Effect of Various Proteins on Different Molecular Weight Proanthocyanidin Fractions of Red Wine during Wine Fining

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Abstract: The effect of several proteins on three main wine proanthocyanidin containing fractions with the mean degree of polymerization (mDP) of 1.5 (FI), 3.4 (FII), and 4.9 (FIII) was studied. Although casein and potassium caseinate showed similar molecular weight (MW) distribution, casein decreased the FI fraction more than the twice as effectively as potassium caseinate. A gelatin with a medium MW polydispersion induced a similar decrease (~20%) in all tannin fractions. A gelatin with low MW primarily removed the tannin fractions of lower mDP (FI and FII), while a gelatin with a higher MW had a minor effect (5%) on the fraction of higher mDP (FIII). Neither of the two studied isinglasses reduced the FII fraction. The tannins of FI and FIII were removed by swim bladder isinglass twice as effectively as by fish skin isinglass. For the mDP of fined wines, egg albumin induced a decrease on mDP of 24% for the more polymerized tannin fraction (FIII); although within all assays there was a decrease ranging from 6 to 14%.

Key words: wine, protein, fining agents, proanthocyanidins

Enological protein fining agents are mainly used in red wine for clarification and for reduction of phenolic compounds. The main protein fining agents used in wine are animal proteins such as gelatin, egg albumin, casein, potassium caseinate, and isinglass. However, in recent years certain proteins of vegetable origin (cereals and legumes) have also been investigated as possible wine fining agents (Marchal et al. 2002, Maury et al. 2003). Proteins used as wine fining agents have distinct physicochemical characteristics such as molecular weight (MW) distribution, isoelectric point, and surface charge density (Paetzold and Glories 1990, Lagune and Glories 1996a, Lamandon et al. 1997, Versari at al. 1998, Marchal et al. 2002, Maury et al. 2003, Cosme et al. 2007).

Studies on wine fining have used two different approaches. In the first, authors have concentrated on the influence of fining proteins on wine composition, not on characterizing the protein fining agents. In the second, relations between physicochemical characteristics (molecular weight and surface charge density) of the fining protein and their effect on wine composition are specified.

Several authors have studied the influence of protein fining agents on wine composition (Ough 1960, Ricardo-da-Silva et al. 1991a, Sims et al. 1995, Machado-Nunes et al. 1998, Lovino et al. 1999, Castellari et al. 2001). Fining a young red wine (Mourvèdre) with gelatin and casein reduced the concentration of total anthocyanins and the absorbance at 420, 520, and 620 nm, but the concentrations of flavan-3-ol monomers and several procyanidin dimers and trimers, either esterified or not esterified with gallic acid, were not affected (Ricardo-da-Silva et al. 1991a). It has been supposed that proteins interact more intensely with more polymerized proanthocyanidins and those esterified with gallic acid (Ricardo-da-Silva et al. 1991a, Sarni-Manchado et al. 1999, Maury et al. 2001, 2003). One study suggests that other, more active, phenolic compounds, namely high MW proanthocyanidins and anthocyanin-proanthocyanidin complexes (polymeric pigments), protect the small oligomeric procyanidins (Ricardo-da-Silva et al. 1991a). Other authors have shown that gelatin selectively decreases the polymerized phenolic compounds (Yokotsuka and Singleton 1987, 1995, Sims et al. 1995). However, in wines with low phenolic compound concentrations, the addition of casein also influences low molecular weight flavonoid composition (Schneider 1988, Machado-Nunes et al. 1998). These authors established the importance of the initial phenolic composition of the wine, mainly anthocyanins and condensed tannins, on the fining process. It was also shown that casein significantly reduced the absorbance at 520 nm and total and poly-

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Acknowledgments: The authors are grateful to the Agro Program (project nº 22) for the financial support of this work.

The authors thank the companies AEB Bioquímica Portuguesa, S.A., Proenol Indústria Biotecnológica, Lda., and Ecofiltra for providing the fining agents.

Manuscript submitted Jan 2008, revised Oct 2008, accepted Oct 2008. Publication costs of this article defrayed in part by page fees.

meric phenolic compounds (Sims et al. 1995). The effect of this protein was attributed to its alternative polar and apolar zones and to hydrophobic and hydrophilic amino acid distribution (Stocké and Ortmann 1999).

However, the relationships between the physicochemical characteristics of protein fining agents and their interaction with wine phenolic compounds has been specified in relatively few studies, primarily on gelatins (Hrazdina et al. 1969, Marchal et al. 1993, Lagune and Glories 1996b, Versari et al. 1998, Sarni-Manchado et al. 1999, Maury et al. 2001, 2003, Braga et al. 2007, Cosme et al. 2008), and vegetable proteins (Marchal et al. 2002, Maury et al. 2003). Some studies have shown that the protein MW distribution of gelatins influences the protein-tannin interaction (Hrazdina et al. 1969, Sarni-Manchado et al. 1999, Maury et al. 2001, 2003). Thus, gelatins with high MW distribution preferentially remove proanthocyanidins rich in epigallocatechin units, while gelatins with low MW distribution selectively deplete the highly polymerized proanthocyanidins (Sarni-Manchado et al. 1999, Maury et al. 2001). Gelatins with low surface charge densities precipitate fewer wine components than gelatins with higher surface charge densities (Versari et al. 1998). In addition, it has been confirmed that gelatins selectively remove proanthocyanidins with high degrees of polymerization (~12 mDP) and galloylated procyanidins (Sarni-Manchado et al. 1999).

An enhanced knowledge of the quantity and type of phenolic compounds remaining in red wine after fining with proteins having specific physicochemical characteristics should help to optimize the fining process. To our knowledge, there are no detailed studies available on the structural composition of flavonoids remaining in wine or on the abundance of the three main tannin fractions after the addition of protein fining agents such as swim bladder isinglass, egg albumin, casein, and potassium caseinate. Therefore, the main objectives of this work were to compare the effects of enological protein fining agents (gelatin, egg albumin, casein, potassium caseinate, and isinglass) and their distinct physicochemical characteristics (molecular weight distribution, isoelectric point, surface charge density) on the structural characteristics of proanthocyanidins, on the three main tannin fractions (monomeric, oligomeric, and polymeric flavan-3-ols), and on color and pigments of red wine after fining.

Materials and Methods

Chemicals. Vanillin was purchased from Merck (Darmstadt, Germany) and toluene-α-thiol from Fluka (Buchs, Switzerland). Solvents and acids used were of HPLC grade.

Protein fining agents. Eight previously characterized fining agents (Cosme et al. 2007) were used in this study: one egg albumin (AS_1), two isinglasses (IL_1, IS_4), one potassium caseinate (CKS_1), one casein (CS_4), and three gelatins (GL_1, GS_2, and GS_4) (Table 1).

Fining experiments. A young blended red wine (vintage 2003) of Castelão, Tinta Roriz, and Caladoc grape varieties from the Estremadura region (north of Lisbon) was used in fining experiments. Treatments were carried out by addition of standard quantities of the protein fining agents (isinglass, casein, potassium caseinate, and gelatin) prepared as recommended by manufacturers (Table 1) to 250 mL wine. Untreated wine was used as a control. The fining agents were thoroughly mixed and allowed to remain in contact with the wines for 7 days at 20°C; samples were then centrifuged at 4,000 rpm for 15 min before analysis. All fining experiments were done in duplicate.

Separation of proanthocyanidins and determination by vanillin assay. Separation of flavan-3-ol was performed using a C_{18} Sep-Pak cartridge (Waters, Milford,

Table 1 Physicochemical characteristics of protein fining agents used in the fining trial (Cosme et al. 2007).

Fining agent (concn)[a]	Molecular weight distribution (kDa)	Surface charge density (meq/g product at pH 3.4)[b]	Protein content as % N x k (%w/w, dry wt)[b,c]	Isoelectric point[b]
IL_1 (50 mL/hL)	Polydispersion below 20.1	0.04 ± 0.00	112 ± 4	4.55 ± 0.02
IS_4 (2.25 g/hL)	Bands >94.0, between 94.0–43.0, and at 20.1	0.41 ± 0.01	73 ± 3	6.48 ± 0.03
CS_4 (40 g/hL)	Band close to 30.0	0.09 ± 0.01	71 ± 1	4.64 ± 0.06
CKS_1 (40 g/hL)	Band close to 30.0	0.04 ± 0.00	85 ± 2	4.51 ± 0.04
AS_1 (12.5 g/hL)	Band close to 43.0	0.73 ± 0.01	78 ± 1	5.00 ± 0.02
GL_1 (50 mL/hL)	Polydispersion <43.0	0.11 ± 0.00	92 ± 2	4.20 ± 0.01
GS_2 (8 g/hL)	Polydispersion >43.0	0.74 ± 0.02	98 ± 1	4.74 ± 0.00
GS_4 (8 g/hL)	No bands between 94.4–14.4	0.26 ± 0.00	91 ± 4	4.50 ± 0.00

[a]Isinglass (IL_1) (from fish skin), isinglass (IS_4) (from fish swim bladder), casein (CS_4), potassium caseinate (CKS_1), egg albumin (AS_1), gelatin (GL_1) (obtained by chemical hydrolysis), gelatin (GS_2), gelatin (GS_4) (high hydrolysis degree).
[b]Mean values of triplicate determinations ± standard deviation (SD).
[c]Multiplication factor (k): 6.68 for egg albumin; 6.25 for isinglass; 6.38 for casein and potassium caseinate; 5.55 for gelatin.

Ireland) according to the degree of polymerization in three fractions: FI (monomeric), FII (oligomeric), and FIII (polymeric) (Sun et al. 1998). Quantification of total flavan-3-ol in each fraction was performed using the vanillin assay (Sun et al. 1998). Quantification was carried out by means of standard curves prepared from monomers (FI), oligomers (FII), and polymers of flavan-3-ol (FIII) isolated from grape seeds, as described previously (Sun et al. 1998).

Acid-catalyzed depolymerization of proanthocyanidins. The oligomeric and polymeric proanthocyanidins were depolymerized in the presence of a nucleophilic agent (toluene-α-thiol) in an acid medium (Maury et al. 2001). Reversed-phase HPLC analysis of the products formed allows determination of the structural composition of proanthocyanidins, which are characterized by the nature of their constitutive extension units (released as their benzylthioethers) and terminal units (released as flavan-3-ols). It also allows calculation of their structural characteristics, such as the mean degree of polymerization (mDP), the average molecular mass (aMW), the *cis* to *trans* ratio (cis:trans), the percentage of prodelphinidins (% prodelph), and the percentage of galloylation (% gal) (Ricardo-da-Silva et al. 1991b, Rigaud et al. 1991, Kennedy et al. 2000).

The acid-catalyzed depolymerization was performed according to a published method (Monagas et al. 2003). The thiolyzed sample was then analyzed directly by HPLC. The HPLC system was comprised of a Uvis 200 UV-vis detector (Konik Instruments, Konik-Tech, Barcelona, Spain) set at 280 nm, a Merck Hitachi intelligent ternary pump (model L-6200A; Tokyo, Japan), with a Rheodyne manual injector (model 7125-A) fitted with a 50 µL loop, coupled to a Konikrom data chromatography treatment system (version 6.2; Konik Instruments), and using a C_{18} Lichrosphere 100 column (250 mm x 4.6 mm, 5 µm) (Merck, Darmstadt, Germany). Separations were performed at room temperature. The linear gradient elution conditions were as follows: 1.0 mL/min flow rate; solvent A (water/formic acid, 98/2, v/v); solvent B (acetonitrile/formic acid/water 80/2/18, v/v/v); 5–30% B from 0 to 40 min, 30–50% B from 40 to 60 min, 50–80% B from 60 to 70 min, 80–100% B from 70 to 75 min, followed by 100% B isocratic from 75 to 97 min. The amount of monomers (terminal units) and toluene-α-thiol adducts (extension units) released from the depolymerization reaction in the presence of toluene-α-thiol was calculated from the areas below the chromatographic peaks at 280 nm by comparison with calibration curves (Kennedy et al. 2000).

Separation of monomeric and oligomeric flavan-3-ols by polyamide column chromatography and quantification by HPLC analysis. Procyanidin separation was performed with a 3 mL red wine volume (Ricardo-da-Silva et al. 1990), using the same HPLC system as in the analysis of the products released by acid-catalyzed depolymerization in the presence of toluene-α-thiol. Elution conditions for monomeric flavan-3-ols were as follows: 0.9 mL/min flow rate; solvent A (water/acetic acid, 97.5/2.5, v/v); solvent B (acetonitrile/solvent A 80/20, v/v); 7–25% B from 0 to 31 min, followed by 100% (methanol/water, 50/50, v/v) from 32 to 50 min and reconditioning of the column from 51 to 65 under initial gradient conditions. The elution conditions for oligomeric procyanidins (dimeric and trimeric) were as follows: 1.0 mL/min flow rate; solvent A (water); solvent B (water/acetic acid, 90/10, v/v); 10–70% B from 0 to 45 min, 70–90% B from 45 to 70 min, 90% B isocratic from 70 to 82 min, 90–100% B from 82 to 85 min, 100% B isocratic from 85 to 90 min, followed by isocratic (methanol/water, 50/50, v/v) from 91 to 100 min and reconditioning of the column from 101 to 120 min under initial gradient conditions. Identification (Ricardo-da-Silva et al. 1991b, Rigaud et al. 1991) and quantification (Ricardo-da-Silva et al. 1990, Dallas et al. 1996) of monomeric flavan-3-ols and oligomeric procyanidins (some dimers and trimers) was performed as described previously.

Color and pigments. Color was determined by measuring absorbance at 620, 520, and 420 nm (1-mm cell) using a UV-vis UV4 spectrophotometer (Unicam, Cambridge, UK) (OIV 1990). The concentration of total and colored anthocyanins and total and polymeric pigments were determined according to the method of Somers and Evans (1977).

Statistical analysis. The data are presented as means ± standard deviation. One-way analyses of variance and comparison of treatment means (LSD, 5% level) were performed using ANOVA Statistica 6 software (StatSoft, Tulsa, OK) in respect to the effect of protein fining agents.

Results and Discussion

Physicochemical characteristics of the fining agents used in this work are summarized in Table 1. The mDP of the FI (monomeric) fraction from the different samples ranged from 1.47 to 1.58. The mDP of the monomeric fraction should be 1; however, the FI fraction also includes two unknown compounds (Sun et al. 1998). It is probable that very few oligomeric proanthocyanidins pass through the C_{18} Sep-Pak during separation.

Decrease of flavan-3-ol fractions affected by fining. Condensed tannins with a mean polymerization degree of 4.9 (fraction FIII), probably associated with astringency, were significantly removed (20 to 28% reduction) by swim bladder isinglass, egg albumin, and the two types of gelatins characterized by a polydispersion on the low molecular weight (Figure 1). The two isinglasses (IL_1 [MW < 20.1 kDa] and IS_4 [with bands at MW > 94.0, 94.0–43.0 and at 20.1 kDa], which removed 13% and 28%, respectively) showed distinct effects on the polymeric flavan-3-ol. Swim bladder isinglass decreased the tannin fraction with mDP 4.9 more than twice as effectively as fish skin isinglass.

The oligomeric flavan-3-ol (fraction FII, mDP 3.4) was extensively decreased by egg albumin, casein, potassium

caseinate, and the three gelatins. Among the gelatins, GS_4 (MW < 14.4 kDa) led to a greater decrease in oligomeric proanthocyanidins (~55%) than the other two gelatins (GS_2, polydispersion >43.0 kDa; GL_1, polydispersion <43.0 kDa). The isinglasses did not lower the concentration of these compounds significantly.

The monomeric flavan-3-ol (fraction FI, mDP ≈ 1.5), generally associated with bitterness, was significantly removed by casein, swim bladder isinglass, and the low MW distribution gelatins (Figure 1). Casein and potassium caseinate showed an electrophoretic profile with similar MW distribution (~30.0 kDa) (Cosme et al. 2007). However, their affinity for monomeric flavan-3-ol (fraction FI) was different. Casein decreased these compounds significantly; this decrease was not observed for potassium caseinate. Notably, swim bladder isinglass, with a high MW distribution, decreased these compounds to a greater degree than fish skin isinglass, with a MW distribution <20.1 kDa. Egg albumin (7% reduction) did not lower the monomeric flavan-3-ol significantly.

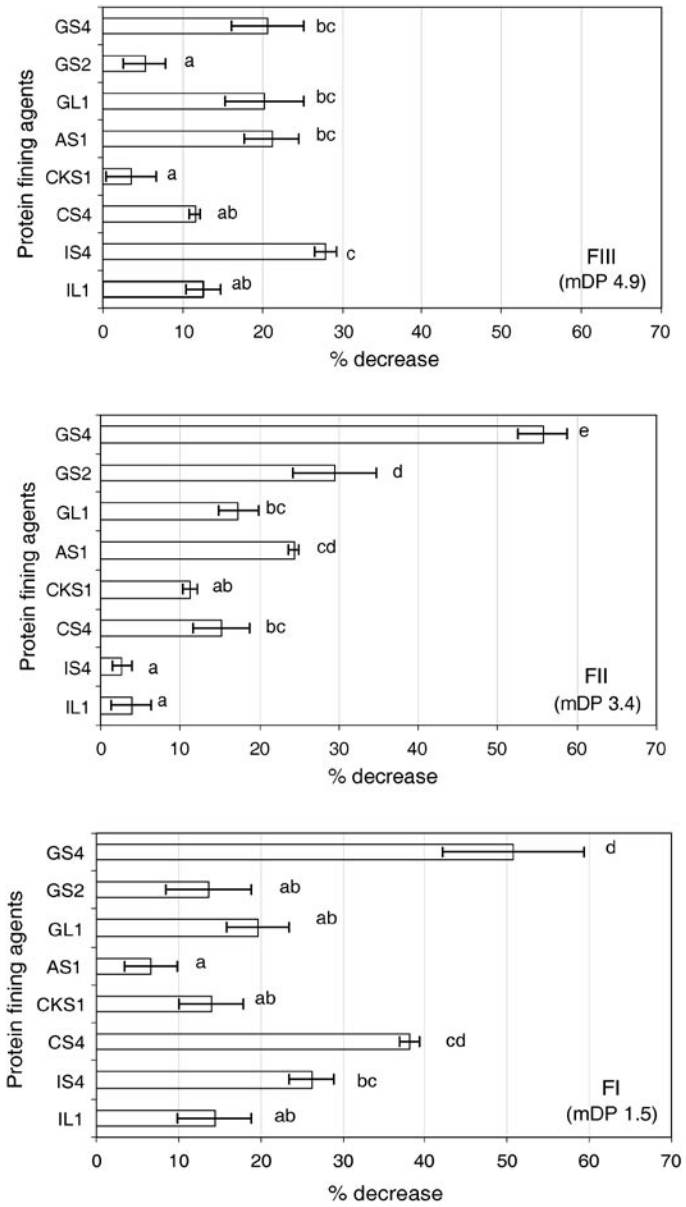

Figure 1 Decrease of the tannic fractions (%) FI, FII, and FIII, with the mean degree of polymerization (mDP) of 1.5, 3.4, and 4.9, respectively, after fining treatment with distinct proteins. Concentration of condensed tannins in unfined wine: FI (15.1 ± 0.7), FII (110.7 ± 15.1), FIII (582.2 ± 52.6), respectively. Error bars represent the standard deviation. Means within a column followed by the same letter are not significantly different (LSD, 5%). (Isinglass (IL1), isinglass (IS4), casein (CS4), potassium caseinate (CKS1), egg albumin (AS1), gelatin (GL1), gelatin (GS2), gelatin (GS4).)

Structural characterization of proanthocyanidin fractions affected by fining. The structural characteristics of wine proanthocyanidins obtained by reverse-phase HPLC of depolymerization products released by thiolysis are presented in Table 2. The mDP of the oligomeric and polymeric proanthocyanidins remaining in the fined red wine decreased in all trials (6 to 24%) compared with the unfined wine, which agrees with other studies conducted with gelatins of different molecular weights (Maury et al. 2001). These results also agree with earlier reports that the largest molecules are precipitated first (Ricardo-da-Silva et al. 1991a). This effect could be due to the higher number of phenolic rings present in more polymerized proanthocyanidins with an accompanying increase in hydrophobicity, allowing the complexes formed with proteins to be removed more effectively (Baxter et al. 1997). However, only egg albumin and swim bladder isinglass led to a significant decrease in the mDP of proanthocyanidins remaining in the fined wine (Table 2), allowing us to predict that these fining agents should selectively remove proanthocyanidins with higher mDP.

The gelatin and egg albumin fining treatments significantly decreased the percentage of galloylation (% gal) in the polymeric proanthocyanidins. The percentage of prodelphinidins (containing epigallocatechin units) within the polymeric proanthocyanidin fraction was significantly lower for all treatments, suggesting that these proteins interact selectively with epigallocatechin units. However, when gelatin GS_4 (MW < 14.4 kDa) was used, the decrease was less. These findings are in accordance with the results obtained by other authors in similar experiments (Sarni-Manchado et al. 1999). The cis:trans ratio was significantly reduced by both isinglass treatments and by potassium caseinate for the oligomeric proanthocyanidin fraction and increased by gelatin treatments for the polymeric proanthocyanidin fraction.

Quantification of some monomeric, dimeric, and trimeric flavan-3-ols affected by fining. A detailed HPLC analysis was conducted of the most important oligomeric proanthocyanidins, such as procyanidin dimers (B1, B2, B3, and B4), trimers (trimer 2 and C1), and dimer gallates (B2-3-*O*-gallate, B2-3'-*O*-gallate, and B1-3-*O*-gallate), included in the FII fraction (Table 3). The three gelatins significantly depressed all of the individual dimeric procyanidins (B1, B2, B3, and B4). In contrast, none of the individual dimeric procyanidins (B1, B2, B3, and B4)

Table 2 Structural characterization of proanthocyanidins (oligomeric and polymeric). Mean degree of polymerization (mDP), percentage of galloylation (% gal), percentage of prodelphinidins (% prodelph), average molecular mass (aMW), and *cis* to *trans* (cis:trans) ratio are reported for both unfined red wine and red wine after different fining treatments (mean ± SD).

	Oligomeric proanthocyanidins (FII)					Polymeric proanthocyanidins (FIII)				
Treatment[a]	mDP	% gal	% prodelph	aMW	cis:trans	mDP	% gal	% prodelph	aMW	cis:trans
T	3.4 ± 0.1 a[b]	13.6 ± 1.8 ab	43.5 ± 5.2 ab	1066 ± 29 a	3.9 ± 0.5 a	4.9 ± 0.0 a	19.3 ± 0.6 a	30.2 ± 2.1 a	1587 ± 1 a	2.8 ± 0.3 ab
IL_1	3.1 ± 0.1 ab	15.2 ± 0.7 b	28.1 ± 3.7 cd	995 ± 24 ab	2.0 ± 0.3 b	4.4 ± 0.6 ab	19.8 ± 1.1 a	18.1 ± 0.6 b	1430 ± 19 ab	2.4 ± 0.1 abc
IS_4	3.0 ± 0.0 b	13.2 ± 0.7 abc	33.1 ± 2.6 acd	936 ± 14 b	2.5 ± 0.2 bc	4.2 ± 0.3 bc	20.2 ± 1.3 a	19.3 ± 2.0 b	1367 ± 10 bc	2.2 ± 0.0 ac
CS_4	3.1 ± 0.0 ab	15.1 ± 1.3 b	29.0 ± 4.1 cd	983 ± 1 ab	3.1 ± 0.6 abc	4.5 ± 0.6 ab	19.5 ± 0.2 a	20.1 ± 2.5 b	1430 ± 20 ab	2.5 ± 0.3 abc
CKS_1	3.1 ± 0.3 ab	14.7 ± 0.9 b	33.0 ± 1.9 acd	990 ± 19 ab	2.7 ± 0.1 bc	4.4 ± 0.4 ab	19.5 ± 1.6 a	18.6 ± 3.7 b	1421 ± 12 ab	2.4 ± 0.2 abc
AS_1	3.2 ± 0.1 ab	12.6 ± 1.4 acd	31.9 ± 2.9 cd	1044 ± 36 ab	3.5 ± 0.8 ac	3.7 ± 0.7 c	13.3 ± 0.6 c	12.2 ± 2.1 c	1148 ± 6 c	2.4 ± 0.1 abc
GL_1	3.0 ± 0.3 ab	12.2 ± 0.3 acd	41.1 ± 4.2 abd	946 ± 21 ab	3.3 ± 0.3 abc	4.5 ± 0.1 ab	16.0 ± 0.1 b	26.0 ± 0.5 d	1397 ± 3 ab	4.3 ± 0.1 d
GS_2	3.0 ± 0.2 ab	10.9 ± 1.0 d	42.2 ± 4.2 ab	935 ± 21 ab	3.1 ± 0.9 ac	4.4 ± 0.0 ab	11.7 ± 0.4 d	25.9 ± 0.5 d	1389 ± 16 ac	3.8 ± 0.3 d
GS_4	3.2 ± 0.1 ab	11.7 ± 0.5 cd	40.3 ± 2.3 ab	1002 ± 30 ab	3.0 ± 0.4 ac	4.6 ± 0.0 ab	15.4 ± 0.3 b	26.5 ± 0.6 d	1455 ± 17 ac	4.3 ± 0.4 d

[a]Unfined (T), isinglass (IL_1), isinglass (IS_4), casein (CS_4), potassium caseinate (CKS_1), egg albumin (AS_1), gelatin (GL_1), gelatin (GS_2), and gelatin (GS_4).
[b]Means within a column followed by the same letter are not significantly different (LSD, 5%).

Table 3 (+)-Catechin, (-)-epicatechin, dimeric, trimeric, and dimeric procyanidins esterified by gallic acid analyzed by HPLC for red wine before and after different fining treatments (mean ± SD).

	Monomers		Dimers					Trimers			Dimer gallates			
Treatment[a]	(+)-Catechin (mg/L)	(-)-Epicatechin (mg/L)	B3 (mg/L)	B1 (mg/L)	B4 (mg/L)	B2 (mg/L)	∑dimeric (mg/L)	Trimer 2 (mg/L)	C1 (mg/L)	∑trimeric (mg/L)	B2-3-*O* gallate (mg/L)	B2-3'-*O*-gallate (mg/L)	B1-3-*O*-gallate (mg/L)	∑gallates (mg/L)
T	14.4 ± 1.7a[b]	2.7 ± 0.7a	5.4 ± 0.5a	31.4 ± 0.4a	11.7 ± 1.1a	11.8 ± 0.6a	60.5 ± 2.8a	5.7 ± 0.0a	1.6 ± 0.3a	7.3 ± 0.3a	6.8 ± 0.4a	1.7 ± 0.2a	1.2 ± 0.1a	9.6 ± 0.1a
IL_1	13.9 ± 0.5a	2.3 ± 0.6ab	5.5 ± 0.0a	28.6 ± 0.3b	7.9 ± 0.2cd	10.4 ± 0.3ab	52.4 ± 0.7c	3.7 ± 0.4bc	1.6 ± 0.1a	5.3 ± 0.3bc	6.4 ± 0.1ab	0.9 ± 0.1bc	0.7 ± 0.1b	8.0 ± 0.2b
IS_4	12.6 ± 0.1ab	2.0 ± 0.0ab	4.7 ± 0.1ab	31.5 ± 0.4a	9.2 ± 0.8bc	11.1 ± 0.5ab	56.5 ± 1.7b	4.7 ± 0.1ab	1.5 ± 0.0ab	6.2 ± 0.1bc	6.0 ± 0.7ab	1.0 ± 0.5abc	0.5 ± 0.0bc	7.6 ± 0.3b
CS_4	7.3 ± 0.2cd	1.7 ± 0.1b	3.5 ± 0.0c	28.3 ± 0.6b	5.7 ± 0.4e	10.8 ± 0.3ab	48.2 ± 1.2d	2.3 ± 0.3c	1.5 ± 0.2ab	3.8 ± 0.5cd	5.2 ± 0.3bc	1.5 ± 0.5ab	0.5 ± 0.1bc	7.2 ± 0.7b
CKS_1	13.0 ± 1.0a	2.7 ± 0.1a	3.8 ± 0.1c	30.2 ± 0.3a	6.0 ± 0.1de	10.7 ± 0.9ab	50.7 ± 1.3cd	3.3 ± 0.2bc	1.7 ± 0.6a	4.9 ± 0.8bc	6.4 ± 0.4ab	1.3 ± 0.2abc	0.7 ± 0.3b	8.3 ± 0.9ab
AS_1	14.4 ± 0.2a	2.4 ± 0.1ab	5.3 ± 0.2a	31.6 ± 0.4a	10.5 ± 0.3ab	11.6 ± 0.4a	58.9 ± 0.5ab	5.7 ± 0.6a	1.5 ± 0.2ab	7.2 ± 0.4ab	6.5 ± 0.2ab	1.0 ± 0.0bc	0.7 ± 0.0b	8.3 ± 1.2ab
GL_1	8.6 ± 0.2c	2.3 ± 0.2ab	3.5 ± 0.1c	28.2 ± 0.6b	5.3 ± 0.4e	10.0 ± 0.3b	47.0 ± 3.4de	4.3 ± 0.8ab	1.3 ± 0.1ab	5.5 ± 1.9bc	5.7 ± 1.0ab	1.6 ± 0.2ab	0.3 ± 0.1c	7.6 ± 0.8b
GS_2	10.9 ± 0.1b	2.5 ± 0.3a	4.4 ± 0.1bc	22.5 ± 0.4d	4.9 ± 0.7e	7.8 ± 0.0c	39.5 ± 0.0f	3.9 ± 0.1b	1.0 ± 0.0b	4.8 ± 0.2d	4.0 ± 0.2c	0.7 ± 0.4c	0.3 ± 0.0c	5.0 ± 0.2c
GS_4	5.9 ± 1.1d	2.3 ± 0.2ab	2.5 ± 0.3d	25.5 ± 0.1c	5.4 ± 0.4e	9.8 ± 0.1b	43.2 ± 0.7ef	3.3 ± 0.2bc	1.2 ± 0.3ab	4.5 ± 0.5cd	5.8 ± 0.7ab	1.4 ± 0.1ab	0.2 ± 0.0c	7.5 ± 1.1b

[a]Unfined (T), isinglass (IL_1), isinglass (IS_4), casein (CS_4), potassium caseinate (CKS_1), egg albumin (AS_1), gelatin (GL_1), gelatin (GS_2), and gelatin (GS_4).
[b]Means within a column followed by the same letter are not significantly different (LSD, 5%).

Table 4 Total pigments (TP), color intensity (CI), hue (H), colored anthocyanins (CA), polymerized pigments (PP), and total anthocyanins (TA) for both unfined red wine and red wine after different fining treatments (mean ± SD).

Treatment[a]	TP (AU)	CI (AU)	H (AU)	CA (AU)	PP (AU)	TA (mg/L)
T	39.76 ± 0.09 a[b]	25.74 ± 0.11 a	0.442 ± 0.01 a	13.03 ± 0.09 ab	3.21 ± 0.04 a	701 ± 3 a
IL_1	39.49 ± 0.04 b	24.80 ± 1.02 ab	0.436 ± 0.01 ab	12.62 ± 0.92 abc	3.09 ± 0.08 abc	699 ± 1 a
IS_4	39.44 ± 0.07 b	24.64 ± 0.43 ab	0.435 ± 0.00 ab	12.56 ± 0.26 abc	3.07 ± 0.03 bcd	699 ± 2 a
CS_4	38.78 ± 0.08 c	23.34 ± 1.31 b	0.442 ± 0.00 a	11.81 ± 0.81 bc	2.95 ± 0.10 de	677 ± 2 c
CKS_1	38.83 ± 0.07 c	24.07 ± 0.71 ab	0.438 ± 0.01 a	12.22 ± 0.38 ac	2.96 ± 0.07 cde	681 ± 2 b
AS_1	39.44 ± 0.07 b	25.60 ± 0.02 a	0.424 ± 0.00 b	13.43 ± 0.03 ab	2.96 ± 0.01 cde	699 ± 1 a
GL_1	38.53 ± 0.07 d	23.40 ± 1.43 b	0.442 ± 0.00 a	11.81 ± 1.00 bc	2.90 ± 0.04 e	698 ± 2 a
GS_2	39.44 ± 0.07 b	24.77 ± 1.01 ab	0.442 ± 0.00 a	12.20 ± 1.08 abc	3.21 ± 0.06 a	697 ± 1 a
GS_4	38.80 ± 0.04 c	23.51 ± 0.31 b	0.442 ± 0.00 a	11.49 ± 0.28 c	3.11 ± 0.04 ab	638 ± 0 d

[a]Unfined (T), isinglass (IL_1), isinglass (IS_4), casein (CS_4), potassium caseinate (CKS_1), egg albumin (AS_1), gelatin (GL_1), gelatin (GS_2), and gelatin (GS_4).
[b]Means within a column followed by the same letter are not significantly different (LSD, 5%).

were lowered significantly by addition of egg albumin. Of the individual trimeric procyanidins (trimer 2 and C1), only gelatin characterized by a polydispersion >43.0 kDa caused a significant decrease in trimer C1. A significant decrease of the three dimeric procyanidins esterified by gallic acid (B2-3-*O*-gallate, B2-3'-*O*-gallate, and B1-3-*O*-gallate) was only seen with gelatin that had a polydispersion >43.0 kDa.

In general, gelatins were the fining agents that most decreased total dimeric (22–35%) and trimeric procyanidins (25–38%), which is in agreement with the results obtained for the oligomeric flavan-3-ol (fraction FII). The effects of casein and potassium caseinate on the amount of total trimeric procyanidins were also important. These concentrations were decreased by 48% and 33%, respectively (Table 3).

Some other studies have shown that tannins esterified by gallic acid seem to complex more easily with proteins (Sarni-Manchado et al. 1999, Maury et al. 2001). Swim bladder isinglass and egg albumin resulted in a greater decrease in the amount of total dimeric procyanidins esterified by gallic acid (21% and 14%, respectively) compared with the corresponding nongalloylated procyanidins (dimeric 7% and 3%, respectively, and trimeric 15% and 1%, respectively). The gelatin with a polydispersion >43.0 kDa also showed a greater effect on this type of molecule, producing a decrease of ~48%, while the reduction in the amount of total dimeric and total trimeric procyanidins was 34%. Nevertheless, this tendency was not observed for all protein fining agents assayed (Table 3).

For the monomeric [(+)-catechin and (-)-epicatechin] flavan-3-ols, the protein fining agents promoted a greater decrease in (+)-catechin than in (-)-epicatechin. No fining agent other than casein decreased (-)-epicatechin significantly. The (+)-catechin was significantly lowered by casein and by the three gelatins tested. Although casein and potassium caseinate presented similar electrophoretic patterns, their affinity to these isomers was different. Casein induced a significant decrease in both isomers that was not observed with potassium caseinate.

Color and pigments. Color intensity and molecules related to wine color (mainly colored anthocyanins and total and polymeric pigments) are less affected by fining with protein agents than are the tannins. However, the addition of casein and gelatins characterized by a polydispersion <43.0 kDa significantly decreased color intensity, whereas the hue remained unchanged after the addition of all the protein fining agents, with the exception of egg albumin. These results are in line with the findings of others (Versari et al. 1998, Lovino et al. 1999) (Table 4).

The gelatin with polydispersion at low MW (GL_1) was the only fining agent that promoted a significant decrease in colored anthocyanins. The three gelatins tested showed different effects on the polymeric pigments. The results reveal that gelatin GL_1 (MW < 43 kDa) induced a significant decrease in polymeric pigments, but such a decrease was not observed for gelatin GS_4 (MW < 14.4 kDa) and GS_2 (MW > 43 kDa) (Table 4).

Conclusion

Results of fined red wine showed that each protein fining agent presented a distinct interaction and precipitation capacity in respect to the different condensed tannin fractions. Even proteins of the same general type can have quite different effects on the various tannic fractions. Gelatin characterized by a polydispersion <43.0 kDa brought about a similar decrease in all three flavan-3-ol fractions. However, gelatin characterized by a polydispersion >43.0 kDa did not remove the polymeric proanthocyanidins and monomeric flavan-3-ol

significantly, while gelatin GS_4 (MW < 14.4 kDa) significantly removed the various tannic fractions (monomers, oligomers, and polymers). Fish swim bladder isinglass (IS_4) showed an affinity for polymeric (mDP = 4.9) and monomeric (mDP ≈ 1.5) proanthocyanidins, while egg albumin (MW ~43.0 kDa) showed an affinity for polymeric (mDP = 4.9) and oligomeric (mDP = 3.4) proanthocyanidins. Casein (MW ≈ 30.0 kDa) selectively removes monomeric flavan-3-ol (mDP ≈ 1.5). Results indicate that the use of a particular fining protein can lead to the reduction of a condensed tannin fraction with a specific mean degree of polymerization.

Gelatin GS_4 (MW < 14.4 kDa) significantly decreased color intensity and colored anthocyanins. Also, color intensity and molecules related to wine color can be selectively decreased by specific fining proteins. These results suggest that the enologist's choice of protein fining agent for clarification and for the reduction of particular phenolic compounds is important and should be very carefully considered.

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