INFLUENCE OF DIFFERENT COMMERCIAL FINING AGENTS ON PROANTHOCYANIDIN FRACTION AND ANTIOXIDANT ACTIVITY OF A RED WINE FROM BAGA GRAPES

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Abstract

Aims: Evaluate the impact of different commercial fining agents (egg albumin, bentonite, gelatine, potassium caseinate and isinglass) and their concentration on proanthocyanidin fraction content (monomeric, oligomeric and polymeric) and antioxidant activity of a red wine from Baga grape variety. Additionally establishment of correlations between the antioxidant activity and the different proanthocyanidin fraction content were also an other aim of this work.

Methods and results: The fractionation of wine proanthocyanidins was made according to their polymerisation degree: catechins (monomers), oligomeric (degree of polymerisation ranging from 2 to 12-15) and polymeric (degree of polymerisation > 12-15). For antioxidant activity of red wine, two analytical methods were used: the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and the ABTS radical cation scavenging assay. In general the different commercial fining agents applied induced a decrease of the wine total phenols and the different proanthocyanidin fraction content studied. The monomeric flavanols were significantly depleted by egg albumin and isinglass (specially using the highest concentration), while for oligomeric fraction only the highest egg albumin, gelatine and isinglass concentration induced a decrease on the values.

Conclusion: The commercial fining agents analyzed in the present study affected the monomeric and the oligomeric proanthocyanidin. At same time some fining agents (specially in high concentrations), such as egg albumin, isinglass and gelatine induced a reduction of antioxidant activity of red wines too.

Significant and impact study: The knowledge of the impact of the commercial fining agents in proanthocyanidin content and antioxidant properties of red wines is important for red wine fining optimisation and consequently for the red wine quality. This study is particular important when the « antioxidant power » of the foods, in this case in red wine, is an expression of its capability both to defend the human organism from the action of the free radicals and to prevent degenerative disorders deriving from persistent oxidative stress.

Key words: antioxidant activity, fining agents, proanthocyanidins, red wine

Résumé

Objectifs : Évaluer l'impact de différents agents de clarification commerciaux (ovalbumine, bentonite, gélatine, caséinate de potassium et ichtyocolle) et de leur concentration sur la teneur de fractions de proanthocyanidines (monomérique, oligomère et polymère) et l'activité antioxydante d'un vin rouge du cépage Baga. On a également établi des corrélations entre l'activité antioxydante et la teneur de différentes fractions de proanthocyanidines.

Méthodes et résultats : Le fractionnement des proanthocyanidines des vins a été fait selon leur degré de polymérisation : catéchine (monomères), oligomères (degré de polymérisation s'étendant de 2 à 12-15) et polymères (degré de 12-15 de polymérisation). Pour l'activité antioxydante du vin rouge, deux méthodes analytiques ont été employées : l'analyse radicale de 2.2 diphényl-1-picrylhydrazyl (DPPH) et l'analyse radicale de cation d'ABTS. Les différents agents commerciaux de clarification induisent une diminution des phénols totaux du vin et de la teneur des différentes fractions de proanthocyanidines étudiées. Les flavanols monomériques ont été significativement réduits par l'ovalbumine et ichtyocolle (particulièrement à la plus forte concentration), alors que pour la fraction oligomère, seule la plus forte concentration d'ovalbumine, de gélatine et d'ichtyocolle entraîne de manière significative une diminution ($p<0.05$) des valeurs du vin clarifié. La fraction polymère n'a pas été diminuée sensiblement par les différents agents de clarification utilisés, à l'exception de l'ovalbumine et à la concentration la plus élevée en gélatine. L'ovalbumine, l'ichtyocolle et la gélatine (pour la concentration la plus forte) entraînent une diminution significative ($p<0.05$) de l'activité antioxydante du vin rouge. Cette diminution a été plus évidente avec la méthode d'ABTS. Les résultats ont également montré de manière significative la corrélation des valeurs entre la teneur de différentes fractions de proanthocyanidines et l'activité antioxydante pour tous les traitements de clarification (excepté pour celui à l'ichtyocolle).

Conclusion : Les agents commerciaux de clarification analysés dans cette étude ont affecté les proanthocyanidines monomériques et oligomères. Quelques agents (particulièrement à fortes concentrations), comme l'ovalbumine, l'ichtyocolle et la gélatine induisent aussi une réduction de l'activité antioxydante des vins rouges.

Signification et impact de l'étude : La connaissance de l'impact des agents de clarification commerciaux sur la teneur en proanthocyanidines et sur les propriétés antioxydantes des vins rouges est importante pour l'optimisation de la clarification des vins rouges et par conséquent pour la qualité du vin. Cette étude est particulièrement importante. Le pouvoir antioxydant des aliments, dans ce cas le vin rouge, est une expression de leur capacité à défendre le corps humain de l'action des radicaux libres et d'empêcher des désordres dégénératifs dérivant du stress oxydatif persistant.

Mots clés : activité antioxydante, agents de clarification, proanthocyanidines, vin rouge

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INTRODUCTION

Clarification techniques are one of the oenological practices that affect the wine quality. Fining is applied to obtain limpid and bright wines, eliminating substances in suspense as well as proteic instability. Fining is also responsible for elimination of some phenolic compounds of colloidal nature (BÁRON *et al.*, 2000), implicated on oxidation phenomena and on the excess astringency of wine, contributing to the improvement of some organoleptic characteristics of wines. At the same time, the fining agents should allow the clarified conditions to be maintained as long as necessary before the wine is consumed.

Several authors (MAURY *et al.*, 2001; GÓMEZ-PLAZA *et al.*, 2000; 2002; COSME *et al.*, 2007) have demonstrated that fining agents such as gelatine, bentonite, egg albumin and casein induce a reduction of phenolic levels and alter the colour in some wines. Additionally other studies (SARNI-MACHADO *et al.*, 1999; MAURY *et al.*, 2003) have been demonstrated a selective action of gelatine and plant protein preparations used as wine fining agents. This selective action induce a preferential removal of highly polymerized and highly galloylated tannins.

In vitro antioxidant activity of wine has been assessed thoroughly employing different methods (TEISSEDRE *et al.*, 1996; FERNÁNDEZ-PACHÓN *et al.*, 2004). Different artificial species have been applied to wine samples, such as 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (MILLER *et al.*, 1993), 1,1'-dihenyl-2-picrylhydrazyl (DPPH) (BRAND-WILLIAMS *et al.*, 1995) and N,N-dimethyl-p-phenylendiamine (DMPD) (FOGLIANO *et al.*, 1999). However, multiple and variable experimental conditions performing the assays such as radical generator, time of measure, expression of results, make it difficult to compare reported data. According to the authors, despite the methodological differences between the different methods used, the results obtained showed similar conclusions, except for DMPD method where the standard deviations found were very high. Additionally, according several authors (FERNÁNDEZ-PACHÓN *et al.*, 2004; KATALINIC *et al.*, 2004; KUSKOSKI *et al.*, 2005) one of the important characteristics of the antioxidant methods, are the capacity to give fast and reproducible results.

The antioxidant potential of red wines is due to the presence of phenolic compounds, which may inhibit platelet aggregation (GRYGLEWSKI *et al.*, 1987), prevent oxidation of the human low-density lipoproteins (LDL) (FRANKEL *et al.*, 1993), and decrease the inflammatory and carcinogenic processes (TAPIERO *et al.*, 2002).

Phenolic compounds are considered free-radical scavengers, and their antioxidant properties depend on their chemical structure. Specifically, it depends on their ability to donate hydrogen or electron and their ability to delocalise the unpaired electron within the aromatic structure. According to BEECHER (2003), flavan-3-ols, flavonols and anthocyanins are the most important compounds that contribute for red wine antioxidant proprieties.

One of the important phenolic compounds present in wines are the proanthocyanidins. These compounds are of undoubted importance in oenology because they contribute to the sensory properties of wine and they have an important role in the process of maturation and ageing of red wines. During wine ageing, these molecules that are highly reactive species, undergo several reactions, such as polymerisation, interactions with polysaccharides, proteins and other substances and can be oxidised too (RICARDO DA SILVA *et al.*, 1991; CHEYNIER *et al.*, 1997). Additionally several authors (DAS *et al.*, 1999; SÁNCHEZ-MORENO *et al.*, 2003; CIMINO *et al.*, 2007) demonstrated that the peroxy radical-scavenging capacities of red wines was related to the amount of total proanthocyanidins. There is not a consensus if the antioxidant properties of wines are linked with the total phenol concentration, rather than individual phenols (ARNOUS *et al.*, 2001). Therefore, studies on the relationship between different proanthocyanidin fractions and antioxidant properties of fined and unfined wines, are scarce. In general, the comparative studies about the impact of the fining agents have focused attention on the general wine phenolic composition, but not at the same time on the different degree of proanthocyanidin polymerisation content and the antioxidant activity values of red wines as function of the type and concentration of fining agents added.

Thus the main goal of this study was, therefore to undertake a comparative study on the effect of five commercial fining agents (egg albumin, bentonite, gelatine, potassium caseinate and isinglass) on the proanthocyanidin fractions (monomeric, oligomeric and polymeric), as well as on the antioxidant activity (evaluated by DPPH and ABTS methods) of a red wine from Baga grape variety.

MATERIALS AND METHODS

2 - Wine sample, reagents and material

Wine was made from Baga red grape variety (*Vitis vinifera* L.) grown on Bairrada region (Centre North region of Portugal) and harvested at the technological state of ripeness in September 2006. Baga is the main red grape variety of Bairrada region, and it is of high production and has late-ripening bunches made up of small berries.

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In general, its thick skins yield dark colour, high tannin content with balance aroma that tends to age well.

The wine was made by classical wine making technology, with a maceration time of 10 days. After fermentation (sugar content below 2.5 g/L), the wine was kept under controlled conditions and was regularly analysed for the level of total and free SO_2. At clarification assays the wine main characteristics were the following: alcohol content 13 % (by volume), pH=3.84, total acidity 6.0 g/L as tartaric acid, volatile acidity 0.38 g/L as acetic acid, total SO_2 45.0 mg/L and free SO_2 20.0 mg/L.

The commercial fining agents commonly used in wine industry (egg albumin, bentonite, gelatine, potassium caseinate and isinglass) were provided by AEB Bioquímica Portuguesa S.A. (Viseu, Portugal). DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) used for antioxidant activity determination and vanillin (used in flavanols fraction quantification) were purchased from Fluka-Biochemika (Buchs, Switzerland). Gallic acid, potassium persulfate (di-potassium peroxdisulfate) and trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) were purchased from Sigma-Aldrich (Steinheim, Germany), while malvidin-3-glucoside was purchased from Polyphenols SA Laboratories (Sandnes, Norway). Spectrophotometric measurement was performed on a Perkin Elmer instrument Lambda 25UV/Vis spectrometer (Norwalk, CT, USA).

2 - Fining experiments

Clarification with fining agents was performed on a laboratory scale similarly to the process carried out in the wine cellar. For each commercial fining agent used, we applied three different concentrations according the technical information received from fining agents producer. The fining agents concentrations applied were: 5, 30 and 50 g/hL for egg albumin, bentonite and potassium caseinate and 5, 30 and 50 mL/hL for gelatine and isinglass (from collagen hydrolysis from fish skin). Egg albumin, bentonite and potassium caseinate were first dissolved in water before added to the wine as suggested by the manufacturer. Gelatine and isinglass were directly added to the wine. The trials were conducted in 500 mL volumes of wine. Untreated wine was used as control. All fining experiments were done in duplicate.

The fining agents were thoroughly mixed and allowed to remain in contact with the wines for 7 days (in the dark) at 20 ºC to obtain a properly clarified and limpid wine. The wine samples were then filtered through a No. 1 Whatman before analysis.

3 - General phenolic composition analysis

Total phenols content were determined by direct reading of the absorbance at 280 nm (RIBÉREAU-GAYON *et al.*, 2000). This is a faster procedure based on the absorbance of the aromatic ring. Most catechins have a maximum absorption at around 280 nm. (+)-Catechin is reported to be the major catechin monomer in all red grape and wine, and is reported to be highly correlated with absorbance at 280 nm (YILMAZ and TOLEDO, 2006). The results were expressed as gallic acid equivalents by means of calibration curves with standard gallic acid. Total anthocyanins, colour density and hue were determined using the analytical methods recommended by the OIV (2006). The results of total anthocyanins were expressed as malvidin-3-glucoside equivalents. All measurements were performed in duplicate.

4 - Fractionation of proanthocyanidins according their polymerisation degree

For fractionation of wine proanthocyanidins according to their polymerisation degree: catechins (monomers), oligomeric (degree of polymerisation ranging from 2 to 12-15) and polymeric (degree of polymerisation > 12-15) fraction using C_{18} Sep-Pack column, a method described by SUN *et al.* (1998 a) was used. Thus, each sample was passed through the two preconditioned neutral Sep-Pack cartridges connected in series. To eliminate phenolic acids, 4 mL de-alcoholized medium was adjusted to pH 7.0 and then passed through the two connected Sep-Pack cartridges preconditioned with 10 mL of water adjusted to pH 7.0.

After drying the column with N_2 elutions were carried out first with 25 mL ethyl acetate to elute catechins and oligomeric proanthocyanidins and then the polymeric fraction was eluted with 10 mL methanol. To separate the monomeric from oligomeric fraction, both fractions were evaporated to dryness under vacuum at 25 ºC, dissolved in distilled water and then redeposited onto the same connected cartridges preconditioned with distilled water. After drying the cartridges with N_2, catechins and oligomeric proanthocyanidins were eluted sequentially with 25 mL diethyl ether (catechins fraction) and finally with 10 mL methanol (oligomeric fraction).

For each fraction obtained previously, the quantification of flavanols was performed by the modified vanillin assay described by SUN *et al.* (1998 b). All quantification were done in duplicate.

5 - Antioxidant activity

The procedure used for antioxidant activity using the DPPH method is described by BRAND-WILLIAMS *et*

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al. (1995). Briefly, 0.1 mL of different sample concentrations were added to 3.9 ml of 2,2-diphenyl-1-picrylhydrazyl (DPPH) methanolic solution (25 mg/L). The DPPH solution was prepared daily and protected from the light. Absorbance at 515 nm was measured after 30 min of reaction in a spectrophotometer. The reaction was carried out in closed Eppendorf tubes shaken at 20 ºC. The blank reference cuvette contained methanol.

For antioxidant activity determination using the ABTS assay, the method used was based in procedure described by RE *et al.* (1999). Thus ABTS was dissolved in potassium persulfate to a 7 mM concentration. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 hours before use. The $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.700 at 734 nm. Each fraction, properly diluted, 0.1 mL was added to 1.7 mL of ABTS solution and stirred. The decrease in absorbance was determined at 734 nm after 15 min.

Trolox was used as standard reference data in the two antioxidant methods used. All measurements were performed in duplicate.

6 - Statistical analysis

In order to study the influence of the different fining agents and concentration applied in phenolic parameters studied and antioxidant activity, an analysis of variance and comparison of treatment means (ANOVA, one-way) was performed using SPSS software program version 11.0 (SPSS Inc. Headquarters, Chicago, Illinois, USA).

RESULTS AND DISCUSSION

I - Effect of fining treatments on general phenolic composition

Total phenols and anthocyanins, colour density and hue of both fined and unfined red wines are present in table 1. The results in table 1, showed clearly that in general the different fining agents applied (especially for the highest fining agent's concentration used) induced a decrease of the total phenols content present in wines. The major significant decrease of the total phenols content was for isinglass (IS50 – 28.3%) followed by egg albumin (EA50 – 27.2%). For EA30 and G50 fining treatment a significant total phenolic content reduction occurred as well. These results, especially for egg albumin and gelatine, are in accordance with previously works published (GÓMEZ-PLAZA *et al.*, 2000; MAURY *et al.*, 2001). In contrast, bentonite (for all concentrations used), isinglass (IS5 and IS30) and potassium caseinate (PC5 and PC 30) were the fining agents used where a less wine total phenol content decrease was quantified.

Regarding the total anthocyanins (table 1), in general all fining agents induced a reduction of these compounds,

Table 1 - Analytical results for total phenol, total anthocyanin, colour density and colour hue of the unfined red wine and the red wine after different fining treatments.

Fining Treatments	Total phenol (mg/L gallic acid equiv.)	Total anthocyanin (mg/L malvidin-3-glucoside equiv.)	Colour density (abs. units)	Colour hue (abs. units)
T	$2980.6^{A} \pm 12.3$	$397.0^{A} \pm 0.3$	$18.9^{A} \pm 0.26$	$0.66^{A} \pm 0.01$
EA5	$2800.9^{B} \pm 10.2$	$363.0^{B} \pm 0.1$	$16.9^{B} \pm 0.24$	$0.65^{A} \pm 0.03$
EA30	$2175.0^{C} \pm 5.4$	$361.8^{B} \pm 0.4$	$17.5^{B} \pm 0.25$	$0.64^{A} \pm 0.02$
EA50	$2169.6^{C} \pm 9.3$	$354.1^{B} \pm 0.2$	$17.2^{B} \pm 0.23$	$0.65^{A} \pm 0.01$
B5	$2784.1^{B} \pm 4.5$	$394.4^{A} \pm 0.6$	$17.8^{B} \pm 0.25$	$0.61^{B} \pm 0.2$
B30	$2753.3^{B} \pm 6.7$	$393.3^{A} \pm 0.1$	$16.9^{B} \pm 0.19$	$0.61^{B} \pm 0.03$
B50	$2743.0^{B} \pm 7.6$	$386.5^{A} \pm 0.2$	$17.2^{B} \pm 0.21$	$0.65^{A} \pm 0.01$
G5	$2891.7^{B} \pm 10.2$	$383.2^{A} \pm 0.4$	$18.4^{A} \pm 0.26$	$0.64^{A} \pm 0.01$
G30	$2794.3^{B} \pm 3.9$	$338.8^{C} \pm 0.1$	$18.3^{A} \pm 0.27$	$0.67^{A} \pm 0.03$
G50	$2253.3^{C} \pm 17.1$	$306.4^{C} \pm 0.4$	$17.8^{B} \pm 0.26$	$0.66^{A} \pm 0.05$
IS5	$2955.8^{A} \pm 8.1$	$374.3^{B} \pm 0.6$	$17.7^{B} \pm 0.27$	$0.67^{A} \pm 0.02$
IS30	$2937.9^{A} \pm 6.5$	$368.3^{B} \pm 0.1$	$17.3^{B} \pm 0.12$	$0.66^{A} \pm 0.02$
IS50	$2135.3^{C} \pm 7.8$	$160.1^{D} \pm 0.7$	$10.7^{C} \pm 0.16$	$0.60^{B} \pm 0.3$
PC5	$2971.2^{A} \pm 4.9$	$384.4^{A} \pm 1.0$	$18.3^{A} \pm 0.23$	$0.64^{A} \pm 0.02$
PC30	$2955.8^{A} \pm 9.3$	$380.4^{A} \pm 0.7$	$17.5^{B} \pm 0.56$	$0.66^{A} \pm 0.01$
PC50	$2845.6^{B} \pm 14.1$	$370.7^{B} \pm 0.3$	$17.4^{B} \pm 0.31$	$0.66^{A} \pm 0.01$

(T) Unfined wine, (EA5) egg albumin 5 g/hL, (EA30) egg albumin 30 g/hL, (EA50) egg albumin 50 g/hL, (B5) bentonite 5 g/hL, (B30) bentonite 30 g/hL, (B50) bentonite 50 g/hL, (G5) gelatine 5 mL/hL, (G30) gelatine 30 mL/hL, (G50) gelatine 50 mL/hL, (IS5) isinglass 5 mL/hL, (IS30) isinglass 30 mL/hL, (IS50) isinglass 50mL/hL, (PC5) potassium caseinate 5 g/hL, (PC30) potassium caseinate 30 g/hL, (PC50) potassium caseinate 50 g/hL. Means within a column followed by the same letter are not significantly different ($p<0.05$). ± Standard deviation.

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except for bentonite (all concentrations used) and gelatine (G5), where the decrease of the total anthocyanins content were not significant. Potassium caseinate only induced a significant decrease of the total anthocyanins in the highest concentration used (PC50). Recently COSME *et al.* (2007) using similar potassium caseinate concentration reported a high decrease of the total monomeric anthocyanins too. Additionally, the highest isinglass concentration applied (IS50), induced the major total anthocyanins reduction (above 50%) in red wines.

The data obtained for colour density of the unfined and fined wines showed that in general there were significant changes after fining. Thus, in all fined wines, except for gelatine (G5 and G30) and potassium caseinate (PC5), the colour density decrease was significant. The greatest decrease was found for wine fined with the highest concentration of isinglass (IS50) with a reduction of 43%. This result is correlated with the strong removal of the total anthocyanins that occurred in the wine fined with high isinglass concentration (IS50). The total anthocyanins and colour density decrease obtained in our study in fined wines was expected due to previous reports of the same effects (SARNI-MACHADO *et al.*, 1999; MAURY *et al.*, 2001; COSME *et al.*, 2007; CASTILLO-SÁNCHEZ *et al.*, 2008).

Regarding to the results obtained for the colour hue, between each fined wine and the unfined wine, only for bentonite (B5 and B30) and isinglass (IS50) a significant colour hue decrease was quantified. For the other wines similar colour hue values were obtained.

2 - Effect of fining treatments on different proanthocyanidin fraction

The effect of the different fining agents used on the different proanthocyanidin fractions quantified in fined wine are present in table 2. Thus in general, it was observed that all fining agents induced a decrease of all proanthocyanidin fractions studied.

The fining agents that removed the monomeric flavanols most strongly were egg albumin (EA5 – 36.3%; EA30 – 54.5% and EA50 – 56.0%) isinglass (IS50 – 52.2%) and gelatine (G50 – 43.1%). Potassium caseinate, bentonite and gelatine (except for G50) had no statisticall different effect ($p<0.05$) on monomeric flavanols. These results are in accordance with previous studies reported by COSME *et al.* (2008). According to the authors the various fining agents commonly used in enology had different efficiencies in removing the monomeric flavanols ((+)-catechin and (-)-epicatechin).

In the case of oligomeric flavanols a significant decrease ($p<0.05$) was observed for the highest protein fining agents employed (EA50 - 31.9%; G50 – 35.0% and IS50 – 56.2%). For all potassium caseinate and bentonite concentration used no statistical differences were observed between unfined and fined wine. Thus, it appears that these mineral nature fining agents do not have an impact on oligomeric flavanols content of fined red wines.

Recently COSME *et al.* (2008), studied the effect of the fining agents in some dimeric procyanidins in white wines. These authors reported that potassium caseinate

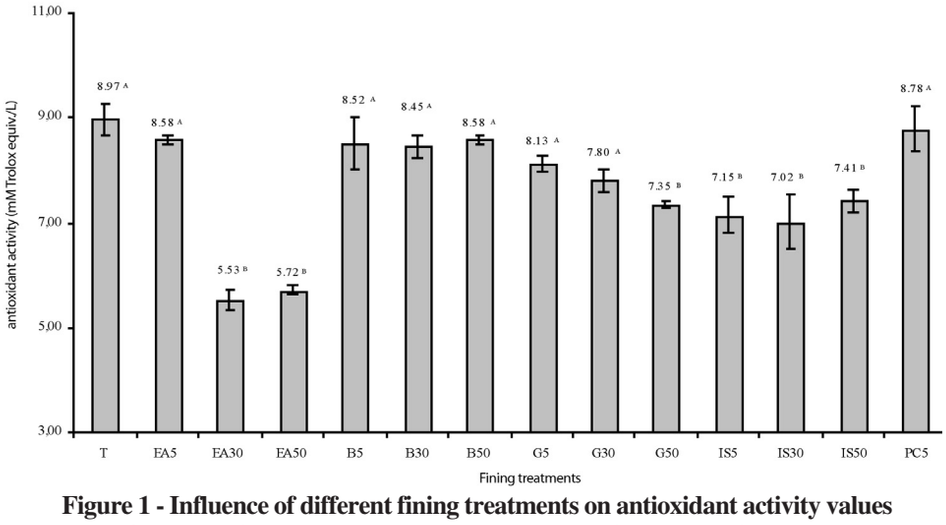


Figure 1 - Influence of different fining treatments on antioxidant activity values of the red wine evaluated by DPPH method radical scavenging assay.

(T) Unfined wine, (EA5) egg albumin 5 g/hL, (EA30) egg albumin 30 g/hL, (EA50) egg albumin 50 g/Hl, (B5) bentonite 5 g/hL, (B30) bentonite 30 g/hL, (B50) bentonite 50 g/hL, (G5) gelatine 5 mL/hL, (G30) gelatine 30 mL/hL, (G50) gelatine 50 mL/hL, (IS5) isinglass 5 mL/hL, (IS30) isinglass 30 mL/hL, (IS50) isinglass 50mL/hL, (PC5) potassium caseinate 5 g/hL, (PC30) potassium caseinate 30 g/hL, (PC50) potassium caseinate 50 g/hL. Means followed by the same letter are not significantly different ($p<0.05$). Error bars indicate standard deviation.

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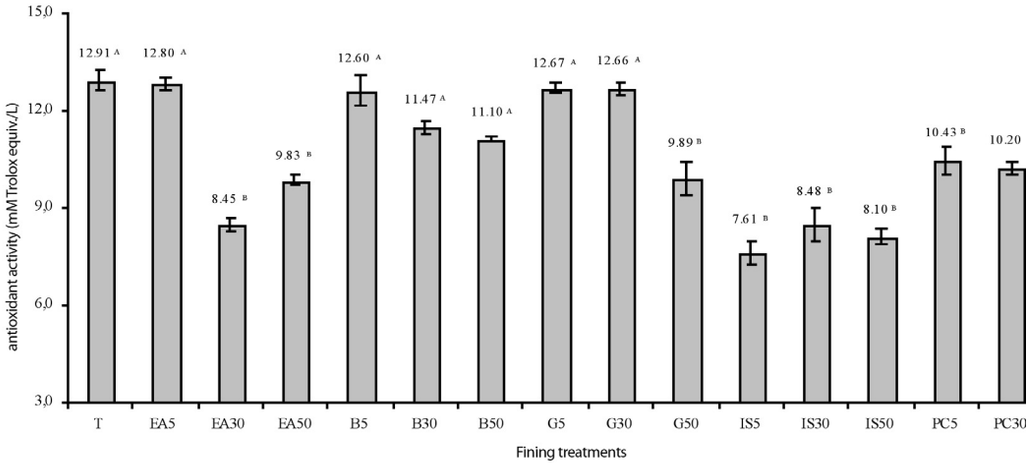


Figure 2 - Influence of different fining treatments on antioxidant activity values of the red wine evaluated by ABTS radical cation scavenging assay.

(T) Unified wine, (EA5) egg albumin 5 g/hL, (EA30) egg albumin 30 g/hL, (EA50) egg albumin 50 g/Hl, (B5) bentonite 5 g/hL, (B30) bentonite 30 g/hL, (B50) bentonite 50 g/hL, (G5) gelatine 5 mL/hL, (G30) gelatine 30 mL/hL, (G50) gelatine 50 mL/hL, (IS5) isinglass 5 mL/hL, (IS30) isinglass 30 mL/hL, (IS50) isinglass 50mL/hL, (PC5) potassium caseinate 5 g/hL, (PC30) potassium caseinate 30 g/hL, (PC50) potassium caseinate 50 g/hL. Means followed by the same letter are not significantly different ($p<0.05$). Error bars indicate standard deviation.

did not influence the individual dimeric procyanidins (B1, B2, B3 and B4) concentration of fined wine, which contrasted with egg albumin, gelatine and isinglass, which induced a significant decrease in all dimeric procyanidins. MACHADO-NUNES *et al.* (1998) also observed that protein fining agents (especially casein) decreased procyanidins in wines. However, JOUVE *et al.* (1989) did not find significant decreases of oligomeric procyanidins (dimeric and trimeirc) with protein fining agents.

The polymeric flavanols were decreased significantly ($p<0.05$) by the two egg albumin concentration (EA30 - 32.2% and EA50 – 35.3%) and by the highest gelatine concentration used (G50 – 30.2%). None of the isinglass decreases of the concentration of these compounds were significant (only from 1.6 to 5.7%). According to BONERZ *et al.* (2004) the proteinaceous removed proanthocyanidins only from wine with a low mean degree of polymerisation. However SARNI-MANCHADO *et al.* (1999) did not find significant decrease of phenolic composition with gelatine. Nevertheless, the polyphenols precipitated were characteristic, because gelatins preferentially removed high molecular weight galloyted.

3 - Effect of fining treatments on ontioxidant activity

Results obtained from antioxidant activity (using the DPPH and ABTS method) of the clarified red wine with the different commercial fining agents and concentrations are shown in figures 1 and 2.

In general, for all fining treatments a reduction of the antioxidant activity values was quantified in both two methods used. The decrease of the values was more evident when the antioxidant activity was evaluated by the ABTS radical cation scavenging assay (figure 2) than by use of the DPPH method radical scavenging assay (figure 1). However, the ranking of the samples was similar for the results obtained in the two antioxidant activity methods used. Thus for example considering the antioxidant values quantified using the ABTS method in red wine fined with isinglass and potassium caseinate, the percentage values decreased from 34.3 to 41.0% and from 19.2 to 34.1%, respectively. In contrast, the antioxidant values quantified by the DPPH method for the same fining agents decreased only from 17.3 to 21.7% and from 2.0% to 8.6%, respectively. As a consequence of the results obtained it is possible to conclude that the ABTS method shows a slightly more sensitive action than the DPPH method. This result is in accordance with the study previously published by VILLAÑO *et al.* (2006). At the same time to understand these differences, it is important to consider that $ABTS^{+}$ and $DPPH^{\bullet}$ radicals have different stereochemical structures and different paths of genesis and thus they give, after the reaction with the antioxidants, qualitatively different responses to the inactivation of each radical.

The statistical analysis results using the ABTS method for the individual effects of each fining agents used (figure 2), show that isinglass induced the major significant reduction ($p<0.05$) of antioxidant activity (IS5 – 41.0%, IS30 – 34.3% and IS50 – 37.2%) followed by egg albumin (EA30 – 34.5% and EA50 – 23.8%). However, between the red wines that were fined with these fining agents no statistical differences occurred. Bentonite and gelatine (except for the highest gelatine

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Table 2 - Monomeric, oligomeric and polymeric fraction of proanthocyanidins in the unfined red wine and the red wine after different fining treatments.

Fining Treatments	Monomeric fraction (mg/L)	Oligomeric fraction (mg/L)	Polymeric fraction (mg/L)
T	13.2 ^{A} ± 0.9	70.2 ^{A} ± 2.1	620.8 ^{A} ± 3.9
EA5	8.4 ^{B} ± 0.5	65.3 ^{A} ± 3.5	550.3 ^{B} ± 7.7
EA30	6.0 ^{C} ± 0.4	50.7 ^{B} ± 2.1	420.9 ^{C} ± 6.9
EA50	5.8 ^{C} ± 0.2	47.8 ^{B} ± 4.5	401.3 ^{C} ± 5.2
B5	11.8 ^{A} ± 1.2	66.1 ^{A} ± 2.2	601.3 ^{A} ± 2.5
B30	11.5 ^{A} ± 0.8	62.3 ^{A} ± 2.8	595.7 ^{A} ± 4.8
B50	11.0 ^{A} ± 0.5	55.5 ^{B} ± 1.2	591.3 ^{A} ± 3.2
G5	11.3 ^{A} ± 0.7	68.3 ^{A} ± 0.9	590.1 ^{A} ± 1.7
G30	11.0 ^{A} ± 1.0	60.7 ^{A} ± 2.4	569.3 ^{B} ± 2.1
G50	7.5 ^{C} ± 0.4	45.6 ^{B} ± 1.7	432.7 ^{C} ± 1.7
IS5	10.8 ^{A} ± 0.9	67.3 ^{A} ± 0.4	610.3 ^{A} ± 0.9
IS30	9.0 ^{B} ± 1.2	63.4 ^{A} ± 0.9	595.7 ^{A} ± 6.3
IS50	6.3 ^{C} ± 0.7	30.7 ^{D} ± 3.2	585.2 ^{A} ± 2.9
PC5	12.5 ^{A} ± 1.2	68.3 ^{A} ± 0.7	613.2 ^{A} ± 3.8
PC30	12.0 ^{A} ± 0.4	64.7 ^{A} ± 1.2	597.1 ^{A} ± 2.9
PC50	12.1 ^{A} ± 0.3	60.3 ^{A} ± 2.3	587.3 ^{A} ± 4.8

(T) Unfined wine, (EA5) egg albumin 5 g/hL, (EA30) egg albumin 30 g/hL, (EA50) egg albumin 50 g/hL, (B5) bentonite 5 g/hL, (B30) bentonite 30 g/hL, (B50) bentonite 50 g/hL, (G5) gelatine 5 mL/hL, (G30) gelatine 30 mL/hL, (G50) gelatine 50 mL/hL, (IS5) isinglass 5 mL/hL, (IS30) isinglass 30 mL/hL, (IS50) isinglass 50mL/hL, (PC5) potassium caseinate 5 g/hL, (PC30) potassium caseinate 30 g/hL, (PC50) potassium caseinate 50 g/hL. Means within a column followed by the same letter are not significantly different ($p<0.05$). ± Standard deviation.

concentration used) didn't induce a significantly antioxidant activity decrease. Concerning the results of the effect of the different fining agents concentration applied by the statistical analysis it can be concluded that the increase of concentrations didn't change the wine antioxidant activity decrease significantly, except for egg albumin and gelatine. This entire tendency was similar when the antioxidant activity was measured using the DPPH method (figure 1).

The major antioxidant activity decreases as a result of these applied fining agents, can be explained by the total phenols and the different proanthocyanidins fraction content decrease reported in tables 1 and 2. Some authors demonstrated a direct correlation between antioxidant activity of red wines and their total phenols content (BURNS *et al.*, 2000; FERNÁNDEZ-PACHÓN *et al.*, 2006; CIMINO *et al.*, 2007). Additionally, the antioxidant activity decrease obtained in our work was quite similar to those of red wines from the Syrah red grape variety clarified with egg albumin, previously reported by VILLAÑO *et al.* (2006). For these authors, the antioxidant activity decrease could be explained by the considerable flavan-3-ols and anthocyanins decrease in wines fined with egg albumin. However, ANOVA analyses showed no significant differences between clarified and non clarified wines.

The correlations between the two antioxidant activity methods used and the different proanthocyanidin fraction (monomeric, oligomeric and polymeric) content for the different fining agents and their concentration were also determined (table 3). In general among the several commercial fining products assayed, high correlations were obtained between the antioxidant activity and the different proanthocyanidin fraction content. However for both methods used the wines fined with isinglass fining agent showed a low R^2 value, especially for the correlations that included the oligomeric and polymeric fraction.

The antioxidant potential of red wines is believed to depend on a great extent on the flavanol content (SÁNCHEZ-MORENO *et al.*, 2000; BURNS *et al.*, 2000; FERNÁNDEZ-PACHÓN *et al.*, 2006), but is also influenced by the relative amounts of the individual flavanols. According to FERNÁNDEZ-PACHÓN *et al.* (2004), fifty percent of the total red wines scavenging

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Table 3 - Correlation between proanthocyanidin fraction and antioxidant activity in the red wines treated with various fining agents (include the different fining concentration).

	Correlation coefficients (R^2)	
Fining agents/Variables	Antioxidant methods	
	DPPH	ABTS
Egg albumin		
MF	0.745	0.640
OF	0.966	0.848
PF	0.947	0.834
Bentonite		
MF	0.741	0.758
OF	0.643	0.899
PF	0.792	0.759
Gelatine		
MF	0.860	0.887
OF	0.753	0.897
PF	0.731	0.966
Isinglass		
MF	0.460	0.520
OF	<0.20	<0.20
PF	<0.20	<0.20
Potassium caseinate		
MF	0.556	0.790
OF	0.972	0.837
PF	0.899	0.827

MF – Monomeric fraction; OF – Oligomeric fraction; PF – Polymeric fraction

radical activity are attributed to polymeric phenolic compounds. This hypothesis is supported by the fact that the different fining agent's natures have a different effect in phenolic composition, especially in flavanol monomers and dimmers. Several authors (SARNI-MACHADO *et al.*, 1999; MAURY *et al.*, 2001; COSME *et al.*, 2008) reported that some fining agents (plant proteins and gelatines) have a selective action, precipitating highly polymerized and highly galloylated tannins from red wines. Probably fining agents have a selective action on individual phenolic composition and at the same time these individual compounds contribute in different ways to the red wine antioxidant activity. Further studies are needed to evaluate the selective action of each fining agent on individual phenolic compounds and relate that selective action with antioxidant capacity.

CONCLUSIONS

In general, the commercial fining agents analyzed in the present study affected the proanthocyanidin content (especially the monomeric and the oligomeric) but did not have great effect on the antioxidant potential of red wines. However, for the highest egg albumin, gelatine and isinglass concentration the results showed a significant decrease of antioxidant activity content. Additionally for all fining agents used (except for isinglass) a high correlation between the different proanthocyanidin fraction and antioxidant activity was obtained.

These results are of great relevance when the clarification of red wine is made using fining agents. Thus considering the importance of antioxidant properties of red wines it will be necessary to make a fining optimization treatment not only considering the taste, colour, bouquet and clarity, but the proanthocyanidin content and the antioxidant activity properties also. Taking this care it will be more important to apply a moderate and/or proper concentration of fining agent to obtain a good red wine clarification and stabilization.

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