GELATINE, CASEIN AND POTASSIUM CASEINATE AS DISTINCT WINE FINING AGENTS: DIFFERENT EFFECTS ON COLOUR, PHENOLIC COMPOUNDS AND SENSORY CHARACTERISTICS

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Abstract

Aims: Describe and compare some characteristics, such as molecular weight (MW) distribution and surface charge density of commercial protein fining agents and to enhance the understanding of their effect on wine chemical and sensory characteristics.

Methods and results: Protein (casein, potassium caseinate and gelatin) MW distribution was characterised by electrophoresis. These proteins were added to a red and a white wine, in order to evaluate its effect on colour, phenolic compounds and sensory attributes.

Conclusion: A band at 30.0 kDa characterised casein and potassium caseinate. Gelatines showed polydispersion on the MW distribution, gelatine GSQ on the higher MW (> 43.0 kDa) and gelatine GL on the lower MW (< 43.0 kDa). Despite the fact that casein and potassium caseinate had similar MW distribution, casein decreased essentially the monomeric ((+)-catechin and (-)-epicatechin) while the potassium caseinate showed a lower influence on these compounds. Also, among the two gelatines used, a different behaviour was observed. The gelatine characterised by a polydispersion below 43.0 kDa depleted more the polymeric tannin fractions than the gelatine characterised by a polydispersion above 43.0 kDa. That gelatine has also decreased colour intensity and coloured anthocyanins of red wine but the hue remains unchanged. Addition of fining agents did not affect greatly the concentration of monomeric anthocyanins. Sensory analysis showed that wines fined with the different proteins presented distinct characteristics.

Significance and impact of study: The knowledge of the physico-chemical characteristics, such as MW distribution and surface charge density, is important for wine fining optimisation and consequently for the wine quality.

Key words: fining agent, anthocyanins, condensed tannins, surface charge density, wine, molecular weight

Résumé

Objectif: Décrire et comparer quelques caractéristiques importantes des colles protéiques, comme la distribution des masses moléculaires (MM) et la densité de charge de surface, pour mieux comprendre son effet sur la composition chimique et l’analyse sensorielle des vins.

Méthodes et résultats: La distribution des masses moléculaires (MM) des protéines (caséine, caséinate de potassium et gélatine), objet de cette étude, a été caractérisée par électrophorèse. Ces protéines ont été appliquées à l’échelle du laboratoire, au collage d’un vin rouge et d’un vin blanc, qui a eu par but d’évaluer l’effet de ces protéines sur la couleur, les composés phénoliques et les caractéristiques sensorielles des vins.

Conclusion: La caséine et le caséinate de potassium possèdent une bande située à 30,0 kDa. Les deux gélatines étudiées (GSQ et GL) ont montré une polydispersion des masses moléculaires (MM) respectivement au-dessus et au-dessous de 43,0 kDa. Bien que la caséine et le caséinate de potassium possèdent une distribution de MM similaire, la caséine a diminué le contenu des vins en (+)-catéchine et (-)-epicatechine, tandis que le caséinate a montré une moindre influence sur ces composés. Aussi, les deux gélatines utilisées ont montré des comportements différents. La gélatine caractérisée par une polydispersion au-dessous de 43,0kDa a éliminé plus fortement les flavanols polyényliques que la gélatine possédant une polydispersion au-dessus de 43,0 kDa. L’intensité colorante du vin rouge a diminué après application de la gélatine avec une MM au-dessous de 43,0 kDa, mais la teinte est restée inchangée. L’addition des colles protéiques n’a pas affecté considérablement la concentration en anthocyanines monomériques. L’analyse sensorielle a montré que les vins collés avec les différentes colles protéiques présentent des caractéristiques distinctes.

Signification et impact de l’étude: La connaissance des principales caractéristiques physico-chimiques des colles protéiques, comme la distribution de masse moléculaire (MM) et la densité de charge de surface, est importante pour l’optimisation de l’opération du collage et par conséquence pour la qualité du vin.

Mots clés: colles protéiques, anthocyanes, tannins condensés, densité de charge de surface, vin, masse moléculaire

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INTRODUCTION

Fining allows wine clarification, stabilisation and the improvement of sensory characteristics. However, it is necessary to know and to understand the fining mechanisms, to reach the intended objectives.

The main protein fining agents used in wine are gelatine, casein, potassium caseinate, egg albumin and isinglass. However, in the recent years, plant proteins (wheat, gluten and other origins) have also been studied for wine fining (MARCHAL et al., 2000a; b; PANERO et al., 2001; MAURY et al., 2003; FISCHERLEITNER et al., 2002, 2003).

Proteins used as wine fining agents present diverse physico-chemical characteristics mainly molecular weight distribution and surface charge density. The knowledge of these characteristics is important for wine fining optimisation and consequently for the wine quality.

The great diversity of gelatines available in the market is a result of the collagen origin and the nature of the production process. Collagen could be found in the skin, bones or cartilages. Hydrolysis of the collagen could be chemical (alkaline or acid) or enzymatic. For the chemical process, the hydrolysis degree is function of the temperature and the time (LAGUNE and GLORIES, 1996a). The gelatines obtained by enzymatic hydrolysis present protein fractions with MW lower than 13.7 kDa (PAETZOLD and GLORIES, 1990); the presence of gelatines with a polydispersion on the higher MW as well as on the lower MW was also verified by several authors (PAETZOLD and GLORIES, 1990; MARCHAL et al., 1993; 2000a; b; 2002; VERSARI et al., 1998; 1999; COSME et al., 2007). The MW distribution of gelatine affects both the quantity and the type of phenolic compounds removed from red wines (HRAZDINA et al., 1969; YOKOTSUKA and SINGLETON, 1987; RICARDO-DA-SILVA et al., 1991a; LAGUNE et al., 1996; SCOTTI and POINSAUT, 1997; VERSARI et al., 1998; LEFEBVRE et al., 1999; SARNI-MANCHEADO et al., 1999; MAURY et al., 2001). According to the type of gelatine and the pH of the medium, surface charge densities ranged from 0.02 to 1.2 mEq/g (PAETZOLD and GLORIES, 1990; LAGUNE and GLORIES, 1996b; LAMADON et al., 1997).

Furthermore, a major band at 30 kDa and other minor bands with lower MW, as well as higher MW characterised casein and potassium caseinate fining agents (MARCHAL et al., 2000a; b; COSME et al., 2007).

Wine phenolic compounds interact with protein fining agents. For example, the two main types of interaction between proteins and tannins are: hydrogen bonds and hydrophobic interactions (MURRAY et al., 1994). The complexes formed could be soluble or insoluble. The precipitation occurs in two steps: association between proteins and tannins leads to the formation of soluble complexes that could, in a following step, aggregate each other and precipitate. This last step depends on the capacity of the tannin to establish linkages between protein molecules (CHEYNIER et al., 1998). Environmental conditions such as pH, alcohol and temperature also influence the formation of tannin-protein complexes (CALDERON et al., 1968, RIBÉREAU-GAYON et al., 1977, 1998).

Thereby, protein fining agents could be used to remove specific phenolic compounds and consequently astringency or bitterness of wines. The sensation of astringency is due to the interaction of salivary proteins (rich in proline) with wine phenolic compounds, mainly condensed tannins (KALLITHRAKA et al., 1998; SAINT-CRICQ-DE-GAULEJAC et al., 1999). However, LEA and ARNOLD (1978) have suggested that not all wine phenolic compounds contribute in the same way to wine astringency. These authors have concluded that the sensation of astringency is essentially due to the more polymerised tannins and those esterified with gallic acid. Gelatines like salivary proteins present higher levels of proline than most of the proteins (LAGUNE and GLORIES, 1996a). Therefore, addition of gelatine to the wine leads to a reduction in the tannin content, mainly concerning the more polymerised tannins and those esterified with gallic acid (SARNI-MANCHADO et al., 1999; MAURY et al., 2001). This indicates that gelatine addition could decrease wine astringency. In a study with flavanol monomers and several flavanol dimers and trimers, esterified or not with gallic acid, RICARDO-DA-SILVA et al. (1991a) have observed that gelatine and casein interact more intensely with the more polymerised proanthocyanidins and also those esterified with gallic acid.

Therefore, the main objective of this work was to describe and compare some characteristics, such as molecular weight distribution, surface charge density and protein content of several distinct commercial protein fining agents (gelatines, casein and potassium caseinate) and to enhance the understanding of their effect on wine (white and red) colour, chromatic characteristics, monomeric anthocyanins, phenolic compounds and sensory characteristics.

MATERIALS AND METHODS

1 - Fining agents characterisation

a) Protein fining agents
In this work, four protein fining agents have been characterised: two gelatines (GSQ, GL), one potassium caseinate (CK) and one casein (CS) (table 1).

b) Total nitrogen

The total nitrogen content was determined by the Kjeldahl method based on mineralisation, distillation and titration with 0.1 N HCl (MANFREDINI, 1989; OIV, 2006b).

c) Protein quantification

The protein content was determined by the Bradford method as modified by READ and NOR THCOTE (1981). Analyses were carried out by adding different proteins [protein fining agents and standard proteins (bovine serum albumin)] to a dye reagent [Coomassie brilliant blue G-250 (Acros Organics, USA), ethanol, phosphoric acid and deionised water], which resulted in an increased absorbance at 595 nm, due to the formation of a protein-dye complex.

d) Protein MW distribution characterised by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The MW distribution of oenological protein fining agents was studied using a SDS-PAGE method as described by LAEMMLI (1970) and adapted for protein fining agents by MARCHAL et al. (2000a; b; 2002). Standard proteins covering a 14.4-94.0 kDa range were used to estimate the MW [Low Molecular Weight (LMW) Amersham Biotech, London, United Kingdom]. Samples and standard proteins were treated with buffer [(0.125 M Tris-Cl, 4 % SDS, 20 % glycerol, 2 % 2-mercaptoetanol, pH 6.8)] (v/v) and denatured at 100 ºC for 5 minutes. The gel with 0.75 mm thickness was run in a mini-vertical gel electrophoresis unit (Mighty-Small II SE 250, Hoefer, San Francisco, USA) at a constant voltage (75 V) at 20 ºC until the bromophenol blue raised the bottom of the gel. After migration, proteins were stained in a solution made up of one part Coomassie blue R-350 (Amersham Biotech, Uppsala, Sweden) and nine parts of a solution with methanol: acetic acid: water (3:1:6) and destained in a mixture of acetic acid: methanol: water (1:2:7) (MARCHAL et al., 2000a; b; 2002).

e) pH

For solid gelatine, pH was measured on a 1 % solution of the initial product (w/v). As concerns solid potassium caseinate, pH was measured on a 5 % solution of the initial product (w/v), and solid casein on a 10 % solution of the initial product (w/v). As regards liquid gelatine, pH was measured directly in the colloidal solution. pH determination was based on the International Codex of Oenology (OIV, 2006b).

j) Weight loss on drying

Weight loss on drying was determined according to the International Codex of Oenology (OIV, 2006 b) at 100-105 ºC on a 2 g sample of the following proteins: casein, potassium caseinate and gelatine. In the case of a colloidal solution of gelatine, a 10 g sample was used, which was dried over water at 100 ºC for four hours, and then dried in an oven at 100-105 ºC for three hours.

g) Surface charge density

The surface charge density was determined with a particle charge detector - produced by MUTEK (Herrsching, Germany) model PCD 03 pH - by titration with a charge compensating polyelectrolyte 0.001 N electropositive-polydiallyldimethylammonium [polyDADMAC (Herrsching, Germany)] or 0.001 N electronegative-sodium polyethylenesulfate [PES-Na (Herrsching, Germany)] (PAETZOLD and GLORIES, 1990; DIETRICH and SCHÄFER, 1991) until the streaming potential is 0 mV, which corresponds to the point where all charges are neutralised. The volume of polyelectrolyte required for the neutralisation allowed to estimate the surface charge density of the product, expressed in milliequivalent of polyelectrolyte per gram of fining agent (mEq/g).

Gelatine fining agents were dispersed in a model wine solution without ethanol (VERNHET et al., 1996).

Casein and potassium caseinate were first dissolved in 0.1 N KOH and subsequently dispersed in the model solution. The surface charge density of these fining agents was measured at pH 3.4 (adjusted with 50 % HCl and centrifuged at 4.000 rpm during 15 minutes).

2 - White and red wine fining trials

a) Chemicals

Vanillin was purchased from Merck (Darmstadt, Germany). Solvents and acids used were of HPLC grade.

b) Wines

White and red wines of the 2003 vintage used in this study were elaborated with different grapes from Vitis vinifera varieties from the Óbidos Region (Adega Cooperativa do Bombarral) and from Lisbon (Tapada da Ajuda - Instituto Superior de Agronomia) respectively. Table 2 shows the analytical composition of both wines before the fining treatment.

c) Fining experiments

Experiments involved the addition of standard quantities of the protein fining agents (gelatines, casein and potassium caseinate) prepared as recommended by...
the producers (table 1). Trials were conducted at the laboratory scale in 1,000 mL volumes of wine. Untreated wine was used as control. The fining agents were thoroughly mixed and allowed to remain in contact with the wine for 7 days; the samples were then centrifuged at 4000 rpm for 15 min. before analysis.

d) Physico-chemical analysis of wine

Alcohol content % (v/v), pH, density, titratable and volatile acidities, free sulphur dioxide, malic acid and residual sugars were analysed according to the Organisation Internationale de la Vigne et du Vin methods (OIV, 2006a).

e) Fractionation of proanthocyanidins according to the degree of polymerisation by C18 Sep-Pak cartridges and determination of the flavan-3-ol content by the vanillin assay

The separation of flavanols was performed on a C18 Sep-Pak cartridge (Waters, Milford, Ireland) according to the degree of polymerisation in three fractions, monomers, oligomers and polymers of flavan-3-ol in agreement with the method described by SUN et al. (1998a). Quantification of the total flavan-3-ol content in each fraction was performed using the vanillin assay according to the method described by SUN et al. (1998a, b). For the monomeric fraction, the absorbance at 500 nm was read after reaction at 30 °C for 15 min. using a Unicam UV-vis UV4 spectrophotometer (Unicam, Cambridge, U.K.). For the oligomeric and polymeric fractions, the reaction was performed at room temperature and left until the maximum absorbance value at 500 nm was reached. Quantification was carried out by means of standard curves prepared from monomers, oligomers, and polymers of flavan-3-ol isolated from grape seeds, as described earlier (SUN et al., 1998a, 2001).

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

Procyanidin separation was performed according to RICARDO-DA-SILVA et al. (1990). High Performance Liquid Chromatography (HPLC) analyses were carried out using a HPLC system including a Konik Instruments (Konik Instruments, Konik-Tech, Barcelona, Spain) UV-vis detector (Uvis 200) 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 Instrument, Konik-Tech, Barcelona, Spain). The column was a reverse-phase C18Lichrosphere 100 (250 mm x 4.6 mm, 5 µm) (Merck, Darmstadt, Germany). Separation was performed at room temperature. The 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 linear gradient from 0 to 31 min. followed by washing (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, (distilled water), solvent B, (water/acetic acid, 90/10, v/v), 10-70 % B linear gradient from 0 to 45 min., 70 - 90 % B linear gradient from 45 to 70 min., 90 % B isocratic from 70 to 82 min., 90-100 % B linear gradient from 82 to 85 min., 100 % B isocratic from 85 to 90 min., followed by washing (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., 1995, DALLAS et al., 1996a, b) of monomeric flavan-3-ols and oligomeric procyanidins (dimeric and trimeric) were performed.

g) Monomeric anthocyanins

Monomeric anthocyanin analysis was carried out by HPLC according to DALLAS and LAUREANO (1994). The equipment used was a Perkin-Elmer (Norwalk, USA) system, equipped with a model L-7100 Lachrom Merck Hitachi-High-Technologies pump (Tokyo, Japan), a model LC-95 UV-Vis detector set at 520 nm coupled to a version 6.2 Konikrom data chromatography treatment system (Konik Instruments, Konik-Tech, Barcelona, Spain). The column was a reverse-phase C18 Lichrosphere 100 (5 µm packing, 250mm x 4.6 mm i.d.) (Merck, Darmstadt, Germany) protected with a guard column of the same
material. The separation was carried out at room temperature. The elution conditions for monomeric anthocyanins were as followed: 0.7 mL/min., flow rate, solvent A was 40 % formic acid, solvent B was CH₃CN and solvent C was bidistilled water. The initial conditions were 25 % of A, 6 % of B and 69 % of C for 15 min. followed by a linear gradient to 25 % of A, 25.5 % of B 49.5 % of C during 70 min., and 20 min. of 25 % A, 25.5 % of B and 49.5 % of C.

Quantification of monomeric anthocyanins in wine was performed by means of standard curves prepared by using different concentrations of malvidin 3-glucoside chloride in methanol 0.1 % HCl. The peak area was converted to mg/L of malvidin 3-glucoside equivalent. Twenty µL of each sample were injected in triplicate.

h) Quantification of non-flavonoid phenols

Determination of the phenolic content of wines was carried out by absorbance measurement 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 non-flavonoid compounds in the wine.

i) Chromatic characterisation, colour and pigments

Absorption spectra of the wine samples were recorded with a Unicam UV-vis UV4 spectrophotometer (Unicam, Cambridge, U.K.), scanned over the range 380 to 770 nm using quartz cells. Data were collected at 10 nm intervals, and referred to a 1-cm path length to calculate L* (lightness), a* (measurement of redness), b* (measurement of yellowness), coordinates using the CIELab method (OIV, 2006a). The spectrophotometer incorporates the software required to calculate the CIELab parameters, directly (Chroma version 2.0 Unicam, Cambridge, United Kingdom). Colour intensity was calculated by summation of the absorbances at three wavelengths 620, 520 and 420 nm (1-mm cell) using a Unicam UV-vis UV4 spectrophotometer (Unicam, Cambridge, U.K.). Hue was expressed as the ratio of absorbance at 420 nm and 520 nm. The content of total and coloured anthocyanins and total and polymeric pigments were determined according to the method proposed by SOMERS and EVANS (1977).

j) Sensory evaluation

The wines were subjected to sensory analysis to assess the differences between the unfined and the fined wines. A panel composed by nine trained members evaluated the wines. The wines were presented in two sessions; one for white wines and another for red ones (unfined and fined wines). Wines were presented to the panel at random. A code with three arbitrary numbers was attributed to each wine. White wines were assessed for limpidity, colour, aromatic intensity and quality, taste intensity and quality, fullness and global appreciation. Red wines were assessed for colour intensity, hue, aromatic intensity and quality, taste intensity and quality, fullness, astringency and global appreciation. There was a structured scale with numbers from 0 to 4 for colour evaluation and from 1 to 7 for the other characteristics.

A principal component analysis (PCA) was carried out on the results of the averages of the sensory analysis data for each attribute. For statistical analysis, the Statistica 6.0 program was used.

## RESULTS AND DISCUSSION

### 1 - Fining agents characterisation

a) Loss during drying, pH, total nitrogen and protein content

### Table 2 - Physico-chemical characteristics of the white and the red wines used before fining treatment.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Red wine</th>
<th>White wine</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>3.53</td>
<td>3.48</td>
</tr>
<tr>
<td>Free sulphur dioxide (mg/L)</td>
<td>16</td>
<td>26</td>
</tr>
<tr>
<td>Volatile acidity (g/L acetic acid)</td>
<td>0.81</td>
<td>0.44</td>
</tr>
<tr>
<td>Titratable acidity (g/L tartaric acid)</td>
<td>6.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Reducing sugars (g/L)</td>
<td>2.8</td>
<td>1.2</td>
</tr>
<tr>
<td>Alcohol content (% v/v)</td>
<td>13.9</td>
<td>10.4</td>
</tr>
<tr>
<td>Density (g/cm³)</td>
<td>0.9960</td>
<td>0.9937</td>
</tr>
<tr>
<td>Malolactic fermentation</td>
<td>Occurred</td>
<td>Occurred</td>
</tr>
</tbody>
</table>
The liquid gelatine (GL) had a loss during drying of 86 % (w/w). As expected, the value was higher than those obtained for fining agents in a solid state [8 - 11 % (w/w)]. Losses during drying are in accordance with the recommendations of the International Codex of Oenology (OIV, 2006b) (table 3).

All of the fining agents analysed had acidic or almost neutral pH (table 3).

Total nitrogen values of solid and liquid gelatines were respectively, 14.0 and 18.9 % (w/w, dry weight) and, for potassium caseinate and casein, the values were 14.5 and 10.7 % (w/w, dry weight), respectively (table 3).

b) Protein molecular weight distribution

The MW distribution of casein and potassium caseinate observed in the SDS-PAGE electrophoretic pattern (figure 1) showed that both fining agents are characterised by a major band at 30.0 kDa. This was also observed by other authors for casein (MARCHAL et al., 2000a; b; COSME et al., 2007) and potassium caseinate (COSME et al., 2007). The gelatines GSQ and GL showed polydispersion according to MW distribution, which was also observed by other authors (MARCHAL et al., 1993; 2000a; b; 2002, COSME et al., 2007). However, gelatine GSQ showed a polydispersion on the higher MW (MW > 43.0 kDa) whereas gelatine GL showed a polydispersion on the low MW (MW < 43.0 kDa) (figure 1). Knowledge of the MW distribution of the protein fining agents is important for tannin-protein interactions (SARNI-MANCHADO et al., 1999; MAURY et al., 2001).

c) Surface charge density

The highest surface charge density was measured in solid gelatine (GSQ) (table 3), which could be related with a lower degree of hydrolysis of these proteins (SCOTTI and POINSAUT, 1997; LAMADON et al., 1997).

As described earlier, casein and potassium caseinate were initially dissolved in KOH and afterwards dispersed in a model solution lacking ethanol at a pH adjusted to 3.4. The surface charge densities of these fining agents were measured at the pH of dissolution (CS - pH 9.7, CK - pH 10.6) and subsequently at pH 3.4. It was observed that the surface charge density of these fining agents changed after pH adjustment (from -1.09 to 0.20 and -1.3 to 0.24 mEq/g of product, respectively).

2 - White wine fining trials

a) Phenolic compounds and chromatic characteristics

The objective of this study was to know which tannin fraction (monomeric, oligomeric and polymeric flavan-3-ol) is quantitatively more depleted after addition of the diverse protein fining agents.

The fining agent that more depleted the monomeric flavanols was casein (58 %), followed by potassium caseinate (29 %) (table 4). This result agrees with those of AMAT et al. (1979) for potassium caseinate (20-34 % decrease in catechin). Gelatine did not considerably remove the monomeric flavanols, which is in accordance with the work done by SARNI-MANCHADO et al. (1999).

All fining agents used in this study decreased the oligomeric flavanol content; however, gelatine with a polydispersion above 43.0 kDa and casein were found to be the fining agents that decreased to a greater extend.

Table 3 - Weight loss on drying, pH, total nitrogen, total protein and surface charge density (mean±SD).

<table>
<thead>
<tr>
<th>Fining agent</th>
<th>Weight loss (% w/w)</th>
<th>pH</th>
<th>Total Nitrogen (% w/w, dry weight)</th>
<th>Protein content by Bradford method (mg BSA/g fining agent)</th>
<th>Surface charge density (mEq/g product, at pH 3.4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSQ</td>
<td>11±0.2</td>
<td>4.2±0.02</td>
<td>14.0±1.1</td>
<td>42.0±1.6</td>
<td>0.98±0.00</td>
</tr>
<tr>
<td>GL</td>
<td>86±0.1</td>
<td>2.5±0.01</td>
<td>18.9±1.6</td>
<td>54.7±2.0</td>
<td>0.52±0.02</td>
</tr>
<tr>
<td>CS</td>
<td>840.0</td>
<td>7.2±0.02</td>
<td>10.7±1.2</td>
<td>101.1±1.4</td>
<td>0.20±0.00</td>
</tr>
<tr>
<td>CK</td>
<td>840.2</td>
<td>6.7±0.02</td>
<td>14.5±1.0</td>
<td>93.3±2.1</td>
<td>0.24±0.00</td>
</tr>
</tbody>
</table>

GSQ: gelatine, GL: gelatine, CS: casein, CK: potassium caseinate

Figure 1 - Electrophoretic patterns of casein - CS, potassium caseinate - CK and gelatine - GSQ, GL. MW standard - P, are given on the left and right side.
these compounds (50%). Gelatine with a polydispersion below 43.0 kDa and potassium caseinate removed oligomeric flavanols, but to a lesser extend (20-25%). It was observed that casein and potassium caseinate showed different affinities for these compounds, despite the similarity of their electrophoretic profiles (MW ≈ 30 kDa) and surface charge densities (≈ 0.20 mEq/g). Casein decreased to a greater extend the oligomeric flavanol content in white wines (table 4) than did potassium caseinate.

The gelatine with a polydispersion below 43.0 kDa removed the polymeric flavanols at a higher quantity (71%), which agrees with the results found by MAURY et al. (2001). These authors showed that proteins of lower MW (16 kDa), presented a greater affinity to polymeric tannins than proteins with higher MW (190 kDa). The other fining agents used in this study removed similar quantities of these compounds (30-45 %).

Proanthocyanidins (oligomeric and polymeric) were more influenced by proteic fining agents than monomeric flavanols, which is probably related to their higher degree of polymerisation. This observation has already been done by ROSSI and SINGLETON (1966), CHEYNIER et al. (1997) and by SARNI-MANCHADO et al. (1999).

According to these results, gelatine with a polydispersion below 43.0 kDa and casein were the fining agents that promote the highest decrease of proanthocyanidins and monomeric flavanols, respectively.

As could be observed in table 4, addition of fining agents diminishes the content of non-flavonoid (20.6-26.3 %) compounds. The results obtained with the CIELab method for determining the chromatic characteristics of the unfined and fined wine with diverse proteins are presented in table 4. In the wine fined with gelatine with a polydispersion below 43.0 kDa (GL), lightness slightly increased (L*); this suggests a clarifying action on this wine. Yellowness (b*) decreased after gelatine and casein addition, as well.

b) Effect of protein fining on sensory evaluation

The wine fined with gelatine with a polydispersion above 43.0 kDa (GSQ), showed a high aroma intensity. Attributes that also were few pointed are colour and taste quality. In contrast, the wine fined with gelatine with a polydispersion below 43.0 kDa, showed the best visual characteristics (colour and limpidity). The wines fined with casein and potassium caseinate showed similar sensory characteristics. These two wines presented the highest values for body and global appreciation (figure 2).

3 - Red wine fining trials

a) Phenolic compounds and colour

Gelatine with a polydispersion below 43.0 kDa was the fining agent that promotes the highest decrease of the oligomeric and polymeric flavanol content. As previously observed by YOKOTSUKA and SINGLETON (1995), the amount of proanthocyanidins removed, diminishes with the increase of the MW of the proteic fining agent.
Gelatine GL was characterised by protein fractions with lower MW distribution (MW<43,0 kDa) whereas gelatine GSQ was characterised by protein fractions with higher MW distribution (MW>43 kDa) (figure 1); thereby the results observed in table 5 are in accordance with those of YOKOTSUKA and SINGLET ON (1995).

The monomeric flavanols [(+) - catechin and (-) - epicatechin] were separated by HPLC (table 6). Analyses evidenced that the diverse proteic fining agents have different efficiencies in depleting these two compounds. Consequently, addition of gelatines did not considerably decrease these compounds, which was in accordance with the data of RICARDO-DA-SILVA et al. (1991a). In contrast, casein and potassium caseinate decreased the concentration of (+) - catechin and (-) - epicatechin.

The dimeric procyanidin B3 was little influenced by protein fining with the exception of casein which removes 40 % of this compound. One can point out that, casein and potassium caseinate despite their similarities concerning surface charge densities and MW distribution (MW = 30.0 kDa), showed different affinity to this compound (procyanidin B3). Addition of gelatines did not influence procyanidins B1. Procyanidin B4 was mainly removed by gelatine with a polydispersion above 43.0 kDa (GSQ) and potassium caseinate; however procyanidin B2 was mainly removed by casein.

Table 5 - Oligomeric and polymeric proanthocyanidin contents of both fined and unfined red wine (means±SD).

<table>
<thead>
<tr>
<th>Fining agent</th>
<th>Oligomeric proanthocyanidins (mg/L)</th>
<th>Polymeric proanthocyanidins (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>280.8±4.7</td>
<td>790.5±22.2</td>
</tr>
<tr>
<td>GSQ</td>
<td>267.2±8.9</td>
<td>569.8±26.2</td>
</tr>
<tr>
<td>GL</td>
<td>163.9±1.2</td>
<td>545.0±38.2</td>
</tr>
<tr>
<td>CS</td>
<td>248.2±7.6</td>
<td>599.1±19.1</td>
</tr>
<tr>
<td>CK</td>
<td>211.1±1.2</td>
<td>743.2±26.4</td>
</tr>
</tbody>
</table>

T: unfined, GSQ: gelatine, GL: gelatine, CS: casein, CK: potassium caseinate

Table 6 - (+) - Catechin, (-) - epicatechin, dimeric, trimeric and dimeric procyanidins esterified by gallic acid (mg/L) as analysed by HPLC for both fined and unfined red wine (means±SD).

<table>
<thead>
<tr>
<th>Fining agent</th>
<th>Monomers</th>
<th>Dimers</th>
<th>Trimers</th>
<th>Dimer gallates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(+) - Catechin</td>
<td>(-) - Epicatechin</td>
<td>B3</td>
<td>B1</td>
</tr>
<tr>
<td>T</td>
<td>31.4±0.3</td>
<td>18.1±0.5</td>
<td>7.0±0.0</td>
<td>32.0±0.7</td>
</tr>
<tr>
<td>GSQ</td>
<td>30.2±0.8</td>
<td>17.7±0.6</td>
<td>5.9±0.2</td>
<td>33.3±0.8</td>
</tr>
<tr>
<td>GL</td>
<td>29.3±0.1</td>
<td>17.5±0.9</td>
<td>6.3±0.6</td>
<td>33.2±0.8</td>
</tr>
<tr>
<td>CS</td>
<td>22.0±0.0</td>
<td>15.8±0.6</td>
<td>4.1±0.1</td>
<td>20.7±0.9</td>
</tr>
<tr>
<td>CK</td>
<td>26.4±0.5</td>
<td>16.0±0.9</td>
<td>6.9±0.5</td>
<td>20.6±0.3</td>
</tr>
</tbody>
</table>

T: unfined, GSQ: gelatine, GL: gelatine, CS: casein, CK: potassium caseinate

These results are not in accordance with those of RICARDO-DA-SILVA et al. (1991a), which verified that none of these procyanidins are influenced by the addition of proteic fining agents in a young red wine. This could probably be explained by the high level of anthocyanins and tannins present in the wine elaborated from the Mourvèdre grapevine variety, as observed by RICARDO-DA-SILVA et al. (1991a). This high phenolic content can protect the smaller wine tannins from the action of fining agents. In contrast, MACHADO-NUNES et al. (1995) also verified that proteic fining agents decreases wine procyanidins.

b) Monomeric anthocyanins, colour, pigments and chromatic characteristics

The following monomeric anthocyanins were separated by HPLC: delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, malvidin-3-glucoside, malvidin-3-acetylglucoside and malvidin-3-p-coumaryl-glucoside (table 7). Addition of casein promotes the highest decrease of monomeric anthocyanins, but this decrease was very low. These findings agree with those of LOVINO et al. (1999) and...
COSME et al. (2007) who observed that fining red wine with casein decreases the level of monomeric anthocyanins. Colour intensity only decreased after addition of gelatine with a polydispersion below 43.0 kDa (GL) and the wine hue was not affected by protein fining (table 8). These results are in accordance with the works of RICARDO-DA-SILVA et al. (1991a), MACHADO-NUNES et al. (1995), VERSARI et al. (1998), LOVINO et al. (1999) and PANERO et al. (2001).

Casein promotes a slight decrease in the total anthocyanin content of wine (table 8). Fining agents also have a little effect on total pigments. Colour intensity and coloured anthocyanins are related, the decrease of coloured anthocyanins leading to a reduction of the colour intensity, as it was observed following the addition of gelatin with a polydispersion below 43.0 kDa (GL) (table 8).

Determination of polymeric pigments gives an indication of the amount of anthocyanins combined with tannins. The lowest contents of polymeric pigments were found in wines fined with gelatine with a polydispersion below 43.0 kDa (GL).

The results obtained with the CIELab method for the chromatic characteristics of the unfined and fined red wine with diverse proteins showed that changes occurred after fining (table 8). In wines fined with gelatine, lightness (L*) increased, which appears to be related with the low redness (a*) values due to the removal of pigments as earlier observed by GIL-MUÑOZ et al. (1997).

c) Effect of proteic fining on sensory evaluation

Figure 3 showed that the global appreciation was strongly correlated with astringency and aroma quality as well as aroma intensity and colour intensity. The wine fined with gelatine with a polydispersion above 43.0 kDa (GSQ) showed a colour hue, a taste intensity and quality that differentiate this wine from the others. However, the wine fined with gelatine with a polydispersion below 43.0 kDa was characterised as being the more astringent, with a higher aroma quality and a better global appreciation. The wine fined with casein showed more colour and more aroma intensity. The appreciation of the wine fined with potassium caseinate was very similar to the wine fined with gelatine with a polydispersion below 43.0 kDa (GL).
The fining agents studied here presented a similar total nitrogen content (11-19 % w/w). However, casein and potassium caseinate showed higher quantities of total protein, when compared to gelatines. They also present different molecular weight distribution. The electrophoretic pattern of gelatines was characterised by a polydispersion. However, gelatine GSQ is characterised by a polydispersion on the high molecular weight (MW > 43.0 kDa), contrary to gelatine GL that was characterised by a polydispersion on the low molecular weights (MW < 43.0 kDa). Casein and potassium caseinate were both characterised by a band at 30.0 kDa.

In white wine, the monomeric and oligomeric flavanol contents decreased after casein addition. However, polymeric proanthocyanidins in white wine and oligomeric and polymeric proanthocyanidins in red wine were more depleted by the gelatine characterised by a polydispersion below 43.0 kDa than by the gelatine characterised by a polydispersion above 43.0 kDa. These results show that, the same type of protein, as was gelatine, could influence in a different way the diverse flavan-3-ols.

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Colour intensity and molecules linked with wine colour are less influenced by protein fining, but they could also be selectively decreased by a specific fining protein. For example, gelatine with a polydispersion below 43.0 kDa diminishes more intensively the colour intensity and the coloured anthocyanins.

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REFERENCES


Protein fining agents: characterisation and effects on wine fining


