Influence of Fining with Plant Proteins on Proanthocyanidin Composition of Red Wines

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Protein fining treatments clarify wine and reduce astringency. Five plant protein preparations (two whole wheat glutens, two hydrolyzed wheat glutens, and one preparation from white lupin, *Lupinus alba*) were studied to evaluate their suitability as wine fining agents with regard to their capacity to precipitate condensed tannins. Fining of two wines and a model wine with these proteins showed that precipitation always occurred, although the level of precipitation was low. The hydrolyzed wheat glutens and the white lupin preparation were the most selective, precipitating highly polymerized and highly galloylated tannins, as did gelatin. Initial results showed that plant proteins can be used as fining agents in enology.

Key words: Wine, fining, plant proteins, condensed tannins, proanthocyanidins, astringency

Protein fining treatment is applied to clarify wine and reduce astringency [25]. In such a process, tannins, which are responsible for astringency [11,14] and haze [1,12,26] in beverages such as wine and beer, interact with fining proteins and are precipitated by them [18,22,31]. A common fining agent is gelatin [19,25]. However, the discovery of the transmissible nature of Bovine Spongiform Encephalopathy (BSE) has raised concerns about the potential hazard of animal protein to humans. The food chain is the most obvious pathway by which BSE might be transmitted from cattle to humans, and enologists are now reluctant to use gelatins for fining treatment. To maintain consumer confidence, it is important to find nonanimal proteins to fine wine, although porcine gelatin is presently used. Before other proteins can be adapted as new fining agents, it is necessary to study their efficiency in clarifying and reducing astringency and their interactions with tannins. Previous data suggest that plant proteins (PP) may be good candidates; malt proteins are able to interact with tannins in beer [1] and sorghum proteins (prolamins) have been reported to interact with tannins and precipitate them [8,10]. The aim of our work was to evaluate the potential of selected plant proteins to decrease astringency by precipitation of tannins relative to a commercial gelatin, gelisol [15]. Five plant preparations (PP) were studied: two glutens from wheat, two hydrolyzed wheat glutens, and one protein from white lupin. Proteins (and proline-rich proteins such as gelatin) are generally reported to have high affinities for polymerized and galloylated tannins [15,20,22,32]. Therefore, our focus was on the characterization of tannins by their quantification in supernatants and in pellets obtained after fining treatment.

Materials and Methods

Materials. Organic solvents and phenylmethanethiol were purchased from Merck (Darmstadt, Germany) and Fluka (Buchs, Schwitzerland), respectively. Sodium dodecyl sulfate (SDS) was supplied by Sigma Chemical Co. (Poole, Dorset, UK).

Wines. The unfiltered wines were wine A, a Merlot (1998, Prignac, Gironde, France), and wine B, a Syrah (1999, Générac, Gard, France). Wines were analyzed and treated as soon as the bottles were opened.

Model wine. A model wine was prepared using a wine polyphenolic powder extract prepared from a Syrah wine (1997, Nîmes, Gard, France) at INRA Unité Expérimentale d'Œnologie de Pech Rouge (Gruissan, Aude, France) using a method adapted from a previous study [8]. A sample of 100 L of wine was loaded onto a 1.70 m x 0.2 m (i.d.) column filled with vinyldivinylbenzene. The resin was washed with 92 L of water to eliminate proteins, residual sugars, organic acids, and ions. Polyphenolic compounds were eluted with 60 L of methanol. The column was regenerated with NaOH and water, and the process was repeated with another 100 L of wine. The two polyphenolic extracts were pooled and concentrated to 4.5 L. This fraction was then atomized with a dryer (model B 190, Büchi, Flawil, Switzerland), yielding 746 g of phenolic powder.

The model wine was prepared just before the experiments by dissolving 3.73 g/L of powder in an aqueous solution containing potassium hydrogen tartrate 0.015 *M*, pH 3.7, and 12.3% ethanol.

Plant proteins. All tested plant proteins (PP) were powders: a preparation from white lupin (WL) (*Lupinus albus*, Fabaceae),

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Acknowledgments: The authors thank G. Guiraud from Suber Œnologie (France) for providing the wines, Martin Vialatte for the plant proteins and gelisol, and Martine Pradal for amino acid determinations.

Manuscript submitted June 2002; revised October 2002, January 2003

two preparations from wheat gluten (G1 and G2), and two hydrolysates of wheat gluten prepared by proprietary methods of enzyme (HG1) and chemical hydrolysis (HG2). These proprietary commercial plant proteins were provided by Martin Vialatte, Station Œnotechnique de Champagne (Epernay, France).

Gelatin. The gelatin used as reference for fining treatment was gelisol (Martin Vialatte, Epernay, France), a commercial liquid product from pigs. Previous Kjeldahl analysis showed that gelisol contained 100 g of protein per liter [15].

Electrophoresis. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), using concentration gradient gels (9 to 19% acrylamide), was performed as described elsewhere [13]. Proteins were then stained with Coomassie blue G-250 [17].

Amino acid determinations. The amino acid composition of the protein preparations was determined by high-performance liquid chromatography (HPLC) analysis using a Biochrom 20 Analyser (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) after hydrolysis with HCl 6 *N* at 112°C for 24 hr, as previously described [4,20]. After samples were dissolved in a lithium citrate 0.2 *M*, pH 2.2 buffer, they were injected onto a lithium cation-exchange column Ultropac 8 (Pharmacia LKB Biotechnology AB, Uppsala, Sweden). The amino acids were eluted by a lithium citrate buffer gradient increasing in ionic strength (0.20 to 1.65 *M)*, pH (2.80 to 3.55) and column temperature (35 to 78°C). A buffer flow rate of 0.42 mL/min and a postcolumn ninhydrin flow rate of 0.33 mL/min (with a coil temperature of 135°C) were maintained throughout the analysis. Detection of the ninhydrin derivatives was performed at 440 nm for proline and hydroxyproline and at 570 nm for the other amino acids. Samples were prepared in triplicate and each sample was analyzed in duplicate. Each amino acid was previously calibrated by using an external standard. Identification of amino acid was performed on the basis of their retention time.

Fining experiments. Plant protein preparations were suspended in water to obtain a final protein concentration of 10 g/L. The PP were relatively insoluble in water at pH 7, requiring agitation to maintain a homogeneous suspension. Fining experiments were performed by addition of 23 µL of gelisol or 230 µL for the PP to 23 mL of wine, yielding a final protein concentration of 0.1 g/L. The mixture was held at 20°C for 48 hr. Samples were then centrifuged at 4°C at 1900 x *g* for 10 min (Sorvall® Ultraspeed Centrifuge RC5 B, rotor SA-600; Dupont de Nemours, Les Ullis, France), giving a pellet and a supernatant (treated wine). All experiments were done in triplicate, and one analysis was performed per sample.

Turbidity. Turbidity measurements were performed using a HACH 2100P turbidimeter (Hach Co., Loveland, CO) on 20 mL withdrawal of unfined and fined wines. Unfined wine A and wine B were settled at 25°C for 48 and 72 hr, respectively. Fining treatment was performed at 25°C on 500 mL of wine A for 48 hr and on 500 mL of wine B for 72 hr.

Polyphenol analysis. A treatment with 10% SDS at 90°C was performed to dissociate soluble and insoluble tannin-protein complexes. Pellets (obtained after fining treatment) were treated with SDS 10% for 5 min and corresponding supernatants for 5 min (gelatin) or 10 min (PP). Unfined wines and the pellets and supernatants of fined wines were chromatographed on a 12 x 1 cm column filled with TSK HW-50F (Tosohaas Corp., Tokyo, Japan) and analyzed as previously described [15]. Before polyphenol elution, a water wash was used to remove proteins and SDS. Simple polyphenols were directly analyzed by HPLC. Proanthocyanidins were characterized and quantified by acid-catalyzed depolymerization in the presence of phenyl-methanethiol (thiolysis), directly followed by reversed-phase HPLC analysis [21]. The principle of this method is that polymeric proanthocyanidins are cleaved into monomeric units, which are released as flavan-3-ol if they are terminal units and as benzyl thioethers if they are upper and extension units [10,30]. Analysis of the products released by thiolysis was performed at 280 nm by external calibration with commercial standards (catechin, epicatechin) and with benzylthioether derivatives purified in our laboratory. The mDP (mean degree of polymerization: the average number of units in the polymer), the percentage of galloylated units, and the percentage of epigallocatechin units in the original polymers were calculated as described elsewhere [21]. The proanthocyanidin content was calculated by summing the concentrations of all released units.

Statistical analysis. One-way analyses of variance was conducted to compare the effectiveness of the different proteins for fining.

Results and Discussion

Protein MW of plant preparations. Protein patterns of PP obtained by SDS-PAGE are shown in Figure 1. Electrophoresis was used to evaluate the apparent molecular weight (MW) of the proteins contained in each fraction (G1, HG1, HG2, and WL). As expected, proteins detected in wheat gluten (line 1) showed higher apparent MW than those observed in the corresponding hydrolyzed fractions (lines 2 and 4). Most proteins present in G1 were detected in the area corresponding to apparent MW from 30 to 65 kDaltons (kDa). These proteins were reported elsewhere [28] as sulfur-poor proteins from 44 to 70 kDa (also called ω-gliadins) and sulfur-rich proteins for those between 30 and 42 kDa. The latter accounted for about 80% of the total prolamins in wheat. The highest MW proteins (100 to 200 kDa) correspond to high molecular weight proteins (HMW). Copurification of albumin is suggested by the presence of the 10 kDa signal. Most proteins present in HG1 and HG2 were approximately 13 kDa. The two hydrolyzed glutens had the same electrophoresis profile; that is, they possessed proteins having identical or close MW. Proteins of WL preparation (line 3) primarily had MW intermediate between those of nonhydrolyzed gluten proteins and hydrolyzed gluten proteins. Consistent with observations of Tai and Bush [29], two main groups of WL proteins were observed; the first was <18.5 kDa and the second group was between 27.5 and 32.5 kDa.

Amino acid composition of gelatin and PP. The amino acid composition of PP preparations and gelisol is shown in Table 1. Gelisol is mainly composed of glycine, alanine, proline, and hydroxyproline residues; in contrast, the amino acid composition of plant proteins is species-dependent. Major amino acids in the

wheat protein preparations are glutamic acid/glutamine and proline, whereas those of lupin are glutamic acid/glutamine and aspartic acid/asparagine. The absence of hydroxyproline was noticed in these extracts, whereas this amino acid is found in high concentrations in gelatin [20]. Different amino acid compositions of fining products are expected to influence the efficiency of the treatment because of the reported affinity of proline for tannins [3,9,16]. As determined from the amino acid compositions, the protein content of WL was low (31%), while that of hydrolyzed wheat gluten was much higher (88 and 86% for HG1 and HG2, respectively). G1 and G2 contained 69 and 85%, respectively, of protein (expressed mg of analyzed amino acids/mg of PP).

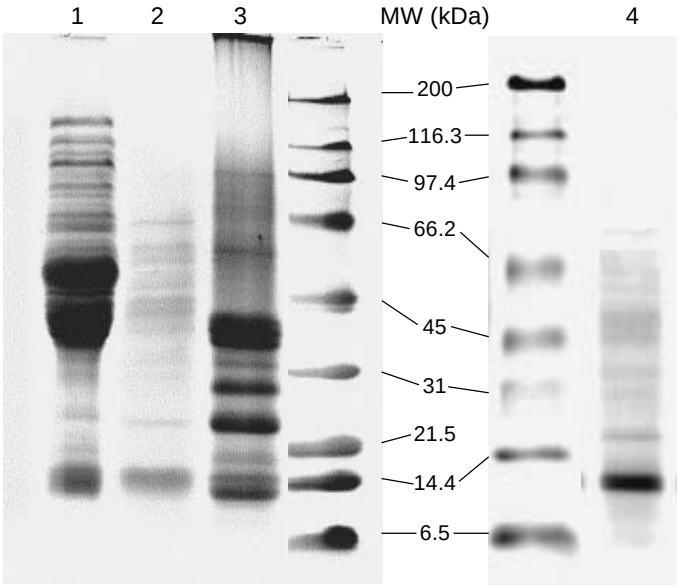


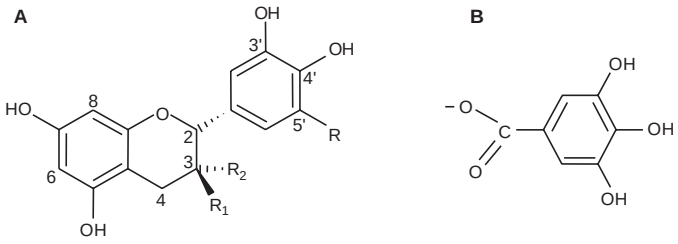
Figure 1 SDS-PAGE pattern of plant protein preparations: line 1: whole gluten, line 2: HG1; line 3: white lupin; line 4: HG2: hydrolyzed gluten; MW: standard proteins.

Table 1 Amino acid composition of gelisol, white lupin, whole gluten (G1, G2), and hydrolyzed gluten (HG1, HG2) preparations. Major amino acids in bold type.

Amino acid origin %	Gelisol	White lupin	Whole gluten		Hydrol. gluten	
			G1	G2	HG1	HG2
Asp+Asn	5	**11**	4	4	3	3
HydroxyPro	**9**	0	0	0	0	0
Glu+Gln	8	**22**	**35**	**35**	**38**	**35**
Pro	**10**	4	**13**	**12**	**13**	**12**
Gly	**33**	**8**	7	6	6	6
α-Ala	**12**	6	3	3	4	4
Ser	4	7	7	7	7	7
Leu	2	7	7	7	7	7
Thr	2	5	4	4	3	4
Phe	1	4	4	4	4	4
Arg	5	8	3	3	2	2
Cys	0	0.1	0.9	0.8	0.8	0.7
Met	0	0.3	0.3	0.2	0.2	0.2
Val	2	4	3	3	4	5
IsoLeu	1	5	3	3	3	4
Tyr	0	2	3	3	2	3
Lys	3	5	2	2	1	2
Hist	1	2	2	2	2	2

Characterization and quantification of wine proanthocyanidins. Proanthocyanidins are characterized by the nature of their extension and terminal flavan-3-ol units (Figure 2) and their mDP [6,21]. The concentrations and the average composition of proanthocyanidins of the three wines are shown in Table 2. The amount of total proanthocyanidins in the model wine (0.93 g/L) was significantly higher than that of wines A and B. With regard to their proanthocyanidin composition, the three wines were different. Wine B had the most polymerized tannins (mDP 10.31) and possessed the highest level of epi-gallocatechin units (19.49%), whereas wine A showed the highest level of galloylated units (8.31%).

Fining treatment with gelatin and plant proteins. The first requirement for a fining agent is to clarify wine (decrease turbidity). As shown in Figure 3, turbidity of untreated wine was spontaneously decreased due to the sedimentation by gravity of the particles in suspension; however, each fining agent yielded a larger decrease in turbidity of wines.



flavanols (a)	R	R_1	R_2
(+)-catechin	H	OH	H
(-)-epicatechin	H	H	OH
(-)-epigallocatechin	OH	OH	H
(-)-epicatechin -3-gallate	H	H	gallic acid (B)

Figure 2 Structure of monomeric units of proanthocyanidins.

Table 2 Characteristics of wines proanthocyanidins. condensed tannins, mDP (mean degree of polymerization), epigallocatechin units (EgC), and galloylated units.

	Wine A	Wine B	Model wine
Condensed tannins (g/L)	0.67 ± 0.05 a[a]	0.85 ± 0.01 b	0.93 ± 0.04 c
mDP	5.81 ± 0.49 a	10.3 ± 0.34 b	8.70 ± 0.34 c
EgC (%)	12.8 ± 2.01 a	19.5 ± 0.36 b	16.47 ± 0.8 b
Galloylated units (%)	8.3 ± 0.10 a	5.1 ± 0.05 b	4.90 ± 0.22 b

[a]Means with different letters in a row differ significantly ($p < 0.05$). Results ± standard deviations are average of three analyses.

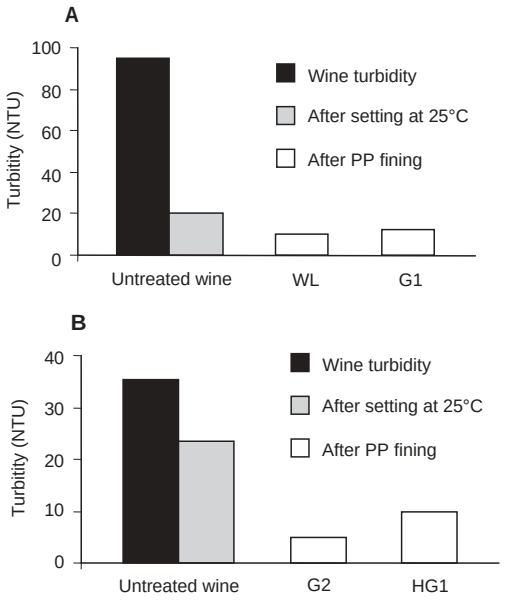


Figure 3 Turbidity of initial wine and after settling at 25°C and after plant protein fining treatment. (**A**) wine A; (**B**) wine B. WL: white lupin; G1 and G2: whole gluten; HG1: hydrolyzed gluten.

As previously reported [15,22,31], after fining, some tannins and probably some proteins remain in the wine supernatant, while precipitated tannin and protein are found in the pellet. Data presented in Table 3 show that some of the tannins present in the original sample were recovered neither in the pellet nor in the supernatant after fining treatment. The unrecovered tannins accounted for 20% of the initial amount, limiting interpretation of tannin characterization data. A 10% SDS treatment (5 min at 90°C) was applied to each compartment (pellet and supernatant) in order to improve tannin recovery. Recovery results for all experiments are summarized in Table 4. The SDS treatment improved the yield up to 97% in the case of gelatin, but did not allow total recovery for PP-treated samples. When SDS treatments were applied for 10 min to PP supernatants, the recovery yields were raised up to 90%. These results suggest that PP complex more tightly with tannins than gelatin. The resulting tannin-protein complexes resistant to SDS and thiolysis could be either soluble or insoluble complexes.

Based on the above recovery data, all analyses were performed on PP treatments using a 5-min SDS treatment for analysis of the pellets and 10-min treatment for supernatants, whereas for gelatin 5-min SDS treatments were used for analyses of both fractions. The composition and characteristics of condensed tannins present in wine and in the pellet and supernatant obtained after fining treatment are presented in Table 5.

Supernatants. Fining with gelisol and PP lowered the tannin content of the wines, as shown by the significantly lower concentration of tannin in the supernatants compared to that of the initial wines (Table 4). For wine A, which had the lowest mDP tannins, the mDP of tannins remaining in solution after fining was not significantly different from that of tannins in the initial wine (Table 5). In contrast, for wine B and model wine when fined with gelisol, and wine B fined with HG1, the mDP of the remaining tannins was significantly lower than that of initial wines. These data suggest that the proteins removed the higher polymerized tannins. The level of galloylation of tannins remaining in solution was not affected by the PP fining treatment, whereas it was decreased when gelisol was used in the case of wines A and B.

Table 3 Proanthocyanidin concentration (g/L) obtained without and with SDS procedure applied to supernatants (S) and pellet (P) from model-wine fining treatment, percentage of precipitated tannins (P/model wine), and percentage of tannin loss calculated from ((S+P)/model wine). Model-wine tannin concentration is 0.93 g/L.

	Gelisol		HG2[a]	
	Without	With SDS (5 min)	With SDS (5 min)	With SDS (5 and 10 min)
Supernatant	0.66	0.79	0.74	0.78
Precipitated tannins (%)	10	12	4	7
Loss (%)	20	3	17	10

[a]For HG2 samples, 5 min is the duration of heating of pellets and 10 min for supernatants.

Table 4 Proanthocyanidin concentration (g/L) of initial wines (in bold), and supernatants obtained after fining treatment with gelisol and five plant proteins, and percentage of precipitated tannins (recovered in pellets after fining treatment) and percentage of tannin loss.

		Tannins		
	Treatment	Supernatants	Precipitated (%)	Loss (%)
Wine A	/	**0.67 ± 0.05 a**[a]	0	/
	Gelisol	0.54 ± 0.01 b	16	4
	WL	0.56 ± 0.03b	5	9
	G1	0.56 ± 0.05 b	2	14
	G2	nr[b]	nr	nr
	HG1	nr	nr	nr
	HG2	nr	nr	nr
Wine B	/	**0.85 ± 0.01 a**	0	/
	Gelisol	0.69 ± 0.02 b	9	3
	WL	nr	nr	nr
	G1	nr	nr	nr
	G2	0.74 ± 0.05 b	1	13
	HG1	0.63 ± 0.02 c	3	22
	HG2	nr	nr	nr
Model wine	/	**0.93 ± 0.04 a**	0	/
	Gelisol	0.82 ± 0.04 b	12	0
	WL	0.80 ± 0.07 c	4	10
	G1	0.80 ± 0.09 c	1	14
	G2	0.79 ± 0.01 c	2	14
	HG1	0.76 ± 0.02 c	7	11
	HG2	0.78 ± 0.04 c	7	10

[a]Within a wine, different letters denote significant differences ($p < 0.05$), results are average of at least three analysis.
[b]nr: no result.

Pellets. No precipitation of simple phenolic compounds resulted from treatment with gelisol, as previously reported [15, 22], or with PP (data not shown). As shown in Table 5, between 1 and 7% of the initial condensed tannin was recovered in the pellet when PP fining was used, whereas a higher level (16%) was found following gelisol treatment. However, the apparently lower efficiency of tannin removal by PP may reflect the incomplete recovery from the pellet or from the supernatant, despite modification of the SDS procedure discussed above. Hydrolyzed glutens precipitated more tannins (7%) than did non-hydrolyzed gluten (1 to 2%), while WL precipitated 4%. Since the glutens have the same amino acid composition but differ in the MW of their proteins, these data suggest that lower MW proteins precipitate tannin more efficiently [15]. Determination of the mDP of the condensed tannins in the pellet shows that WL, HG1, and HG2 selectively precipitated highly polymerized tannins, as did gelisol (Table 5), whereas G1 and G2 were less selective for higher molecular weight proanthocyanidins. For G1 and G2, this lack of selectivity has to be considered in light of incomplete tannin recovery. The recovery of the higher mDP proanthocyanidins was lower in wine B treated with G2. The mDP of supernatant tannins was 8.31 and that of precipitated tannin was 8.65, whereas the mDP of the initial wine B tannins was 10.31. It may be due to a stronger interaction of the high polymerized tannins with the high molecular weight proteins, making them resistant to the SDS procedure. As well as chain length, the nature of the flavanol subunits of condensed tannins may be important in determining interaction with proteins. Some dependence on subunit structure has been described [7]. Formation of insoluble complexes has been reported to increase with the extent of galloylation [2,20,22,23,27]. The galloyl rings (Figure 2B) provide supplementary aromatic cycles and may also favor hydrophobic complexation via π-π interactions, whereas the additional hydroxyl groups are sites for hydrogen bond formations. Therefore, we have examined the nature of the precipitated tannins with regard to proportion of these quantifiable constitutive flavanol units. The comparison of the average level of galloylation (Table 5) showed that precipitated proanthocyanidins were significantly different from those of the initial wines. Gelisol, a gelatin product, removes the highly galloylated tannins as already demonstrated for partly purified gelatins [15]. With wheat proteins, the precipitated tannins are more galloylated than those precipitated by gelisol, whereas WL was less selective for these tannin units

Table 5 Polymeric proanthocyanidin composition of initial wines (in bold), pellets, and supernatants obtained after fining treatment with gelisol and five plant proteins.

Characteristics[a]		Treatment	Wine A	Wine B	Model wine
mDP		**Initial**	**5.81 ± 0.49 a**[b]	**10.31 ± 0.34 a**	**8.74 ± 0.34 a**
	Pellets	Gelisol	11.47 ± 0.42 b	22.24 ± 2.46 b	15.21 ± 0.44 b
		WL	10.44 ± 0.35 c	nr[c]	15.30 ± 1.67 b
		G1	7.40 ± 0.81 d	nr	8.49 ± 1.18 ac
		G2	nr	8.65 ± 0.65 c	9.30 ± 1.83 c
		HG1	nr	16.84 ± 2.25 d	15.33 ± 0.71 b
		HG2	nr	nr	15.56 ± 0.06 b
	Supernatants	Gelisol	5.68 ± 0.05 a	8.97 ± 0.30 b	7.20 ± 0.13 b
		WL	5.80 ± 0.09 a	nr	7.33 ± 1.22 a
		G1	6.18 ± 0.22 a	nr	7.78 ± 0.33 b
		G2	nr	8.30 ± 0.70 b	8.41 ± 0.48 a
		HG1	nr	9.58 ± 0.16 b	8.36 ± 0.25 a
		HG2	nr	nr	8.22 ± 0.10 a
EgC (%)		**Initial**	**12.80 ± 2.01 a**	**19.49 ± 0.36 a**	**16.47 ± 0.77 a**
	Pellets	Gelisol	14.70 ± 2.12 a	20.15 ± 0.09 b	18.00 ± 0.38 a
		WL	16.17 ± 2.48 b	nr	17.36 ± 1.01 a
		G1	9.49 ± 0.51 b	nr	11.06 ± 0.99 bc
		G2	nr	nd c	9.19 ± 1.60 c
		HG1	nr	14.89 ± 2.69 d	14.33± 1.87 ab
		HG2	nr	nr	14.70 ± 1.69 d
	Supernatants	Gelisol	14.68 ± 0.76 a	17.46 ± 0.25 b	15.25 ± 1.05 b
		WL	12.62 ± 1.10 a	nr	13.90 ± 0.82 a
		G1	11.67 ± 1.55 a	nr	15.60 ± 0.47 ab
		G2	nr	16.31± 0.84 c	15.63 ± 1.45 ab
		HG1	nr	17.51 ± 1.54 abc	15.31 ± 0.96 b
		HG2	nr	nr	16.12 ± 1.10 b
Galloyl. tannins (%)		**Initial**	**8.31 ± 0.10a**	**5.09 ± 0.05 a**	**4.90 ± 0.22 a**
	Pellets	Gelisol	10.52 ± 0.24 b	6.57 ± 0.52 b	6.51 ± 0.22 b
		WL	9.41 ± 0.55 c	nr	5.88 ± 0.13 c
		G1	12.03 ± 0.72 d	nr	6.87 ± 0.77 cd
		G2	nr	8.35 ± 0.57 c	5.28 ± 1.81 ac
		HG1	nr	6.73 ± 0.25 b	7.20 ± 0.16 d
		HG2	nr	nr	7.17 ± 0.07 d
	Supernatants	Gelisol	7.87 ± 0.04 b	4.95 ± 0.10 b	4.94 ± 0.07 a
		WL	8.35 ± 0.09 a	nr	5.19 ± 0.40 a
		G1	8.59 ± 0.17 c	nr	5.09 ± 0.10 a
		G2	nr	5.29 ± 0.27 a	5.18 ± 0.15 a
		HG1	nr	5.21 ± 0.11 a	5.31 ± 0.16 a
		HG2	nr	nr	5.03 ± 0.18 a

[a]mDP: mean degree of polymerization; EgC: epigallocatechin units.
[b]Supernatants and pellets considered independently and each compared with initial wine. Within a column, different letters denote significant differences ($p < 0.05$); results are average of at least three analyses.
[c]nr: no result; nd: not detected.

than gelisol. The tested PP selectively precipitated highly galloylated tannins. These data were in agreement with mass spectrometry study, which shows that in a flavanol-peptide model blend, the flavanols and the peptides are held together by relatively weak interactions and that galloylated compounds are better ligands than nongalloylated ones [24]. In contrast, it was not possible to detect any evident relationship between the level of epigallocatechin units and precipitation.

Our previous work showed that for four different red wines the low molecular weight gelatin (16 kD) precipitated more highly polymerized and more galloylated tannins than did the higher molecular weight gelatin (190 kD) [15]. Within the plant proteins studied, analysis of the precipitated tannins showed that hydrolyzed glutens, low MW protein fractions, were the most selective and precipitated highly polymerized and highly galloylated tannins. Their behavior in fining treatment is obviously close to that of gelatins [15] concerning precipitated tannin nature, but their tannin precipitation level was lower.

Conclusion

Analysis of polyphenols from untreated and fined wines showed that plant preparations do not precipitate simple phenolic compounds but selectively precipitate condensed tannins, as do gelatins. Data for a model wine was comparable with that for genuine wines (wines A and B), showing that the model wine is a relevant model and could be used to compare fining treatments. The rather poor tannin recoveries despite SDS treatment suggest that plant proteins interact with tannins, in particular with highly polymerized tannins, more strongly than gelatins. Amino acid composition is not the major factor influencing interaction. The hydrolyzed and nonhydrolyzed glutens had identical amino acid composition. The hydrolyzed glutens HG1 and HG2, which had lower MW proteins than the nonhydrolyzed G1 and G2, precipitated the higher polymerized tannins more effectively. Additionally, white lupin, which has an amino acid composition different from gluten and has lower MW proteins, was almost as selective as hydrolyzed gluten in precipitating high mDP tannins. This study of fining by plant proteins confirms that MW of proteins is an important factor in tannin precipitation and has significant effects on fining. Although the gelatin treatment removed more tannins than the PP, the hydrolyzed glutens and one of the two nonhydrolyzed glutens precipitated highly galloylated tannins to the same extent as gelatin, indicating that some PP preparations have the same selectivity as gelatin. The difference in behavior between the preparations suggests the importance of the choice of proteins in fining treatments, but indicates the need to test a large number of different PP preparations to find those which lead to optimal treatments.

Literature Cited

1. Asano, K., K. Shinagawa, and N. Hashimoto. Characterization of haze-forming proteins of beer and their roles in chill haze formation. J. Am. Soc. Brew. Chem. 40:147-154 (1982).

2. Bacon, J.R., and M.J.C. Rhodes. Development of a competition assay for the evaluation of the binding of human parotid salivary proteins to dietary complex phenols and tannins using a peroxidase-labeled tannin. J. Agric. Food Chem. 46:5083-5088 (1998).

3. Baxter, N.J., T.H. Lilley, E. Haslam, and M.P. Williamson. Multiple interactions between polyphenols and a salivary proline-rich protein repeat result in complexation and precipitation. Biochem. 36:5566-5577 (1997).

4. Benson, J.V., J.R. Manuel, J. Gordon, and J.A. Patterson. Accelerated chromatographic analysis of amino acids in physiological fluids containing glutamine and asparagine. Anal. Biochem. 18:228-240 (1967).

5. Caderni, G., S. Remy, V. Cheynier, G. Morozzi, and P. Dolara. Effect of polyphenols on colon carcinogenesis. Eur. J. Nutr. 38:126-132 (1999).

6. Czochanska, Z., L.Y. Foo, R.H. Newman, and J.L. Porter. Polymeric proanthocyanidins. Stereochemistry, structural units and molecular weight. J. Chem. Soc., Perkin Trans. 1:2278-2286 (1980).

7. Hagerman, A.E. Chemistry of tannin-protein complexation. *In* Chemistry and Significance of Condensed Tannins. R.W. Hemingway and J.J. Karchesy (Eds.), pp. 323-331. Plenum Press, New York (1989).

8. Hagerman, A.E., and L.G. Butler. Condensed tannin purification and characterization of tannin-associated proteins. J. Agric. Food Chem. 28:947-952 (1980).

9. Hagerman, A.E., and L.G. Butler. The specificity of proanthocyanidin-protein interactions. J. Biol. Chem. 256(9):4494-4497 (1981).

10. Hagerman, A.E., and C.T. Robbins. Implications of soluble tannin-protein complexes for tannin analysis and plant defense mechanisms. J. Chem. Ecol. 13(5):1243-1259 (1987).

11. Haslam, E., and T.H. Lilley. Natural astringency in foodstuffs. A molecular interpretation. Crit. Rev. Food Sci. Nutr. 27:1-40 (1988).

12. Heatherbell, D.A. Haze and sediment formation in clarified apple juice and apple wine. II. The role of polyvalent cations, polyphenols and proteins. Food Technol. New Zeal. 17-23 (1976).

13. Laemmli, U.K. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 227:680-685 (1970).

14. Lea, A.G.H. Bitterness and astringency: The procyanidins of fermented apple ciders. *In* Developments in Food Science 25. R.L. Rouseff (Ed.), pp. 123-143. Elsevier, New York (1990).

15. Maury, C., P. Sarni-Manchado, S. Lefebvre, V. Cheynier, and M. Moutounet. Influence of fining with different molecular weight gelatins on proanthocyanidin composition and perception of wines. Am. J. Enol. Vitic. 52:140-145 (2001).

16. Mehansho, H.L., G. Butler, and D.M. Carlson. Dietary tannins and salivary proline-rich proteins: Interactions, induction, and defense mechanisms. Ann. Rev. Nutr. 7:423-440 (1987).

17. Neuhoff, V., N. Arold, D. Taube, and W. Ehrhardt. Improved staining of proteins in polyacrylamide gels including isoelectric focusing gels with clear background at nanogram sensitivity using Coomassie brillant blue G-250 and R-250. Electrophoresis 9:255-262 (1988).

18. Puig-Deu, M., E. Lopez-Tamames, S. Buxaderas, and M.C. Torre-Boronat. Quality of base and sparkling wines as influenced by the type of fining agent added pre-fermentation. Food Chem. 66:35-42 (1999).

19. Ribéreau-Gayon P., Y. Glories, A. Maujean, and D. Dubourdieu. Les traitements de clarification et de stabilisation: Le collage des vins. Chimie du vin, stabilisation et traitement. *In* Traité d'Œnologie, pp. 343-381. Dunod, Paris (1998).

20. Ricardo da Silva, J.M., V. Cheynier, J.M. Souquet, M. Moutounet, J.C. Cabanis, and M. Bourzeix. Interaction of grape seed procyanidins with various proteins in relation to wine fining. J. Sci. Food Agric. 57:111-125 (1991).

21. Rigaud, J., X. Perez-Ilzarbe, J.M. Ricardo da Silva, and V. Cheynier. Micro method for the identification of proanthocyanidin using thiolysis monitored by high-performance liquid chromatography. J. Chromatogr. 540:401-405 (1991).

22. Sarni-Manchado, P., A. Deleris, S. Avallone, V. Cheynier, and M. Moutounet. Analysis and characterization of wine condensed tannins precipitated by protein used as fining agent in enology. Am. J. Enol. Vitic. 50(1):81-86 (1999).

23. Sarni-Manchado, P., V. Cheynier, and M. Moutounet. Interactions of grape seed tannins with salivary proteins. J. Agric. Food Chem. 47:42-47 (1999).

24. Sarni-Manchado, P., and V. Cheynier. Study of noncovalent complexation between catechin derivatives and peptide by electrospray ionization-mass spectrometry (ESI-MS). J. Mass Spectrom. 37:609-616 (2002).

25. Scotti, B., and P. Poinsaut. Le collage à la gélatine: Entre science et tradition. Rev. Œnol. 85 (Sept.):1-7 (1997).

26. Siebert, K.J., A. Carrasco, and P.Y. Lynn. Formation of protein-polyphenol haze in beverages. J. Agric. Food Chem. 44:1997-2005 (1996).

27. Siebert, K.J., N.V. Troukhanova, and P.Y. Lynn, Nature of polyphenol-protein interactions. J. Agric. Food Chem. 44:80-85 (1996).

28. Shewry, P.R., A.S. Tatham, J. Forde, M. Kreis and B.J. Miflin. The classification and nomenclature of wheat gluten proteins: A reassessment. J. Cereal Sci. 4:97-106 (1986).

29. Tai, H.H., and R.S. Bush. Analysis of lupin seed protein digestibility using gel electrophoresis and immunoblots. J. Anim. Sci. 75:1934-1940 (1997).

30. Thompson, R.S., D. Jacques, E. Haslam, and D.J.N. Tanner. Plant proanthocyanidins. Part I. Introduction: The isolation, structure, and distribution in nature of plant procyanidins. J. Chem. Soc., Perkin Trans. 1:1387-1399 (1972).

31. Yokotsuka, K., and V.L. Singleton. Interactive precipitation between phenolic fractions and peptides in wine-like model solutions: Turbidity, particle size, and residual content as influenced by pH, temperature and peptide concentration. Am. J. Enol. Vitic. 46:329-338 (1995).

32. Zhu, M., D. Phillipson, P.M. Greengrass, N.E. Bowery, and Y. Cai. Plant polyphenols: Biologically active compounds or nonselective binders to protein? Phytochemistry 44:441-447 (1997).