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Interactions between protein fining agents and proanthocyanidins in white wine

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Abstract

A comparative fining trial was conducted in a laboratory scale to study the influence of protein fining agents on proanthocyanidins, colour and browning potential of white wine. The monomeric flavanols were significantly depleted by casein, and gelatine with low molecular weight (MW) distribution, and isinglass obtained from fish swim bladder (MW > 94.0, containing some bands in the range 94.0–43.0 and at 20.1 kDa). However, the other gelatines and isinglass with a MW polydispersion below 20.1 kDa did not interact significantly ($P < 0.05$) with these compounds. In contrast, the oligomeric compounds were not decreased by swim bladder isinglass. It was also observed that neither of the isinglasses decreased the polymeric flavanols significantly ($P < 0.05$). Although casein and potassium caseinate had similar MW distributions and isoelectric points, potassium caseinate decreased the polymeric flavanols, whereas casein did decrease monomeric, oligomeric and polymeric flavanols significantly ($P < 0.05$). The degree of polymerisation of polymeric proanthocyanidins that remained in the fined wine decreased significantly ($P < 0.05$) after addition of protein fining agents except when potassium caseinate was used. Casein, potassium caseinate and swim bladder isinglass induced a significant ($P < 0.05$) decrease in wine colour (A_{420nm}), a decrease in browning potential and a decrease in turbidity.

Keywords: White wine; Fining; Protein; Fining agents; Polyphenols; Proanthocyanidins; Thiolysis; Turbidity; Colour; Browning

1. Introduction

Proteins have been used in white wine as fining agents for a long time. The various protein fining agents can behave differently, depending on their composition, their origin and their preparation condition. Nowadays, a wide range of protein fining agents are used, including: gelatine, casein, potassium caseinate, egg albumin or isinglass and, more recently some proteins of vegetable origin. In white wine, fining is frequently employed for clarification and/or for improved stabilisation.

The browning of white wine, is a process related to oxidation and represents an important stability problem in white wine. The presence of large quantities of phenolic compounds enhances susceptibility to oxidation, leading to a decrease of the wine's visual and sensory qualities. This is due primarily to the oxidation of phenolic compounds including catechins, proanthocyanidins and hydroxycinnamic acids present in the wine. Barroso, López-Sánchez, Otero, Cela, and Pérez-Bustamente (1989) established a link between susceptibility to browning and the quantity of phenolic compounds present. Spagna, Barbagallo, and Pifferi (2000), therefore recommended the removal of polyphenols to stabilise white wines and reduce the potential for browning. Browning in white wines is usually minimised by the addition of potassium caseinate, which is a

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very effective fining agent for polyphenols (Amati, Galassi, & Spinabelli, 1979; Manfredini, 1989).

The comparative effects of other fining agents such as gelatine, isinglass, potassium caseinate and casein, on the phenolic compounds of white wine have been studied by several authors (Amati et al., 1979; Castino, 1992; Fischerleitner, Wendelin, & Eder, 2002; Fischerleitner, Wendelin, & Eder, 2003; Gorinstein et al., 1993; Jouve et al., 1989; Machado-Nunes, Laureano, & Ricardo-da-Silva, 1998; Sims, Eastridge, & Bates, 1995). All these studies have focussed attention on the wine phenolic composition, but not on characterising the protein fining agents. Furthermore, as far as we can determine there is a lack of information on the structural characteristics (mean degree of polymerisation, galloylation, cis/trans ratio and the percentage of prodelphinidins) of oligomeric and polymeric proanthocyanidins remaining in white wine after fining as a function of the type of fining protein added. A better knowledge of all the molecules involved in fining could lead to an enhanced control and thus to an optimisation of this treatment.

The main goal of this study was, therefore to undertake a comparative study on the effect of eight commercial protein fining agents [gelatine (x3), isinglass (x2), casein (x1), potassium caseinate (x1) and egg albumin (x1)] on the structural characteristics of proanthocyanidins, as well as on the monomeric flavan-3-ol, and also on flavonoid and non-flavonoid phenolic compounds, chromatic characteristics, turbidity and browning potential of white wine after fining.

2. Materials and methods

2.1. Reagents

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

2.2. Protein fining agents

The fining agents previously characterised by Cosme, Ricardo-da-Silva, and Laureano (2007) were used in this work: one egg albumin (AS_1), two isinglasses (IL_1, IS_4), one potassium caseinate (CKS_1), one casein (CS_4) and three gelatines (GL_1, GS_2 and GS_4) (Table 1).

Table 1
Fining agents employed in this study

Fining agents	Code	Concentration	Producer information
Isinglass	IL_1	50 mL/hL	Collagen hydrolysis from fish skin
Isinglass	IS_4	2.25 g/hL	From fish swim bladder
Casein	CS_4	40 g/hL	–
Potassium caseinate	CKS_1	40 g/hL	–
Egg albumin	AS_1	12.5 g/hL	–
Gelatine	GL_1	50 mL/hL	High concentration
Gelatine	GS_2	8 g/hL	–
Gelatine	GS_4	8 g/hL	High degree of hydrolysis

2.3. Fining experiments

Young white wine of vintage 2004 was used in this study made from various white grapevine varieties (all *Vitis vinifera*, L.) from the Estremadura Region, Portugal. It presented the following characteristics: alcohol content 12.0% (v/v), density (ρ_{20}) 0.9961 g/cm^3, titratable acidity 6.8 g/L (expressed as tartaric acid), volatile acidity 0.36 g/L (expressed as acetic acid), pH 3.41, free sulphur dioxide 9 mg/L and total sulphur dioxide 48 mg/L.

Experiments involved the addition of standard quantities of the protein fining agents (isinglass, casein, potassium caseinate and gelatine) prepared as suggested by the manufacturers (Table 1). The trials were conducted at laboratory scale in 250 mL volumes of wine. Untreated wine was used as control. The fining agents were thoroughly mixed and allowed to remain in contact with the wines for 7 days at 20 °C, the samples were then centrifuged at 537.6 g for 15 min before analysis. All experiments were duplicated.

2.4. Phenolic compounds analysis

2.4.1. Separation of proanthocyanidins according to degree of polymerisation by C_{18} Sep-Pak cartridges and determination of the flavan-3-ol content by the vanillin assay

The separation of flavanols was performed using a C_{18} Sep-Pak cartridge (Waters, Milford, Ireland) according to the degree of polymerisation in three fractions FI (monomeric), FII (oligomeric) and FIII (polymeric) in line with the method described by Sun, Leandro, Ricardo-da-Silva, and Spranger (1998a). Quantification of the total flavan-3-ol in each fraction was carried out using the vanillin assay according to the method described by Sun et al. (1998a) and by Sun, Ricardo-da-Silva, and Spranger (1998b). For the FI fraction, the absorbance at 500 nm was read after a reaction with vanillin at 30 °C for 15 min using a Unicam UV–vis UV4 spectrophotometer (Unicam, Cambridge, UK). For the FII and FIII fractions the reaction was at room temperature and left until the maximum absorbance value at 500 nm was achieved (approximately between 20 and 35 min). Quantification was carried out by means of standards curves prepared from monomers (FI), oligomers (FII), and polymers of flavan-3-ol (FIII) isolated from grape seeds, as described earlier (Sun et al., 1998a, 1998b; Sun, Spranger, Roque-do-Vale, Leandro, & Belchior, 2001).

2.4.2. Characterisation of wine proanthocyanidins (fractionated by C_{18} Sep-Pak cartridges) by acid-catalysed depolymerisation in the presence of toluene-α-thiol followed by reversed-phase HPLC analysis

The proanthocyanidins were depolymerised in the presence of a nucleophilic agent (toluene-α-thiol) in an acid

medium. Depolymerisation allows the distinction between terminal units, which are released as flavan-3-ols, and extension units released as their benzyl thioethers (Maury, Sarni-Manchado, Lefebvre, Cheynier, & Moutounet, 2001; Souquet, Cheynier, & Moutounet, 2000). Reversed-phase HPLC analysis of the products formed allows determination of the structural composition of proanthocyanidins, which are characterised 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 polymerisation (mDP), the average molecular mass (mM), the cis:trans ratio, the fraction of prodelphinidins (prodelph%) and the fraction of galloylation (gal%) (Kennedy, Matthews, & Waterhouse, 2000; Prieur, Rigaud, Cheynier, & Moutounet, 1994; Ricardo-da-Silva, Rigaud, Cheynier, Cheminat, & Moutounet, 1991b; Rigaud, Perez-Ilzarbe, Ricardo-da-Silva, & Cheynier, 1991).

To carry out the acid-catalysed degradation, 100 μL of sample were placed in a glass tube with a hermetic seal together with 100 μL of a solution of toluene-α-thiol in methanol containing HCl (0.2 M). After closing, the mixture was mixed gently and incubated at 55 °C for 7 min by which time the depolymerisation yield was around 70% (Monagas, Gómez-Cordovés, Bartolomé, Laureano, & Ricardo-da-Silva, 2003). The thiolysed sample was cooled and then analysed by reversed-phase HPLC. The HPLC system used included a Waters 2487 dual λ absorbance detector set at 280 nm, and a Merck Hitachi Intelligent pump model L-6200A (Tokyo, Japan), coupled to a Konikrom data chromatography treatment system version 6.2 (Konik Instruments, Konik-Tech, Barcelona, Spain). The column was a reversed-phase C_{18}Lichrosphere 100 (250 mm × 4.6 mm, 5 μm) (Merck, Darmstadt, Germany), and the separation was performed at room temperature. The elution condition 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 linear from 0 to 40 min 30–50% B linear from 40 to 60 min, 50–80% B linear from 60 to 70 min, followed by washing (acetonitrile/formic acid/water 80/2/18, v/v/v) and reconditioning of the column from 75 to 97 min. The amounts of monomers (terminal units) and toluene-α-thiol adducts (extension units) released from the depolymerisation reaction in the presence of toluene-α-thiol, were calculated from the areas of the chromatographic peaks at 280 nm by comparison with calibration curves (Kennedy et al., 2000; Prieur et al., 1994; Rigaud et al., 1991).

2.4.3. Separation of monomeric and small oligomeric flavan-3-ols (dimers and trimers) by polyamide column chromatography and quantification by HPLC analysis

Procyanidins separation was performed according to Ricardo-da-Silva, Rosec, Bourzeix, and Heredia (1990). The HPLC system used was the same as that employed for the HPLC analysis of the products released by acid-catalysed depolymerisation in the presence of toluene-α-thiol. The elution conditions for monomeric flavan-3-ols were as follows: 0.9 mL/min flow rate, solvent A (distilled water/acetic acid, 97.5/2.5, v/v), solvent B (acetonitrile/solvent A 80/20, v/v), 7–25% B linear from 0 to 31 min followed by washing (methanol/distilled water, 50/50, v/v) from 32 to 50 min and reconditioning of the column from 51 to 65 min under initial gradient conditions. The elution conditions for oligomeric procyanidins (dimeric and trimeric) were as follows: 1.0 mL/min, flow rate, solvent A (distilled water), solvent B (distilled water/acetic acid 90/10, v/v), 10–70% B linear from 0 to 45 min, 70–90% B linear from 45 to 70 min, 90% B isocratic from 70 to 82 min, 90–100% B linear from 82 to 85 min, 100% B isocratic from 85 to 90 min, followed by washing (methanol/distilled 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 (Dallas, Ricardo-da-Silva, & Laureano, 1995; Dallas, Ricardo-da-Silva, & Laureano, 1996a; Dallas, Ricardo-da-Silva, & Laureano, 1996b; Ricardo-da-Silva et al., 1990) of monomeric flavan-3-ols and oligomeric procyanidins (dimeric and trimeric) was carried out.

2.4.4. Quantification of flavonoid phenols and non-flavonoid phenols

Determination of the phenol content of the wines was carried out using the absorbance at 280 nm before and after precipitation of the flavonoids through reaction with formaldehyde, according to Kramling and Singleton (1969), leading to a quantification of flavonoid, non-flavonoid and total phenols in the wines.

2.5. Turbidity

Turbidity was evaluated by measuring the optical density at 650 nm before and after centrifugation as described by Feuillat and Bergeret (1966).

2.6. Test for browning potential

Test tubes were filled to 75% with the wine to be tested. Controls were sparged thoroughly with nitrogen and test samples sparged with oxygen. All tubes were sealed hermetically and maintained at 55 °C for 5 days. This test was conducted on treated and untreated wine and allows calculation of the difference of browning values measuring the increase in A_{420nm} as recommended by Singleton and Kramling (1976).

2.7. Chromatic characterisation

The absorption spectra of the wine samples were recorded with a Unicam UV–vis UV4 spectrophotometer (Unicam, Cambridge, UK), scanned over the range 380–770 nm using quartz cells of 1-cm path length. Data were collected at 10 nm intervals, and referenced to 1-cm path length, to calcu-

late L^* (lightness), a^* (measure of redness), b^* (measure of yellowness), coordinates using the CIELab method (OIV, 2006). The spectrophotometer incorporates the software required to calculate the CIELab parameters directly (Chroma version 2.0 Unicam, Cambridge, United Kingdom). The Chroma $[C^* = [(a^*)^2 + (b^*)^2]^{1/2}]$ and the hue-angle $[h^o = \text{tang}^{-1}(a^*/b^*)]$ were also calculated. To differentiate the colour more precisely, the colour difference was obtained using the following expression: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$, in CIELab units. It quantifies the overall colour difference of a sample when compared to a reference sample (untreated sample). Two colours can be distinguished by the human eye when the difference between ΔE^* values is greater than two units (Spagna et al., 1996).

2.8. Colour

Colour was determined by measuring absorbance at 420 nm (10-mm cell) using a Unicam UV–vis UV4 spectrophotometer (Unicam, Cambridge, UK) in line with OIV (2006).

2.9. Analysis of conventional oenological parameters

Alcohol content (v/v%), pH, density, titratable and volatile acidities, free and total sulphur dioxide were measured according to Organisation International de la Vigne et du Vin methods (OIV, 2006).

2.10. Statistical analysis

The data are presented as means ± SD. One-way analysis of variance and comparison of treatment means (LSD, 5% level) were performed using ANOVA Statistica 6.1 software (StatSoft, Tulsa, OK, USA) in respect of the effect of protein fining agents.

3. Results and discussion

The physico-chemical characteristics of the fining agents used in this study are summarised in Table 2, and the structural characteristics of the unfined wine proanthocyanidins are presented on the first lines of Tables 3–5.

The mDP of the fraction FI, the "monomeric fraction", was close to 1.5. The mDP of the monomeric fraction should be 1, but the FI fraction also includes two unknown compounds as shown by Sun et al. (1998a). It is probable that very few oligomeric proanthocyanidins pass through the C_{18} Sep-Pak during separation.

3.1. Effect of the fining agents on the flavan-3-ol fractions

The fining agents that removed the monomeric flavanols (fraction FI) most strongly were casein (46%), gelatine with low molecular weight distribution (GS_4 –31%) and swim bladder isinglass (IS_4 – 28%). Casein and potassium caseinate showed an electrophoretic profile with similar MW distribution (MW ≈ 30.0 kDa) (Cosme et al., 2007). However, their affinity for monomeric flavanols was different. Only casein lowered these compounds significantly, whereas this effect was not observed for potassium caseinate. The two isinglasses (IL_1, IS_4) also showed different behaviours in relation to the monomeric flavanols. Of these two proteins, only the isinglass obtained from fish swim bladder decreased these compounds significantly (Table 3).

Table 3
Monomeric flavanols (FI), oligomeric proanthocyanidins (FII) and polymeric proanthocyanidins (FIII) for both unfined white wine and white wine after different fining treatments (mean ± SD)

Fining treatment	F1 (mg/L)	FII (mg/L)	FIII (mg/L)
T	5.3 ± 0.1a	35.1 ± 0.4a	82.8 ± 0.5a
IL_1	4.2 ± 0.1abc	15.8 ± 0.8d	81.8 ± 0.9a
IS_4	3.8 ± 0.2bc	27.9 ± 2.5abc	81.8 ± 0.9a
CS_4	2.9 ± 0.2c	21.1 ± 2.5cd	42.9 ± 0.5c
CKS_1	4.2 ± 0.3abc	32.6 ± 3.1ab	62.8 ± 4.8b
AS_1	4.7 ± 0.2ab	27.8 ± 3.4bc	39.2 ± 2.9cd
GL_1	4.7 ± 0.3ab	24.9 ± 1.7c	35.1 ± 3.8d
GS_2	4.5 ± 0.2ab	25.4 ± 3.9c	35.1 ± 6.3d
GS_4	3.6 ± 0.7bc	21.2 ± 3.5cd	34.5 ± 2.9d

Unfined (T), isinglass (IL_1), isinglass (IS_4), casein (CS_4), potassium caseinate (CKS_1), egg albumin (AS_1), gelatine (GL_1), gelatine (GS_2), gelatine (GS_4). Means ($n = 2$) within a column followed by the same letter are not significantly different (LSD, 5%).

Table 2
Physico-chemical characteristics of the protein fining agents employed on the fining trial (Cosme et al., 2007)

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

Isinglass (IL_1), isinglass (IS_4), casein (CS_4), potassium caseinate (CKS_1), egg albumin (AS_1), gelatine (GL_1), gelatine (GS_2), gelatine (GS_4). k – Multiplication factor, which was 6.68 for egg albumin; 6.25 for isinglass; 6.38 for casein and potassium caseinate; 5.55 for gelatine.

[a] Mean values of three determinations ± standard deviation (SD).

Table 4
Structural characterisation of proanthocyanidins (oligomeric and polymeric), mean degree of polymerisation (mDP), fractions of galloylation (%), fractions of prodelphinidins (% prodelph), average molecular mass (mM) and the cis/trans (cis:trans) ratio for both unfined white wine and white wine after different fining treatments (mean ± SD)

Fining treatment	Oligomeric proanthocyanidins (FII)					Polymeric proanthocyanidins (FIII)				
	mDP	%gal	%prodelph	mM	cis:trans	mDP	%gal	% prodelph	mM	cis:trans
T	2.9 ± 0.2a	12.0 ± 0.8ab	26.9 ± 4.5a	889 ± 63a	2.5 ± 0.2a	3.8 ± 0.2a	13.1 ± 0.0ab	18.6 ± 3.4a	1200 ± 51a	3.1 ± 0.3a
IL_1	2.2 ± 0.0b	13.3 ± 0.2b	25.8 ± 0.9ab	694 ± 25b	2.0 ± 0.1b	3.0 ± 0.2b	6.4 ± 0.3c	15.4 ± 3.0ab	926 ± 60b	2.4 ± 0.0b
IS_4	2.6 ± 0.3ab	12.0 ± 0.1ab	11.8 ± 2.3c	815 ± 86ab	2.4 ± 0.2ab	3.1 ± 0.2b	9.5 ± 1.7ac	17.5 ± 0.4ab	946 ± 63b	2.3 ± 0.1b
CS_4	2.7 ± 0.4a	11.0 ± 1.3ac	16.8 ± 5.9bc	831 ± 123ab	2.4 ± 0.3ab	3.1 ± 0.4b	8.5 ± 1.4c	15.9 ± 4.6ab	940 ± 110b	2.7 ± 0.5ab
CKS_1	2.7 ± 0.1a	9.2 ± 2.3c	14.2 ± 1.8c	812 ± 35ab	2.5 ± 0.0a	3.4 ± 0.3ab	9.9 ± 1.2ac	20.0 ± 1.1a	1021 ± 80ab	2.5 ± 0.1ab
AS_1	2.6 ± 0.0ab	13.0 ± 1.3ab	15.7 ± 6.0bc	821 ± 5ab	2.5 ± 0.2a	2.8 ± 0.4b	14.5 ± 1.8b	11.1 ± 2.4b	861 ± 103b	2.5 ± 0.3ab
GL_1	2.8 ± 0.0a	12.9 ± 0.6ab	16.7 ± 0.5bc	884 ± 12a	2.7 ± 0.2ac	3.0 ± 0.3b	14.4 ± 2.1b	15.8 ± 4.4ab	952 ± 103b	2.9 ± 0.7ab
GS_2	2.7 ± 0.0a	12.5 ± 0.3ab	13.2 ± 2.2c	827 ± 11ab	2.1 ± 0.2b	3.1 ± 0.1b	9.4 ± 1.7c	18.4 ± 2.4a	955 ± 35b	2.8 ± 0.6ab
GS_4	2.8 ± 0.2a	11.9 ± 0.5ab	16.3 ± 7.4bc	876 ± 73a	3.0 ± 0.1c	2.9 ± 0.3b	15.4 ± 2.5b	14.4 ± 0.7ab	917 ± 92b	2.2 ± 0.1b

Unfined (T), isinglass (IL_1), isinglass (IS_4), casein (CS_4), potassium caseinate (CKS_1), egg albumin (AS_1), gelatine (GL_1), gelatine (GS_2) and gelatine (GS_4). Means ($n = 2$) within a column followed by the same letter are not significantly different (LSD, 5%).

Table 5
Monomeric flavan-3-ols, dimeric, trimeric and dimeric procyanidins esterified by gallic acid, analysed by HPLC for both unfined white wine and for white wine after different fining treatments (mean ± SD)

Fining treatment	Monomers		Dimers				∑dimeric (mg/L)	Trimers		∑trimeric (mg/L)	Dimer gallates			∑galates (mg/L)
	(+) Catechin (mg/L)	(−) Epicatechin (mg/L)	B3 (mg/L)	B1 (mg/L)	B4 (mg/L)	B2 (mg/L)		T2 (mg/L)	C1 (mg/L)		B2-3-O gallate (mg/L)	B2-3′-O-gallate (mg/L)	B1-3-O-gallate (mg/L)	
T	5.6 ± 0.1a	1.7 ± 0.1ab	2.2 ± 0.2a	9.4 ± 0.1a	1.5 ± 0.1a	2.7 ± 0.1a	15.76 ± 0.14a	3.1 ± 0.0a	1.1 ± 0.0a	4.11 ± 0.04a	0.5 ± 0.1a	0.2 ± 0.1a	0.3 ± 0.1a	1.04 ± 0.24a
IL_1	4.4 ± 0.1b	1.3 ± 0.0ac	1.4 ± 0.0d	5.4 ± 0.1d	1.4 ± 0.0a	1.5 ± 0.0cd	9.73 ± 0.02c	1.1 ± 0.0f	0.4 ± 0.0d	1.52 ± 0.04e	0.2 ± 0.0d	0.2 ± 0.0ab	0.2 ± 0.0b	0.53 ± 0.03c
IS_4	4.2 ± 0.1c	1.1 ± 0.0c	1.7 ± 0.3cd	7.0 ± 0.1bc	1.1 ± 0.2bc	2.1 ± 0.1b	11.87 ± 0.54b	3.1 ± 0.1a	0.9 ± 0.2ab	3.98 ± 0.22a	0.4 ± 0.0ab	0.2 ± 0.0a	0.3 ± 0.1a	0.94 ± 0.12ab
CS_4	3.4 ± 0.0f	1.4 ± 0.2abc	1.9 ± 0.1abc	7.5 ± 0.5b	0.9 ± 0.1bc	1.8 ± 0.3bc	12.11 ± 1.05b	1.4 ± 0.0de	0.6 ± 0.0cd	1.96 ± 0.05cd	0.3 ± 0.0cd	0.1 ± 0.1d	0.2 ± 0.0ab	0.53 ± 0.11c
CKS_1	4.4 ± 0.0b	1.1 ± 0.1ac	2.2 ± 0.0ab	8.9 ± 0.3a	1.2 ± 0.1ab	2.4 ± 0.1a	14.62 ± 0.51a	3.0 ± 0.1a	0.8 ± 0.1abc	3.77 ± 0.21a	0.5 ± 0.1a	0.2 ± 0.0abc	0.3 ± 0.0a	0.95 ± 0.11ab
AS_1	4.5 ± 0.1b	1.7 ± 0.1abc	1.8 ± 0.2cd	7.0 ± 0.5bc	0.9 ± 0.1bc	2.0 ± 0.2b	11.75 ± 0.99b	1.5 ± 0.1cd	0.8 ± 0.2abc	2.32 ± 0.34bc	0.4 ± 0.1ab	0.1 ± 0.0d	0.2 ± 0.1ab	0.70 ± 0.22bc
GL_1	3.6 ± 0.1d	1.7 ± 0.1ab	1.8 ± 0.2bc	7.0 ± 0.5bc	0.9 ± 0.2bc	2.0 ± 0.2b	11.80 ± 1.07b	1.8 ± 0.2b	0.8 ± 0.0abc	2.58 ± 0.22b	0.4 ± 0.0abc	0.1 ± 0.0cd	0.2 ± 0.0ab	0.71 ± 0.02bc
GS_2	3.1 ± 0.0g	1.2 ± 0.0ac	1.6 ± 0.1cd	6.8 ± 0.1c	1.0 ± 0.0bc	2.0 ± 0.0b	11.35 ± 0.12b	1.7 ± 0.0bc	0.7 ± 0.2bc	2.42 ± 0.18b	0.4 ± 0.1bc	0.1 ± 0.0bcd	0.2 ± 0.1ab	0.65 ± 0.13bc
GS_4	1.7 ± 0.0h	1.4 ± 0.0abc	1.5 ± 0.0d	5.1 ± 0.2d	0.8 ± 0.1c	1.5 ± 0.1d	8.87 ± 0.11c	1.2 ± 0.1ef	0.7 ± 0.2bcd	1.82 ± 0.20de	0.3 ± 0.0bcd	0.1 ± 0.0d	0.2 ± 0.1ab	0.57 ± 0.10c

Unfined (T), isinglass (IL_1), isinglass (IS_4), casein (CS_4), potassium caseinate (CKS_1), egg albumin (AS_1), gelatine (GL_1), gelatine (GS_2) and gelatine (GS_4). Means ($n = 2$) within a column followed by the same letter are not significantly different (LSD, 5%).

In the case of oligomeric flavanols (fraction FII, mDP = 2.9) the greatest decrease was observed with isinglass IL_1 (55%), gelatine with low molecular weight distribution (GS_4 – 40%) and casein (CS_4 – 40%). Isinglass (IL_1) and gelatine (GS_4) were characterised by a polydispersion of the low molecular weights (<20.1 kDa). For the oligomeric flavanols, casein and potassium caseinate, despite the similarity of their electrophoretic profiles (MW ≈ 30.0 kDa) (Cosme et al., 2007), their affinities for these compounds were quite different. Again, casein decreased these compounds significantly. Isinglass with MW distributions below 20.1 kDa (IL_1) decreased these compounds significantly but no statistical differences were observed with swim bladder isinglass (IS_4) (Table 3).

The polymeric flavanols (fraction FIII, mDP = 3.8) were decreased significantly by the three gelatines (58%). Neither of the isinglasses decreased the concentration of these compounds significantly (1%). Bonerz et al. (2004) observed that a proteinaceous fining agent extracted from fish skin selectively removed proanthocyanidins with lower mDP. Casein (48%) decreased these compounds more than the twice as effectively as potassium caseinate (24%) (Table 3).

3.2. Effect of the fining agents on the structural characteristics of proanthocyanidin fractions

The data regarding the structural characteristics of wine proanthocyanidins obtained by reversed phase HPLC of the depolymerisation products released by thiolysis are presented in Table 4.

Fining with protein fining agents lowered the mDP of oligomeric and polymeric proanthocyanidins remaining in fined white wine compared to the unfined wine. These results are in accordance with previous reports, which suggest that the largest proanthocyanidin molecules are precipitated first in fining experiments (Ricardo-da-Silva et al., 1991a). This effect could be due to the higher number of phenolic rings present in the more polymerised proanthocyanidins with an increase in hydrophobicity, rendering their complexes more effectively removed (Baxter, Lilley, Haslam, & Williamson, 1997). Nevertheless, wine fined with potassium caseinate did not show statistical differences in mDP for the polymeric proanthocyanidins remaining in the fined wine. In contrast, only isinglass characterised by a polydispersion below 20.1 kDa brought about a significant decrease in the mDP of oligomeric proanthocyanidins (Table 4). However, only this isinglass did not significantly reduce the percentage of prodelphinidin (epigallocatechin units) within the oligomeric proanthocyanidin fraction.

3.3. Effect of the fining agents on some monomeric, dimeric and trimeric flavan-3-ols molecules

A detailed HPLC analysis 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) (Table 5) was also performed.

It was observed that the egg albumin, the swim bladder isinglass and the three gelatines, decreased all of the individual dimeric procyanidins (B1, B2, B3 and B4), significantly. In contrast, none of the individual dimeric procyanidins (B1, B2, B3 and B4), were significantly decreased by the addition of potassium caseinate. Regarding the individual trimeric procyanidins (trimer 2 and C1), only swim bladder isinglass and potassium caseinate did not bring about a significant decrease in either of the trimers. The isinglass with a low molecular weight polydispersion (MW < 20.1 kDa), brought about a significant decrease of the dimeric procyanidin esterified by gallic acid B1-3-O-gallate. The three gelatines tested significantly decreased the dimeric procyanidin esterified by gallic acid B2-3′-O-gallate, however only the gelatine characterised by a polydispersion below 43.0 kDa did not significantly reduce the dimeric procyanidin esterified by gallic acid B2-3-O-gallate (Table 5).

Treatment with gelatine (GS_4) and with isinglass (IL_1) significantly depressed the amount of total dimeric procyanidins (44% and 37%, respectively), the total trimeric procyanidins (56% and 63%, respectively) and the total content of dimer gallates (46% and 50%, respectively) – all compared with untreated wine (Table 5). These fining agents were characterised by low MW polydispersions (<20.1 kDa). Potassium caseinate had no statistically different ($P < 0.05$) effect on these compounds which contrasted with casein, which induced significant decreases in all oligomeric procyanidins (total dimers 7% and 23%, total trimers 8% and 52% and total dimer gallates 9% and 49%, respectively). As expected, these observations are in accordance with the results obtained for the oligomeric flavanols (FII). Machado-Nunes et al. (1998) also observed that casein decreased procyanidins in white wines. However, Jouve et al. (1989) did not find significant decreases of oligomeric procyanidins (dimeric and trimeric) with casein.

HPLC analyses of the isomers (+) catechin, and (−) epicatechin, showed that the various fining agents had different efficiencies in removing these two compounds (Table 5). These are actually isomers differing only on the spatial position of one OH group which is either ‘up’, or ‘down’ with respect to the ring. In the event, (−) epicatechin was only significantly removed by swim bladder isinglass, whereas (+) catechin was significantly removed by all of the protein fining agents tested and especially by the gelatines and casein.

3.4. Effect of the fining agents on flavanoid and non-flavanoid compounds, colour, chromatic characteristic, limpidity and browning potential

The function of protein fining is mainly to clarify and to remove by adsorptive precipitation those compounds that lead to turbidity or to changes in colour. The results showed that protein fining decreased the amount of

flavonoid (0.1–7.1%) and non-flavonoid (0.3–3.0%) compounds. As was shown by Lee and Jaworski (1988) the phenolic compounds are not all subjected to oxidation equally. In general the monomeric catechins and the dimeric procyanidins brown more intensely than other phenolics. The flavonoid compounds most important in white wine oxidation are also most easily removed by fining. However, significant decreases were observed only with casein (7.1%) and with potassium caseinate (2.8%) (Table 6). The results for flavonoids agree with those of other authors (Amati et al., 1979; Machado-Nunes et al., 1998; Puig-Deu, López-Tamames, Buxaderas, & Torre-Boronat, 1996), indicating that the protein fining agents have a greater effect on flavonoids than on other polyphenols. For non-flavonoid compounds, the other fining agents studied did not show significant effects with the exception of swim bladder isinglass and of potassium caseinate (Table 6).

White wine colour (expressed as the absorbance at 420 nm) and browning potential both showed a significant decrease with casein and with potassium caseinate as well as with swim bladder isinglass (Table 6). Similar observations have been reported by Schneider (1988), Castino (1992) and Sims et al. (1995) for casein and by Amati et al. (1979) for potassium caseinate. The wines fined with casein, potassium caseinate and swim bladder isinglass were more stable to oxidation. The increase of absorbance (A_{420nm}) produced by the browning test was less in these wines. This effect is probably related to the fact that swim bladder isinglass and potassium caseinate reduced the non-flavonoid compounds significantly, while casein reduced the level of flavonoid compounds significantly (Table 6). In contrast, the loss in white wine colour (A_{420nm}) was not significant for the gelatines. Sims et al. (1995) reported similar results. The reduction of polyphenols was very low with gelatine, which agrees with Sims et al. (1995) and Fischerleitner et al. (2002, 2003).

The results obtained with the CIELab method for the chromatic characteristics of the unfined and fined wine with different proteins, showed that they changed after fining (Table 6). In the wines fined with casein, potassium caseinate, isinglasses, egg albumin and gelatine with a polydispersion on the low molecular weight, lightness (L^*) increased significantly, suggesting a clarifying action. These results fit in with the turbidity data. The values of chroma (C^*) decreased significantly after the addition of casein and potassium caseinate. Also, hue-angle ($h°$) values increased after addition of these two fining agents. Higher values of $h°$ are due to lower absorbance at 420 nm (yellow pigments – 90°). This observation on $h°$ values could indicate that some yellow pigments were removed after addition of casein and potassium caseinate. The values obtained for colour difference (ΔE), between each fined and unfined wine (Table 6), all show values higher than 2 CIELab units, indicating that these colour differences can be discriminated visually (Spagna et al., 1996). The largest values for colour variation ΔE^* were found for potassium caseinate and for casein, followed by both isinglasses and

Table 6
Non-flavonoids, flavonoids, total phenols, turbidity, browning potential, chromatic characteristics and colour A_{420}, of both fined and unfined white wine (mean ± SD)

Fining treatment	Non-flavonoid phenols (mg/L gallic acid)	Flavonoid phenols (mg/L gallic acid)	Total phenols (mg/L gallic acid)	Turbidity	Browning potential[b]	Chromatic characteristics					Colour[a] $A_{420\ nm}$
						L^*(%)	a^*	b^*	C^*	$h°$	ΔE^*	
T	167 ± 1a	332 ± 3a	499 ± 3a	7.1 ± 0.4a	0.023	90.5 ± 0.0a	−0.63 ± 0.09a	15.21 ± 0.06ab	15.22 ± 0.06ab	92.32 ± 0.35ab	–	0.329 ± 0.006a
IL_1	167 ± 1a	332 ± 3a	498 ± 2a	3.8 ± 0.1c	0.013	93.9 ± 0.5de	−0.67 ± 0.04a	15.37 ± 0.45bd	15.38 ± 0.44bd	92.50 ± 0.23ab	3.49 ± 0.37a	0.311 ± 0.006ab
IS_4	162 ± 2c	332 ± 1a	495 ± 5a	3.8 ± 0.4c	0.005	94.7 ± 0.6e	−0.76 ± 0.07ab	14.36 ± 0.55a	14.39 ± 0.55a	93.03 ± 0.40a	4.35 ± 0.40b	0.288 ± 0.005b
CS_4	166 ± 2a	309 ± 3c	475 ± 1c	1.8 ± 0.1d	0.005	95.5 ± 0.3e	−0.94 ± 0.04b	13.08 ± 0.38c	13.11 ± 0.38c	94.11 ± 0.07c	5.52 ± 0.35c	0.224 ± 0.002c
CKS_1	162 ± 2bc	323 ± 2b	485 ± 9b	2.0 ± 0.3d	0.005	95.4 ± 0.0e	−0.90 ± 0.03b	13.24 ± 0.28c	13.27 ± 0.29c	93.89 ± 0.04c	5.35 ± 0.12c	0.225 ± 0.003c
AS_1	165 ± 2abc	331 ± 3a	499 ± 3a	3.9 ± 0.2c	0.020	92.5 ± 0.9cd	−0.57 ± 0.15ac	16.22 ± 0.44de	16.23 ± 0.43de	92.00 ± 0.58bd	2.34 ± 0.49d	0.318 ± 0.002a
GL_1	165 ± 0abc	332 ± 2a	499 ± 1a	6.8 ± 0.6a	0.018	90.1 ± 1.2a	−0.07 ± 0.16d	18.21 ± 0.64g	18.21 ± 0.64g	90.36 ± 0.33e	3.18 ± 0.62ae	0.328 ± 0.004a
GS_2	165 ± 1ab	332 ± 1a	498 ± 2a	5.5 ± 0.3b	0.019	90.8 ± 1.3ab	−0.19 ± 0.07d	17.85 ± 0.23fg	17.85 ± 0.23fg	90.61 ± 0.21e	2.84 ± 0.31de	0.329 ± 0.005a
GS_4	166 ± 1a	332 ± 1a	496 ± 2a	5.3 ± 0.2b	0.016	92.3 ± 0.3bc	−0.41 ± 0.04c	17.00 ± 0.06ef	17.00 ± 0.06ef	91.37 ± 0.11d	2.56 ± 0.21d	0.305 ± 0.006ab

Unfined (T), isinglass (IL_1), isinglass (IS_4), casein (CS_4), potassium caseinate (CKS_1), egg albumin (AS_1), gelatine (GL_1), gelatine (GS_2) and gelatine (GS_4); L^* – lightness, a^* – redness, b^* – yellowness, C^* – chroma, $h°$ – hue angle, ΔE – total colour difference. The values corresponding to ΔE^* were obtained taking as a reference the unfined wine (T). Means ($n = 2$) within a column followed by the same letter are not significantly different (LSD, 5%).

[a] Absorbance unit.

[b] Difference of the increase of absorbance A_{420} between the wine with and without nitrogen, after 5 days at 55 °C.

all detectable by eye. The results also show that the values for b^* decreased with casein or potassium caseinate. These fining agents all reduced the yellow intensity.

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