowledged for critical review of the manuscript and Wes Pearson for assistance with sensory evaluation. The work was conducted at The Australian Wine Research Institute, a member of the Wine Innovation Cluster at the Waite Precinct in Adelaide, and is supported by Australian grape growers and winemakers through their investment body, the Grape and Wine Research and Development Corporation, with matching funds from the Australian Government.

■ ABBREVIATIONS USED

A280, absorbance measured at 280 nm; A520, absorbance measures at 520 nm; GPC, gel permeation chromatography; MCP tannin assay, methyl cellulose precipitable tannin assay; mDp, mean degree of polymerization; PLS, partial least squares; WCD, wine color density

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