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Application of Insoluble Fibers in the Fining of Wine Phenolics

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ABSTRACT: The application of animal-derived proteins as wine fining agents has been subject to increased regulation in recent years. As an alternative to protein-based fining agents, insoluble plant-derived fibers have the capacity to adsorb red wine tannins. Changes in red wine tannin were analyzed following application of fibers derived from apple and grape and protein-based fining agents. Other changes in wine composition, namely, color, monomeric phenolics, metals, and turbidity, were also determined. Wine tannin was maximally reduced by application of an apple pomace fiber and a grape pomace fiber (G4), removing 42 and 38%, respectively. Potassium caseinate maximally removed 19% of wine tannin, although applied at a lower dose. Fibers reduced anthocyanins, total phenolics, and wine color density, but changes in wine hue were minor. Proteins and apple fiber selectively removed high molecular mass phenolics, whereas grape fibers removed those of both high and low molecular mass. The results show that insoluble fibers may be considered as alternative fining agents for red wines.

KEYWORDS: *fiber, fining, red wine, tannins, phenolics, gel permeation chromatography, molecular mass*

INTRODUCTION

Fining describes the deliberate addition of an adsorptive compound that is followed by the settling or precipitation of partially soluble components from the wine. The products used for this purpose are referred to as fining agents. These include animal proteins such as casein, gelatin, egg albumin, and isinglass[1,2] or plant proteins such as wheat glutens[3,4] lupin proteins,[4] and, more recently, patatin.[5] Additionally, polyvinylpolypyrrolidone (PVPP), bentonite,[1,2] and polysaccharides extracted from seaweeds[6] are used as fining agents.

The ability of tannins (condensed tannins or proanthocyanidins) to strongly bind proteins constitutes one aspect of red wine fining. From various studies it has emerged that the action of proteins on wine phenolics influences clarity, sensory characteristics, and aging capacity. Total phenolics, anthocyanins, tannin, color, and astringency have been the attributes of wine most often studied in response to fining,[7−11] and the extent of their modification depends on the fining agent used and the phenolic profile of the wine itself.

The use of animal-derived proteins as fining agents has some undesirable consequences. Concerns have been raised about the addition of animal proteins as enological fining agents due to the disease known as bovine spongiform encephalopathy. This resulted in the banning of some fining agents from foods and beverages (e.g., blood albumin). More recently, legislation has been passed that requires certain protein-based fining agents to be declared on bottle labels due to their classification as potential allergens. This could limit marketability of wine to certain consumer segments and has led to research into the use of alternative fining agents from plant sources.[4]

A recent review of the literature on grape tannin extractability has drawn attention to the potential for cell wall−tannin interactions to remove soluble tannin from wine during the fermentation process.[12] Model experiments have verified that cell wall-derived fiber can remove a significant portion of tannins from the wine during vinification. The fining actions of commercially produced proteins and plant-derived insoluble fibers for wine tannin have been compared in model experiments.[13] This work demonstrates the potential use of fibers as an alternative to fining with proteins in winemaking. It has been suggested that fiber has the potential to regulate the mouthfeel properties of wine through interactions with wine tannins.[14]

The purpose of this investigation was to compare the effect of fiber-based fining agents on wine tannin concentration and color properties, with a specific focus on variability in terms of the fiber source. A comparison with commercial protein fining agents was included as a reference method. Changes in the molecular mass distribution of wine phenolics in response to fining were explored using gel permeation chromatography (GPC).

MATERIALS AND METHODS

Chemicals. Chromatographic solvents were of high-performance liquid chromatography (HPLC) grade, and chemicals were of analytical reagent grade. Acetonitrile, acetone, methanol, formic acid (98−100%), glacial acetic acid (98%), and hydrochloric acid (32%) were from Merck (Darmstadt, Germany). Absolute ethanol was from Rowe Scientific (Adelaide, Australia). Acetaldehyde (99.5%) was sourced from Chem Supply (Adelaide, Australia). Methyl cellulose (M-0387, viscosity of 2% aqueous solution at 20 °C = 1500 cP), ammonium sulfate crystals (A4915), and L-(+)-tartaric acid (99% T400) were sourced from Sigma-Aldrich (St. Louis, MO, USA). Sodium metabisulfite (Univar, A1184) was obtained from Ajax Finechem (Sydney, Australia).

Instrumentation. An Agilent chromatograph, model 1100 HPLC (Agilent Technologies Australia Pty Ltd., Melbourne, Australia), was used with Chemstation software for chromatographic analyses.

Preparation of Fiber Extracts from Grape and Apple Pomace. The procedure for the preparation of crude fiber extracts has been previously published.[13] Fibers were isolated from grape pomace postfermentation and apple pomace postjuicing. Pomaces were frozen at −20 °C prior to processing. For frozen apple pomace,

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the material was pre-extracted for the removal of soluble polysaccharides.[15] Apple pomace was homogenized at 8000 rpm in a Retsch Grindomix GM200 (Retsch GmbH & Co, Haan, Germany) with the addition of 40 mM ice-cold 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) buffer (Boehringer Mannheim, Germany), pH 7. Solids were separated from juice by centrifugation and were re-extracted in HEPES buffer for 30 min at 4 °C. Buffer-insoluble solids were collected by centrifugation. Thereafter, HEPES-extracted apple materials and untreated grape pomace were prepared in the same manner.

Cabernet Sauvignon (CAS) pomaces were obtained following pressing of wines produced using a small-scale winemaking technique (WIC Winemaking Service, Adelaide, Australia). The grape source for winemaking was from a single vineyard block of a commercial producer (Pernod Ricard/Orlando Wyndham) in the Langhorne Creek growing region of South Australia. The original grape sources for the pomaces were from different treatments, namely, two irrigation levels, various ripeness levels, and two consecutive seasons (Table 1).

Table 1. Description of Fining Materials Used: Cabernet Sauvignon (CAS) Pomace Fiber Where Grapes Were from Different Seasons, Irrigation Levels, and Maturity Levels (G1−G6); Apple Pomace Fiber (AP); and Commercial Protein Fining Agents (CP, IC, AD, GL1, GL2, and GL3)

code		description
		Fiber Fining Agents
	G1	2009 CAS pomace, 4 ML/ha irrigation picked at 23 °Brix
	G2	2009 CAS pomace, 2 ML/ha irrigation picked at 23 °Brix
	G3	2010 CAS pomace, 4 ML/ha irrigation picked at 23 °Brix
	G4	2010 CAS pomace, 2 ML/ha irrigation picked at 23 °Brix
	G5	2010 CAS pomace, 2 ML/ha irrigation picked at 24 °Brix
	G6	2010 CAS pomace, 2 ML/ha irrigation picked at 26 °Brix
	AP	apple pomace postjuicing
		Protein Fining Agent
solids		
	CP	commercial potassium caseinate
	IC	commercial isinglass
	AD	commercial egg albumin
	GL1	commercial powdered cold-soluble gelatin (porcine)
liquids		
	GL2	commercial liquid gelatin (porcine)
	GL3	high surface charge density commercial liquid gelatin (porcine)

This was done to determine whether tannin adsorption properties differed between grape pomace fibers due to viticultural parameters, as too great a variability would prevent commercialization. The seeds of grape pomaces were manually separated from the skins prior to extraction.

Untreated frozen grape pomace skins and HEPES-extracted apple pomace were extracted in 70% v/v acetone for 18 h to remove residual tannin. Acetone-extracted residues (fiber) were washed in additional 70% v/v acetone, followed by Milli-Q water (Millipore Corp., Billerica, MA, USA). Insoluble residue from grape pomace that had not undergone initial homogenization was frozen at −20 °C and homogenized at 8000 rpm in a Retsch Grindomix GM200. Recovered fiber from both crude grape and apple pomace extracts was ground in a mortar and pestle under liquid nitrogen, lyophilized, passed through a 0.5 mm sieve, and stored at −20 °C until used. Fibers were added directly into wine (Table 2).

Wine Fining Experiments. The literature was reviewed to determine a fining protocol with conditions similar to those of a commercial application.[4,10,16−18] Fiber dose was varied to give a similar tannin reduction after fining by comparison with traditional protein-based fining agents. An unfined commercial wine was obtained through consultation with the producer and was a 2008 Cabernet Sauvignon, of 14% v/v ethanol, from the Coonawarra region (Southeastern Australia). This wine was selected following a survey of 20 commercial wines due to its high tannin concentration of ≅2.6 g/L (data not shown). Each individual fining experiment was conducted in triplicate using wine from a single bottle. A 10 mL aliquot of wine was transferred into a 12 mL centrifuge tube. Protein fining agents were donated in powder or liquid form by a commercial

Table 2. Tannin Concentration in Cabernet Sauvignon Wine[a] and Tannin Loss after Application of Maximum, Medium, and Minimum Doses of Grape Pomace Fibers (G1−G6), Apple Pomace Fiber (AP), and Commercial Protein Fining Agents (CP, IC, AD, GL1, GL2, and GL3) (n = 3)

sample	dose category	dose (units[b]/mL)	tannin loss by dose (mg/unit[b])	tannin reduction[c] (%)	RSD (%)
		Fiber Fining Agents			
G1	min	5	52	10.2	0
	med	10	66	25.7	4
	max	15	54	31.3	3
G2	min	5	51	9.9	4
	med	10	58	22.8	2
	max	15	48	28.0	8
G3	min	5	43	8.4	2
	med	10	50	19.5	6
	max	15	62	36.6	3
G4	min	5	107	21.1	5
	med	10	78	31.0	4
	max	15	64	38.2	3
G5	min	5	96	18.7	6
	med	10	61	23.7	15
	max	15	60	35.3	17
G6	min	5	73	14.3	4
	med	10	71	27.7	2
	max	15	61	35.9	2
AP	min	5	185	37.1	7
	med	10	104	41.7	4
	max	nd[d]			
		Protein Fining Agent			
solids					
CP	min	0.30	1107	13.1	1
	med	0.45	976	17.4	8
	max	0.60	782	18.6	8
IC	min	0.02	509	0.4	3
	med	0.02	8265	6.5	6
	max	0.03	7091	8.4	10
AD	min	0.06	1720	3.7	1
	med	0.08	2460	7.0	4
	max	0.10	2878	10.3	2
GL1	min	0.08	4239	12.1	1
	med	0.09	4331	14.0	2
	max	0.10	4487	16.1	4
liquids					
GL2	min	0.4	667	9.5	10
	med	0.7	389	9.8	6
	max	1.0	366	13.1	7
GL3	min	0.3	739	7.9	3
	med	0.45	651	10.5	1
	max	0.6	511	11.0	3

[a]Tannin concentration in the original wine was ≅2.6 g/L; a separate control was used for each experiment. [b]Dose units were in milligrams for all applications apart from liquid fining agents, which were in microliters. [c]Tannin reduction following fining as a percentage of the control. [d]nd = not determined.

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supplier (Laffort, Adelaide, Australia) (Table 1). Commercial proteins and fiber extracts were added into wine in the concentration range described in Table 2. Fibers were tested over a range of doses from 5 to 25 mg/mL, and commercial fining agents were applied at the maximum, medium, and minimum doses recommended by the manufacturer. In the case of the protein fining agents, 1 mL of prepared solution was added into wine. To compensate for concentration differences, wine controls and fiber addition experiments were diluted with 1 mL of water. Samples were sealed under nitrogen and placed on a suspension mixer for 48 h at 4 °C. Afterward, the wines were centrifuged at 2770*g* for 5 min at 20 °C and decanted prior to analysis. Experiments were conducted in triplicate.

Turbidity. The evolution of turbidity (as NTU) following fining was determined in 100 mL of wine using a Hach 2100N Turbidimeter (Hatch Pacific, Australia). Fining agents selected were fibers G4, G6, and AP and the protein-based fining agent CP (Table 1). Fining agents were added to wine at the minimum concentration previously described (Table 2). Samples were maintained sealed, under nitrogen, until the turbidity measure stabilized (17 days at 4 °C). Wines were sampled daily, ultrasonicated, conditioned to room temperature for 20 min, and analyzed without prior centrifugation. Experiments were conducted in triplicate, and a control was run with no fining addition.

Isolation of Wine Tannin and Characterization. Wine tannin was isolated from unfined wine as described by Kennedy and Jones[19] with some modifications. A 60 mL bed volume Toyopearl TSK HW 40-F (180 × 25 mm) was used as stationary phase following the manufacturer's instructions. The column was equilibrated with water containing 0.1% v/v formic acid, and 50 mL of wine was applied to the column using a peristaltic pump. Acidified water (150 mL, 0.1% v/v formic acid) was used to re-equilibrate the column. Low molecular mass phenolics were then eluted with 250 mL of H_2O/MeOH (1:1) containing 0.1% v/v formic acid equivalent to 7 bed volumes, and wine tannin was eluted with 150 mL of acetone/water (2:1) containing 0.1% v/v formic acid, equivalent to approximately 3 bed volumes. The acetone eluant was concentrated under reduced pressure at 35 °C to remove acetone, frozen at −80 °C, and lyophilized to a dry powder. The wine tannin isolate was characterized by phloroglucinolysis and GPC[19] to determine subunit composition, mean degree of polymerization (mDP), and molecular mass distribution with the high-throughput method outlined in Bindon and Kennedy.[14] For phloroglucinolysis, in a 0.2 mL PCR tube (Eppendorf, Hamburg, Germany), 25 μL of tannin in methanol was added to an equal volume of 0.2 N HCl, 100 g/L phloroglucinol (Sigma-Aldrich), and 20 g/L ascorbic acid (Sigma-Aldrich) in methanol to give a final maximum tannin concentration of 5 g/L. The phloroglucinolysis reaction was then run at 50 °C for 25 min, cooled, and then neutralized with 150 μL of aqueous sodium acetate (70 mM, Merck) and analyzed by RP-HPLC using (−)-epicatechin (Sigma-Aldrich) as the quantitative standard. For GPC analysis, isolated tannin in methanol (10 g/L) was diluted with 4 volumes of the HPLC mobile phase and then injected to GPC (20 μL). Preveraison skin tannin fractions of known mDP (by phloroglucinolysis) were used as standards for calibration as reported previously by Bindon et al.[20] For calibration, a second-order polynomial was fitted against the tannin retention time at 50% elution for each standard.

Analysis of Wine Color, Metals, and Phenolic Composition. Changes in the concentration of tannin, selected phenolics, and color were determined in wines after fining. Tannin concentration was analyzed using methyl cellulose precipitation (MCPT), and wine color was determined using the modified Somer's assay, following the high-throughput approach described by Mercurio et al.[21] The MCPT assay gave the tannin concentration of each wine in epicatechin equivalents (mg/L) using (−)-epicatechin (Sigma-Aldrich) as the quantitative standard. For the analysis of selected phenolics, the HPLC method described by Cozzolino et al.[22] was followed. Briefly, a Phenomenex Synergi Hydro-RP column (Phenomenex, Australia) (4 μm particle size, 80 Å pore size, 150 mm × 2 mm) was used at 25 °C. Solvents were (A) 1% v/v acetonitrile/1.5% v/v phosphoric acid in water and (B) 20% v/v solvent A/80% v/v acetonitrile for gradient elution at a flow rate of 0.4 mL/min. The injection volume used was 20 μL. Phenolic compounds were detected by a photodiode array detector at 280, 370, and 520 nm. Flavan-3-ols were analyzed at 280 nm and quantified as (−)-epicatechin (Sigma-Aldrich) units. Flavonols were analyzed at 320 nm and quantified as quercetin-3-glucoside (Sigma-Aldrich). Polymeric pigment was analyzed at 520 nm and quantified as malvidin-3-glucoside (Polyphenols Laboratories, Norway) units. Phenolics were identified according to the retention time of known standards and their UV−visible spectrum.[23] For analysis of the molecular mass distribution of wine phenolics, the GPC method developed by Kennedy,[24] and modified by Bindon and Kennedy,[14] was followed. Comparison of the GPC elution profiles of purified wine tannin (devoid of monomers) and whole wine (Figure 1) showed good agreement within the polymeric range (eluting at 12−16 min). On the basis of this comparison, the decision was made to directly inject whole wine for GPC analysis, which also enabled selectivity for the monomeric fraction to be compared between fining experiments (Figure 1). Wine samples before and after fining were centrifuged 16000*g* for 20 min, and 40 μL was directly injected for GPC analysis. To compare wine metal composition before and after fining, samples were analyzed by inductively coupled plasma atomic absorption spectroscopy (ICPOES) [25] by an external provider (Waite Analytical Services, School of Agriculture and Wine, University of Adelaide, Australia).

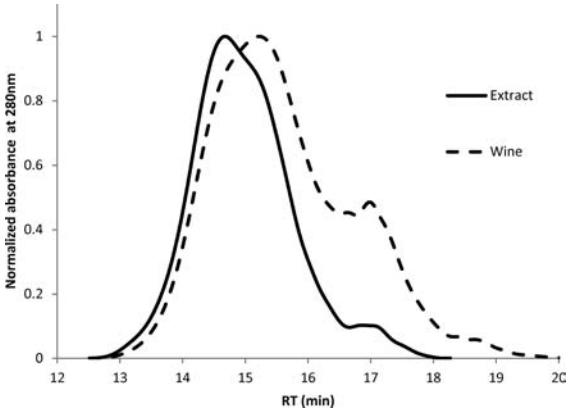

Figure 1. Comparison of the elution profiles by gel permeation chromatography of a purified Cabernet Sauvignon wine tannin isolate (Extract) and whole wine by direct injection (Wine). Absorbance at 280 nm is normalized for comparison of overlaid elution profiles (n = 3). RT = retention time.

Statistical Analysis. Significant differences were determined using a one-way analysis of variance (ANOVA), followed by a Tukey−Kramer HSD test. The JMP 5.0.1 statistical software package (SAS, Cary, NC, USA) was used.

RESULTS AND DISCUSSION

Effect of Fining on Tannin Concentration. To determine the tannin-removal effect over a range of fiber doses, G6 fiber was applied to red wine with a tannin concentration of ≅2.6 mg/mL at doses between 5 and 25 mg/mL (Figure 2). At lower doses the use of G6 fiber reduced wine tannin concentration progressively in a linear manner (Figure 2A), but at higher doses (>20 mg/mL) a plateau was reached. Analysis of the adsorption capacity of fiber for tannin indicated that at higher fiber doses, adsorption of tannin per milligram of fiber decreased significantly (Figure 2B). Tannin concentration was determined, and the reduction in tannin in comparison with the unfined control was calculated as a percentage for the range of doses outlined in Table 2. Maximum tannin reduction was achieved by apple fiber AP and grape fiber G4, with 42 and 38% of tannin removed, respectively (Table 2). With regard to

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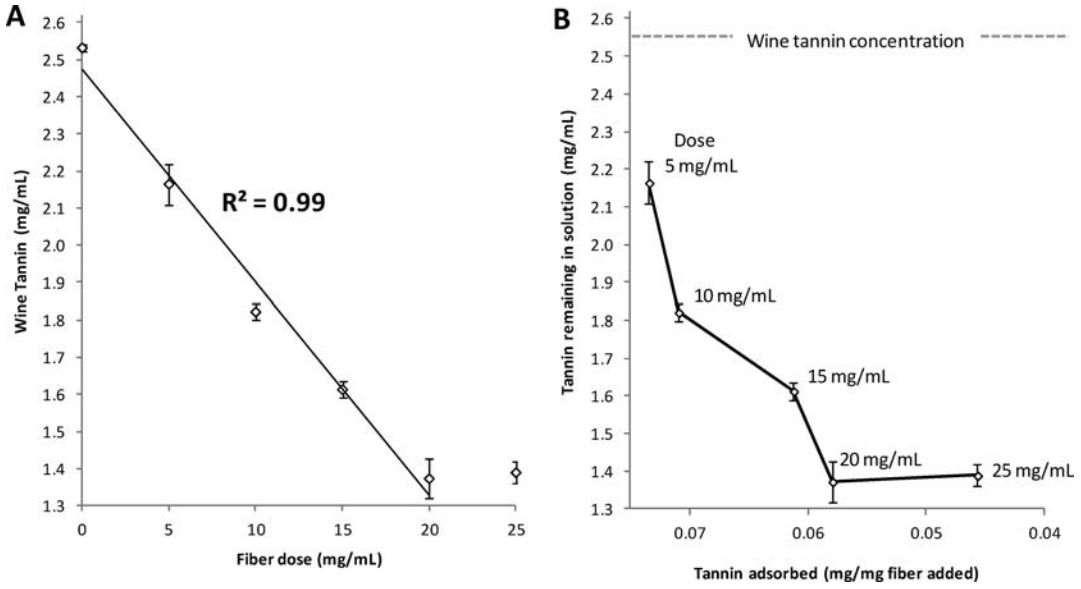

Figure 2. (A) Effect of increasing grape fiber dose (G6) on wine tannin concentration. (B) Dose-dependent adsorption of tannin as a function of wine tannin remaining in solution (n = 3, ±SD).

the commercial fining agents, casein CP was the most effective, with 19% tannin reduction. Fibers reduced tannin within a range from 8 to 37% at the 5 mg/mL dose and between 20 and 42% at 10 mg/mL. Commercial protein-based fining agents, CP and GL1, resulted in the highest tannin reduction from 10 to 20%. The most significant fining effect that has been described in the literature was a 26% tannin reduction with gelatin at a 0.2 mg/mL dose in a wine of 2.3 mg/mL tannin concentration.[16] In contrast, fining with low molecular weight gelatin has also been reported to induce no significant tannin loss.[26]

Apple fiber AP showed a higher affinity for tannin compared to the other fining agents tested. When applied at a 5 mg/mL dose, it reduced tannin concentration by 37% compared to a maximum of 21% for the most effective grape fibers. Higher apple fiber doses were not tested because the reduction in tannin was >40% at 10 mg/mL. It has been demonstrated that grape pulp (mesocarp) fiber had a greater affinity for grape and wine tannin than fiber from grape skin.[27] For apple fiber application, we have shown in model experiments that the affinity for wine tannin is initially greater than for grape fibers, but the response is not linear.[13] At apple fiber doses of >10 mg/mL, the tannin removal effect plateaus. From previous work, it has been proposed that compositional differences in fibers may drive the adsorption effect. Apple fiber has a lower degree of lignification, a higher polysaccharide content, and a higher surface area/volume ratio in aqueous solution than grape pomace fiber,[13] which might explain the higher affinity for wine tannin observed in the current study.

To generalize the findings, differences were observed between the applied grape fibers, but these were minor considering the variability of fining effects observed using commercially available products.[26] The reduction of wine tannin using grape pomace fiber was between 8 and 38%. The reduction of tannin was between 9 and 20% at 5 mg/mL, between 20 and 30% at 10 mg/mL, and between 28 and 38% at 15 mg/mL (Table 2). Although the fibers were prepared from grape pomaces that had variable grape sources (different seasons, maturities, and irrigation levels), there were no clear trends observed in terms of viticultural effects on the affinity of grape fibers for wine tannin.

Wine Turbidity and Elemental Composition in Response to Fining. An experiment was carried out in 100 mL of red wine to study the evolution of turbidity after fining with the selected grape fibers G4 and G6, apple fiber AP, and casein agent CP (Figure 3). G4 resulted in the highest increase

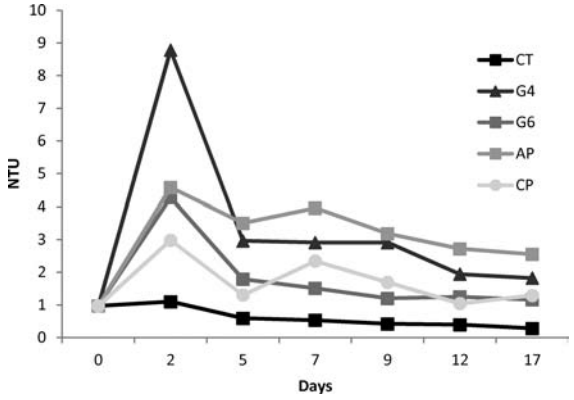

Figure 3. Turbidity evolution after fining Cabernet Sauvignon wine with grape fiber (G4 and G6) and apple fiber (AP) at a 5 mg/mL dose and with potassium caseinate (CP) at a 0.30 mg/mL dose (n = 3).

in turbidity after addition. However, turbidity decreased and slowly stabilized over the time course analyzed. After 17 days, all treated wines ranged between 0 and 3 NTU. A turbidity range between 5.6 and 190 NTU has been described for commercial wines.[28] Hence, we concluded that fiber addition did not appear to cause turbidity problems in wine. However, the starting NTU of the control wine was low, and as such the comparative effects of protein and fiber addition on turbidity reduction were not further explored.

The effect of fining on wine metals was also studied. It is an important consideration because Na^+, K^+, Ca^{2+}, Mg^{2+}, and especially Fe^{3+} cations form negatively charged tannin−-metal complexes that favor the flocculation and the precipitation of

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tannins and positively charged proteins.[29] A wide range of metals were analyzed after fining with G6 and CP samples (Table 3). With regard to B, Cu, Zn, Mg, Na, P, and S concentrations, no significant differences were found. In general, fiber decreased metal concentration in wine. Interestingly, Ca content was raised when fiber was added, which may indicate some loss of fiber-bound Ca, potentially as calcium tartrate. However, the increase was <3 mg/L, a minor effect. CP significantly decreased Fe concentration in treated samples, as would be expected. Removal of metals may be an interesting property for further investigation, because this might protect wine from casse formation.[2]

Table 3. Effect of Grape Fiber (G6) and Potassium Caseinate (CP) Fining at 5 and 0.30 mg/mL, Respectively, on Metal Concentration of a Cabernet Sauvignon Wine (CT)[a]

metal (mg/L)	CT	G6	CP
Fe	1.65 ± 0.02 a	0.83 ± 0.02 c	1.38 ± 0.01 b
Mn	0.80 ± 0.01 a	0.77 ± 0.01 b	0.80 ± 0.0 a
B	7.80 ± 0.16	7.58 ± 0.05	7.77 ± 0.05
Cu	0.04 ± 0.0	0.09 ± 0.0	0.03 ± 0.0
Zn	0.79 ± 0.01	0.77 ± 0.0	0.80 ± 0.0
Ca	58.80 ± 1.23 b	61.38 ± 0.48 a	58.30 ± 0.31 b
Mg	124.5 ± 2.82	121.7 ± 0.80	122.9 ± 0.51
Na	28.54 ± 0.61	27.86 ± 0.21	28.77 ± 0.02
K	1016 ± 11.55 a	980 ± 10.0 b	1016 ± 11.55 a
P	243.3 ± 5.77	233.3 ± 5.77	240.0 ± 0
S	202.9 ± 6.14	193.5 ± 1.80	194.3 ± 0.61
Al	0.35 ± 0.02 a	0.30 ± 0.01 b	0.33 ± 0.01 a

[a]Statistical analysis: ANOVA and Tukey–Kramer HSD test ($p < 0.01$). Different letters indicate statistical differences.

Fiber is an insoluble solid in wine that could be eliminated by decanting or filtering wine after fining application. Fiber addition to wine resulted in NTU levels comparable with those observed following protein addition. It is therefore unlikely that fiber application would result in undesirable changes in turbidity or filterability in wine. It has been shown previously that there is a potential wine volume loss through lees production, which is dependent upon the pomace type, with grape pomace fiber having lower volume and swelling capacity than that from apple on a mass unit basis.[13] It will therefore be an important step in the development of fiber materials as alternative fining agents that the postfining processing step is optimized to minimize wine volume loss.

Selectivity of Fining Agents for Phenolics. Due to their high tannin fining capacity, grape pomace fibers G4 and G6, apple pomace fiber (AP), and casein (CP) were selected to further study their effect on wine color and phenolics. The doses selected were 5 mg/mL for fibers and the maximum recommended dose of 0.6 mg/mL for potassium caseinate. Total phenolics were reduced approximately 10% in wine after fining with grape fibers (Table 4), and this reflected reductions in anthocyanin, flavan-3-ol monomers, and flavonols. Flavan-3-ol monomers were the phenolic group that was least affected by fining. Flavonol concentration was influenced only by the addition of fibers. However, it is important to mention that results in other wines may also be dependent upon the composition of the selected wine.

Higher reductions in anthocyanins and total phenolics, 18 and 16%, respectively, were observed for apple fiber addition. Apple fiber removed 185 mg of tannin and 9 mg of anthocyanins per milligram of fiber at the 5 mg/mL dose. Previous studies using apple cell wall extracts have shown that they have limited affinity for monomeric phenolics.[30] This was confirmed in this study only for flavan-3-ol monomers, although for flavonols, apple fiber showed a lower affinity than did grape fibers. Protein-based fining agents have been shown to remove anthocyanins.[16,26,31−33] Fining a young wine with gelatin or casein has been shown to reduce the concentration of total anthocyanins.[34] It is possible that secondary interactions between colloidal precipitates and low molecular mass phenolics such as anthocyanins might be involved in this reduction. A direct interaction with grape cell walls and monomeric anthocyanin has not yet been shown in research, but has been demonstrated for carrot cell wall analogues.[35] According to that study, anthocyanin reacts with cell walls in a two-stage process, initially binding to the polysaccharide structure via ionic and hydrophobic interactions, with an apparent delayed phase involving stacking of further anthocyanin to the bound layer. This is similar to the observations for apple proanthocyanin interactions with apple cell walls.[30] To use these examples toward understanding the response of fiber addition to wine, anthocyanin may non-covalently interact with the hydroxylated elements of polysaccharide (or protein) or, alternately, interact with either bound anthocyanin or bound tannins via stacking.

Table 4 shows that color density decreased after all fining agent applications, whereas wine hue was minimally affected except for CP addition, where hue decreased. The color outcomes for grape fiber fining were similar to those for CP. AP addition resulted in the greatest wine color density decrease, but this material also had a relatively higher affinity for tannin.

Table 4. Effect of Fining with Grape Fiber (G4 and G6) and Apple Fiber (AP) Applied at 5 mg/mL and of Potassium Caseinate (CP) Applied at 0.60 mg/mL on Phenolics, Color, Nonbleachable Pigments, and Polymeric Pigments

sample	anthocyanin (mg/L)	A:T[a] (mg/mg)	total phenolics (Abs◊)	flavan-3-ols (mg/L)	flavonols (mg/L)	color density (Abs[b])	hue (Abs[b])	nonbleachable pigment (Abs[b])	polymeric pigment (mg/L)
CT	249 ± 1 a	0.10 b	65.3 ± 0.2 a	114.2 ± 1.6 a	19.1 ± 1.0 a	36.35 ± 0.75 a	0.88 ± 0 bc	13.12 ± 0.31 a	29.72 ± 1.46 a
G4	220 ± 5 b	0.11 ab	57.9 ± 0.1 b	107.5 ± 2.5 bc	14.5 ± 0.7 c	30.51 ± 1.50 b	0.88 ± 0 b	10.90 ± 0.22 c	19.57 ± 1.36 b
G6	220 ± 5 b	0.11 ab	58.4 ± 1.7 b	106.2 ± 1.0 c	14.2 ± 0.6 c	30.32 ± 0.66 b	0.88 ± 0 b	10.75 ± 0.20 c	18.77 ± 1.36 b
AP	203 ± 5 c	0.13 a	54.7 ± 1.0 c	115.8 ± 1.8 a	15.6 ± 0.7 b	26.62 ± 0.43 c	0.89 ± 0 a	9.72 ± 0.30 d	12.56 ± 1.10 c
CP	233 ± 4 b	0.11 b	60.1 ± 0.9 b	113.8 ± 2.6 ab	20.5 ± 1.3 a	31.49 ± 0.58 b	0.86 ± 0 c	11.46 ± 0.05 b	19.29 ± 1.22 b

[a]Anthocyanin/tannin ratio calculated by dividing anthocyanin concentration (mg/L) by tannin concentration (mg/L) after fining protocol. CT, control; G4, grape cell wall material; G6, cell wall material; AP, apple cell wall material; CP, casein commercial fining agent. [b]Absorbance units. All data are expressed as the average values of three replicates ± standard deviation ($n = 3$). Statistical analysis: ANOVA and Tukey–Kramer HSD test ($p < 0.01$). Different letters indicate statistical differences.

By comparing the anthocyanin/tannin ratio (A:T) with the control (Table 4) the proportional losses of tannin and anthocyanin following fining could be determined. The wine used in this study showed an A:T ratio of 0.10. Ratios >0.10 after fining indicate a higher removal of tannin relative to anthocyanin. Treatments with grape fibers affected both components and, therefore, maintained A:T. AP increased A:T to 0.13, meaning that the anthocyanin proportion in comparison to tannin was higher in AP-treated wine. Apple fiber also showed the highest affinity for polymeric pigments, reducing the concentration to 12.6 mg/L, compared to 18−20 mg/L using the other fining agents. The effect on wine color density is potentially related to both the reduction in anthocyanin and nonbleachable pigments after fining, which reflects losses in pigmented wine tannin (polymeric pigment). These results are in agreement with other reports for protein fining agents.[26,31−33] Fining of a young wine with gelatin or casein has been shown to result in lower total anthocyanins and wine color density, similar to that reported here for the addition of fibers.

Color effects are an important consideration. Losses in color density following grape fiber addition were comparable with casein addition, so it is unlikely that wine color would be negatively affected in the use of fibers as fining agents. However, per unit tannin removed, more anthocyanin was removed following grape fiber application, and this was greater than for casein addition. The effect of a higher selectivity for anthocyanin on the resultant wine color would be dependent upon the timing of application. For example, during fermentation or in very young wines with a higher anthocyanin/polymeric pigment ratio, an early reduction in the anthocyanin pool may be detrimental to wine color stability. Hence, fiber fining may not be advisable before stable color adducts have developed. To our knowledge, no research has been reported on the potential impact of fiber addition on wine aroma, and this is an additional pertinent consideration in the development of fibers as an alternative fining agent.

Molecular Mass Distribution of Wine Phenolics. The compositional characteristics of tannin isolated from the untreated wine were determined by analysis of the subunit composition and molecular mass distribution. Phloroglucinolysis results (Table 5) showed that the wine tannin (mDP 8) was within the average range observed for wine, which is from 6 to 12 units,[36] but had a low mass conversion of 24%. Low tannin mass conversion means that the use of phloroglucinolysis provides information on only a fraction of the material, and care must be taken in the interpretation of this data. Due to correlation between the molecular mass information provided by phloroglucinolysis and GPC and the observation that GPC provides accurate results even at low tannin concentrations,[13] it was decided to use GPC on whole wines rather than isolated tannin to explore the response to fining treatments. A comparison of normalized (280 nm) GPC elution profiles of isolated wine tannin and whole wine (Figure 1) shows the inclusion of lower molecular mass material in the whole wine sample, which would otherwise be removed in the tannin isolation step. The inclusion of the lower molecular mass phenolic material allows for selectivity of fining agents for monomers and oligomers to be studied, as well as for polymeric tannin. However, we note that the elution profile of purified wine tannin was biased slightly toward higher molecular mass distribution (early eluting fraction) than that of whole wine, potentially due to enrichment of this fraction as a result of the isolation procedure. GPC was used to explore changes in the mass distribution of fined and untreated wines. The average molecular mass across cumulative elution "slices", or cutoff points (referred to as percent elution), was summarized to enable a statistical comparison between treatments (Table 6). Wine molecular mass was reduced across the whole mass distribution after the application of fibers and potassium caseinate, which indicates that both high and low molecular mass materials were removed. The loss of some lower molecular mass phenolics (10−30% elution) is in agreement with the finding by HPLC that some monomeric flavonols and anthocyanin were removed, as discussed previously. In general, the greatest loss in 280 nm material from the elution profile was at 70 and 90% elution for all treatments (Table 6), although the loss was greater for apple fiber and casein than for grape fibers. The changes in molecular mass distribution were confirmed by overlaying the elution profiles of fined wines with the unfined control (Figure 4). Grape fibers were the least selective for phenolics by molecular mass (Figure 4A,B), removing both high and low molecular mass materials, but with consistently greater affinity for tannin in the intermediate molecular mass range (14−15 min elution) but not the highest molecular mass range (<13 min). By comparison, the potassium caseinate treatment (Figure 4C) was highly selective for high molecular mass phenolics (<13 min). In the case of apple fiber AP (Figure 4D), both high and low molecular mass phenolics were removed, but the preferential removal of high molecular mass phenolics shifted the elution profile toward a lower average molecular mass. This confirms our previous observations in model experiments in which proteins (including potassium caseinate) removed higher molecular mass tannins, whereas a Cabernet Sauvignon pomace fiber (corresponding to G6 in the current study) was less selective, rendering tannin average molecular mass either unchanged or slightly increased.[13] Nevertheless, the current study shows that although the protein-based fining agent and apple fiber were more selective

Table 5. Subunit Composition and Molecular Mass of Isolated Tannin from a Cabernet Sauvignon Wine before Fining (n = 3)

	extract
extension subunits[a]	
EGC-P (%)	28.7 ± 0.1
C-P + EC-P (%)	54.8 ± 0.1
ECG-P (%)	4.0 ± 0.0
terminal subunits[a]	
C (%)	6.3 ± 0.2
EC (%)	5.3 ± 0
ECG (%)	0.8 ± 0.4
% trihydroxylation	28.7 ± 0.1
% galloylation	4.8 ± 0.3
mDP (n)[b]	8.0 ± 0.1
MM (g/mol)[c]	2425 ± 39
MM by GPC (extract) 50% (g/mol)[d]	2327 ± 0.0
mass conversion[e] (%)	24.0 ± 1.5

[a]Composition of tannin subunits as molar percentage with the following subunit abbreviations: (-P), phloroglucinol adduct of extension subunit; EGC, (−)-epigallocatechin; C, (+)-catechin; EC, (−)-epicatechin; ECG, (−)-epicatechin-3-*O*-gallate. [b]Mean degree of polymerization in epicatechin units. [c]Molecular mass as determined by phloroglucinolysis. [d]Molecular mass as determined by GPC at 50% tannin elution. [e]Mass conversion as percent recovery gravimetically.

Table 6. Cumulative Molecular Mass Distribution of Cabernet Sauvignon Wines before and after Fining with Grape Fiber (G4 and G6) and Apple Fiber (AP) Applied at 5 mg/mL and with Potassium Caseinate (CP) Applied at 0.30 mg/mL[a]

	cumulative mass distribution (g/mol)				
sample	10%	30%	50%	70%	90%
CT	234 ± 1 a	696 ± 5 a	1367 ± 7 a	2257 ± 12 a	4024 ± 25 a
G4	233 ± 1 b	676 ± 3 b	1328 ± 4 b	2166 ± 8 b	3836 ± 16 b
G6	231 ± 0 b	669 ± 2 b	1315 ± 2 b	2140 ± 4 c	3787 ± 8 c
AP	220 ± 1 d	593 ± 2 d	1197 ± 4 d	1912 ± 10 e	3305 ± 24 e
CP	226 ± 1 c	630 ± 6 c	1265 ± 8 c	2052 ± 11 d	3604 ± 15 d

[a]The molecular mass average is determined as cumulative elution slices (percent elution) by gel permeation chromatography. CT, control; G4, grape cell wall material; G6, cell wall material; AP, apple cell wall material; CP, casein commercial fining agent. All data are expressed as the average values of three replicates (n = 3). Statistical analysis: ANOVA and Tukey–Kramer HSD test (p < 0.0001). Different letters indicate statistically significant differences.

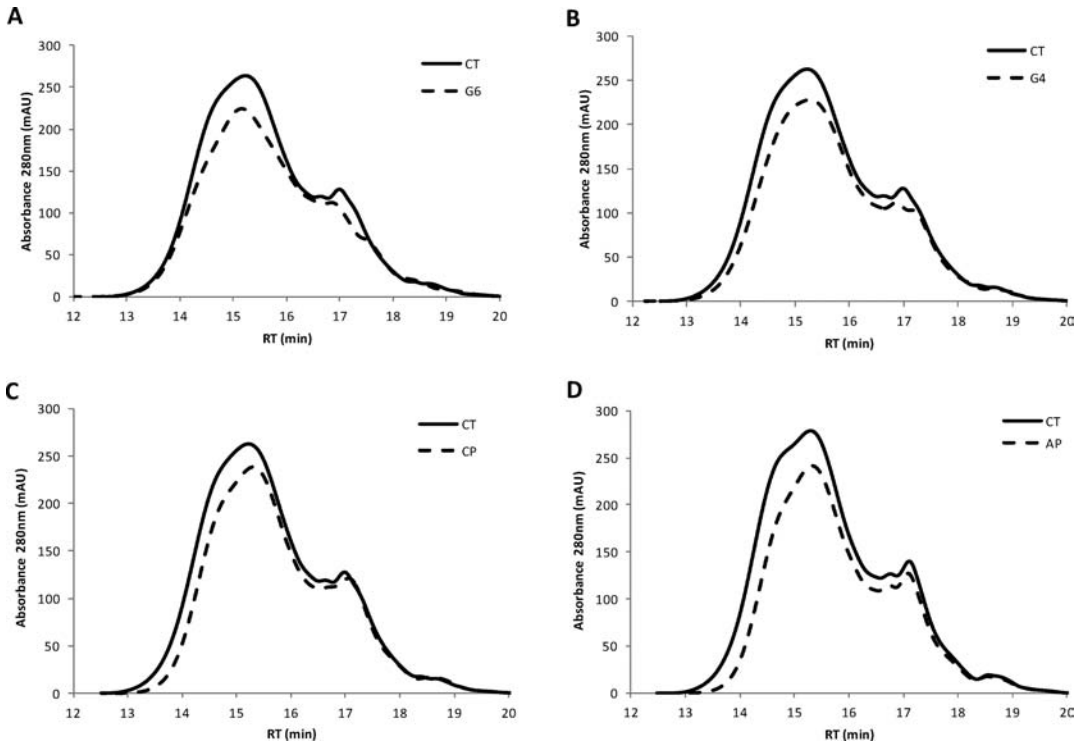

Figure 4. Analysis of the molecular mass distribution of wine phenolics before and after fining using gel permeation chromatography (n = 3): (A) 5 mg/mL grape fiber G4; (B) 5 mg/mL grape fiber G6; (C) 0.3 mg/mL potassium caseinate CP; (D) 5 mg/mL apple fiber AP. RT = retention time.

for high molecular mass phenolics, all fining agents shifted the phenolic profile to a lower average molecular mass.

The use of fibers as an alternative to proteins in red wine fining may improve various aspects of the winemaking process, including wine stability and sensory attributes, the reduction of potential allergenic problems, and the facilitation of the reuse of waste materials. Fining agents are commonly used in wine production to clarify, to control browning, and to improve wine stability. Nevertheless, the impact of fining agents on wine sensory properties is an important consideration.[37,38] Mouthfeel properties of grape seed and skin proanthocyanidins have been examined,[39] and it was shown that astringency increased with the proanthocyanin mDP, if considered independently of other structural factors. Kallithraka et al.[40] suggest that perceived astringency could be highly correlated to the amount of flavan-3-ols that are not precipitated by salivary proteins. It was emphasized that mDP appeared to be the most discriminatory structural variable affecting astringency. Kennedy and Taylor[24] have shown that mDP positively correlates with both the peak area of isolated tannin at 280 nm by GPC, as well as with astringency. Different wine GPC profiles are therefore expected to influence wine mouthfeel properties. Grape and apple fibers affected the molecular mass distribution in a different manner from casein (and potentially other protein fining agents). Therefore, they may produce somewhat different mouthfeel attributes in the fined wine by modulating its phenolic composition. It should be also considered that traces of protein fining agents may remain in the wine after fining and thus directly interfere with the interaction between wine and salivary protein, thereby modulating astringency perception.

Another important benefit to the use of fibers as alternatives to proteins in commercial red wine fining is that they potentially avoid allergen-related effects as they are insoluble, relatively inert, polysaccharide-based materials. These results suggest that the use of grape and apple fiber could be an alternative for proteins. In recent decades, for economic as well as sustainability objectives, there has been a growing pressure to recover and derive value from food wastes.[41] Large quantities of

both liquid and solid wastes are produced annually by the food processing industry, and their disposal creates serious environmental issues. Wine industry wastes include lees, pomace, and stems and may account on average for almost 30% (w/w) of the grapes used for wine production.[42] The use of fiber as a fining agent may add value to materials that previously may have been considered waste by the winery.

We have demonstrated that grape material from different vintages or viticultural treatments decreased tannin reduction in wine similarly in a dose-dependent way, which means that a commercial product with predictable adsorption properties may be possible. Nevertheless, we have also shown significant differences between fibers derived from grape and apple residues, revealing that fibers of different origin or composition may produce various fining effects in wine. This highlights the possibility to develop fining products that can target certain wine phenolic fractions, for example, high or low molecular mass, for the modulation of wine sensory properties.

The results of the current study have shown that grape and apple fiber may be considered as an alternative to commercial protein-based fining agents. Apple fiber had strong adsorption properties for high molecular mass tannin. Grape fibers reduced both high and low molecular mass tannin and achieved similar color characteristics to wine treated with a potassium caseinate fining agent. Further investigation will target how fiber addition affects the wine mouthfeel and flavor and determine long-term effects on wine stability.

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