FINING OF RED WINES: EFFECTS ON THEIR ANALYTICAL AND SENSORY PARAMETERS

Asimenia KARAMANIDOU[1], Stamatina KALLITHRAKA[2]
and Efimia HATZIDIMITRIOU[1],*

1: Laboratory of Food Chemistry and Technology, School of Chemistry, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece
2: NAGREF, Wine Institute of Athens, 1 S. Venizelou St., Lykovrisi, 14123 Athens, Greece

Abstract

Aim: The aim of the present study was to examine the influence of protein fining on selected quality parameters of wines made from indigenous Hellenic red grape varieties.

Methods and Results: Three different commercial hydrolyzed gelatins and egg albumin were added to two young red wines at three concentrations commonly used in winemaking. The cultivars selected were Hellenic native *V. vinifera* species used for the production of high quality Appellation of Origin wines (Agiorgitiko and Xinomavro). All the quality parameters studied (anthocyanin concentration, color intensity and hue, ionization degree, total phenolic content, DPPH radical scavenging activity, tannin and polysaccharide concentration, gelatin and HCl indexes, as well as individual phenolic content) were significantly decreased after the addition of the fining agents. The decrease observed was mainly dependent on grape variety, which genetically determines the wine's phenolic composition, and to a lesser extent on the fining agent used and the dosage applied. In general, treated wines obtained better scores in sensory analysis as compared to untreated ones, although statistically significant differences were only obtained in the Agiorgitiko wine regarding color intensity and hue, acidity, balance, aftertaste, and overall impression.

Conclusion: Wine quality parameters after fining were mainly influenced by the wine's initial phenolic composition, which is determined mostly by grape variety.

Significance and impact of the study: The outcomes of such study might be of practical interest to winemakers since they could optimize red wine production technology by selecting the appropriate fining agent according to the specific phenolic profiles of the produced wines and thus improve their quality.

Keywords: red wine, protein fining, gelatin, egg albumin, phenolic compounds, polysaccharides

Résumé

Objectifs: La présente étude vise à examiner l'influence du collage avec des protéines, sur des paramètres de qualité sélectionnés de vins élaborés à partir de cépages indigènes helléniques rouges.

Méthodes et résultats: Trois différentes gélatines commerciales hydrolysées et de l'albumine d'œuf ont été ajoutés à deux jeunes vins rouges, à trois concentrations couramment utilisées en vinification. Les cépages sélectionnés étaient des espèces *V. vinifera* helléniques indigènes utilisés pour la production des vins d'Appellation d'Origine de haute qualité (Agiorgitiko et Xinomavro). Tous les paramètres de qualité étudiés (concentration en anthocyanes, intensité de la couleur et teinte, degré d'ionisation, teneur en composés phénoliques totaux, activité de piégeage du radical DPPH, concentration en tanins et polysaccharides, indices de gélatine et HCl, ainsi que concentration en composés phénoliques individuels) ont significativement diminué après l'addition des agents de collage. La diminution observée est essentiellement dépendante du cépage, qui détermine génétiquement la composition phénolique du vin, et dans une moindre mesure de l'agent de collage utilisé et la dose appliquée. En général, les vins traités ont obtenu de meilleurs scores dans l'analyse sensorielle comparés aux vins non traités, même si des différences statistiquement significatives ont été obtenues uniquement pour le vin Agiorgitiko en ce qui concerne l'intensité de la couleur et de la teinte, l'acidité, l'équilibre, l'arrière-goût et l'impression générale.

Conclusion: Les paramètres de qualité des vins après collage ont été principalement influencés par la composition phénolique initiale du vin, déterminée essentiellement par le cépage.

Importance et impact de l'étude: Les résultats de cette étude peuvent être d'intérêt pratique pour les vinificateurs, car ils pourraient optimiser la technologie de production de vins rouges en sélectionnant l'agent de collage approprié selon les profils spécifiques phénoliques des vins produits et améliorer ainsi leur qualité.

Mots clés: vin rouge, collage, gélatine, albumine d'œuf, composés phénoliques, polysaccharides

manuscript received 10 February 2010 - revised manuscript received 15th November 2010

*Corresponding author: effiehd@chem.auth.gr

J. Int. Sci. Vigne Vin, 2011, **45**, n°1, 47-60

INTRODUCTION

Phenolic compounds represent an important group of wine constituents. They are responsible for the major differences between white and red wines regarding color, taste and aging ability (Ribéreau-Gayon *et al.*, 1998a). Moreover, the beneficial effects of wines on health and more specifically the antioxidant properties related to the « French paradox » observed in France in the early 90's are attributed to their phenolic composition (Soleas *et al.*, 1997). Polysaccharides form another major group of macromolecules present in wines. They are of particular technological interest due to their contribution to the mouth-feel of the wine (Vidal *et al.*, 2004), their action as « protective colloids », their interaction with tannins (Riou *et al.*, 2002) and volatile compounds (Chalier *et al.*, 2007), and finally their contribution to protein and tartaric stabilization (Ledoux-Moine, 1996).

Fresh wine is a turbid, complex hydroalcoholic solution, microbiologically and physicochemically unstable. Its limpidity, highly appreciated by the consumer, is obtained by natural sedimentation of suspended particles by gravity and, alternatively or additionally, by processes like filtration and centrifugation. Nevertheless, a limpid wine has to remain that way throughout the whole period of bottle aging and storage, under any conditions. Clarification and stabilization of red wines is mainly achieved through the addition of proteins, a process known as « fining ». Fining describes the deliberate addition of an adsorptive compound that is followed by the settling or precipitation of partially soluble components from the wine. The products used for this purpose are referred to as fining agents (Boulton *et al.*, 1996). They include animal proteins such as gelatin, casein and egg albumin (Boulton *et al.*, 1996 ; Ribéreau-Gayon *et al.*, 1998b) ; plant proteins such as wheat glutens (Marchal *et al.*, 2002 ; Maury *et al.*, 2003) and lupin proteins (Maury *et al.*, 2003) ; polyvinylpolypyrrolidone (PVPP) and bentonite (Boulton *et al.*, 1996 ; Ribéreau-Gayon *et al.*, 1998b) ; and even polysaccharides extracted from seaweeds (Cabello-Pasini *et al.*, 2005).

Gelatin, one of the most commonly used fining agents with a wide range of applications, is a mixture of polypeptides derived from collagen, the primary protein component of animal connective tissues (bones, skin and tendons), by partial acid, alkaline or enzymatic hydrolysis (Poppe, 1999). Egg albumin, the oldest product used in wine fining, is also composed of various proteins, ovalbumin being the major one. It is usually recommended for the clarification of high quality, tannin-rich red wines as it selectively removes the astringent tannins and enhances the « mellowness » without altering the rest of the wine's sensory characteristics (Ribéreau-Gayon *et al.*, 1998b). Compared to other proteins, gelatin contains high concentrations of three non-polar amino acids : proline (13 %), hydroxyproline (11 %) and glycine (21 %) (Scotti and Poinsaut, 1997), and this composition is reported to be closely related to its fining potential (Singleton, 1967). Molecular weight distribution, isoelectric point and surface charge density are some other characteristics susceptible to influence the properties of fining agents (Lagune and Glories 1996a, b ; Versari *et al.*, 1998).

The ability of tannins to bind strongly to proteins, the most prominent physicochemical property of these molecules (Calderon *et al.*, 1968 ; Hagerman and Butler 1981), constitutes the basis of red wine fining. Protein fining is considered an essential clarifying process. Nevertheless, from various studies it emerges that the primary action of proteins on wine phenolics secondarily influences its sensory characteristics as well as its aging capacity, with total phenols (Yokotsuka and Singleton, 1987 ; Sims *et al.*, 1995 ; Yokotsuka and Singleton, 1995 ; Villaño *et al.*, 2006), anthocyanins (Ricardo-da-Silva *et al.*, 1991 ; Sarni-Manchado *et al.*, 1999 ; Stankoviç *et al.*, 2004 ; Castillo-Sánchez *et al.*, 2006, 2008), tannins (Yokotsuka and Singleton, 1987 ; Yokotsuka and Singleton, 1995 ; Maury *et al.*, 2001), color (Sims *et al.*, 1995 ; Stankoviç *et al.*, 2004 ; Castillo-Sánchez *et al.*, 2006, 2008) and astringency (Sims *et al.*, 1995 ; Maury *et al.*, 2001) being the components mostly studied. The extent of these modifications depends on the fining agent used and the wine's phenolic profile.

Among the Hellenic native red grape varieties, *Vitis vinifera* L. cv. Agiorgitiko and Xinomavro represent the characteristic varieties of Southern and Northern Greece, respectively. Agiorgitiko wines are generally characterized by their « intense red color with violet nuances, balanced acidity and soft tannins » while Xinomavro wines are presented with « rather light color, high acidity and fairly astringent tannins » (Spinthiropoulou, 2000). In the last years, a lot of research has been conducted concerning the flavonoid composition of these wines (Kallithraka *et al.*, 2001, 2005 ; Makris *et al.*, 2006). However, no data is available regarding the influence of some essential technological processes, like protein fining, on these wines' analytical composition and sensory character.

The primary objective of this study was to evaluate the use of four fining agents (three gelatins and egg albumin) in Agiorgitiko and Xinomavro winemaking by measuring certain red wine quality parameters. These were color parameters (anthocyanin concentration and ionization degree, intensity and hue), phenolic content (total phenols, individual phenols, tannins, gelatin and HCl indexes) and sensory characteristics (appearance, taste and appreciation). Possible influence on polysaccharide content and antioxidant activity was also studied.

MATERIALS AND METHODS

1. Wine samples

Two red experimental wines, vinified in 2006, were analyzed. The cultivars used were *Vitis vinifera* species, Agiorgitiko and Xinomavro, grown in the vineyards of Nemea and Naoussa, respectively. Grapes were destemmed and crushed before the addition of SO_2 (50 mg/L). Alcoholic fermentations were carried out at controlled temperature (28-32 °C) using indigenous microflora in both cases. After completion of malolactic fermentation, the wines were racked and SO_2 level was adjusted. The wine samples were analyzed six months after the end of malolactic fermentation.

2. Chemicals

Gallic acid (99.5 %) was from Sigma Chemical Co. (Germany). D-(+) galactose, absolute ethanol, methanol (97 %), sulphuric acid (95-97 % w/w) and hydrochloric acid (37 % w/w) were from Merck (Darmstadt, Germany). HPLC grade acetonitrile (MeCN) was from Mallinckrodt Baker (Deventer, Holland). $NaHSO_3$ (solution 38-40 %) and anhydrous Na_2CO_3 were from Riedel-de Haën (Seelze, Germany). DPPH (1,1-Diphenyl-2-picrylhydrazyl) free radical (90 %) was from Sigma Chemical Co. (St. Louis, MO, USA), Folin-Ciocalteu and crystallized phenol were from Panreac Quimica S.A. (Barcelona, Spain), and gelatin (Solugel) was from Martin Vialatte Œnologie (Epernay, France).

3. Protein fining agents

Four commercially available fining agents were used: three enological gelatins (noted G1, G2, and G3) and egg albumin (noted EA). Gelatins G2 and G3 were supplied in the form of aqueous solutions (250 and 200 g/L, respectively). Gelatin G1 was supplied as powder; therefore, a fresh solution (50 g/L) was prepared in hot water (60 °C) under continuous stirring as suggested by the manufacturer. Similarly, a solution of egg albumin (250 g/L) was prepared in cold water under gentle stirring and addition of anhydrous Na_2CO_3 (2 g/L) to facilitate dissolution. Characteristics of the fining agents are given in table 1. The dosages applied were 0.05, 0.1 and 0.2 g of fining agent/L of wine, representing the lowest and highest recommended dosage, in addition with an intermediate one, commonly used in red winemaking.

4. Fining experiments

For the fining experiments conducted in laboratory scale, 375 ml aliquots of wine of each grape variety were weighed (367 ± 0.2 g) and transferred in a 500-mL beaker. Each one of the four fining agents was added separately under intense stirring for better aeration and incorporation. Afterwards, wine samples were transferred into 375 ml glass bottles, sealed and left to settle for 5 days at 10 °C. The wines were then racked, centrifuged at 3500 rpm for 20 minutes and used for chemical and sensory analysis. Each fining trial was performed in duplicate. For each series of experiments, a control wine sample (375 ml) was prepared in exactly the same way, but without the addition of fining agents.

5. Analytical determinations

Ethanol content (% v/v), specific gravity (g/mL), titratable (g of tartaric acid/L) and volatile acidity (g of acetic acid/L), pH, reducing sugars (g/L), tartaric acid (g/L) and potassium (mg/L) were determined by FTIR (Fourier Transform Infrared) spectroscopy using WineScan™ FT120 Basic (FOSS, Denmark). Turbidity was determined as the ratio of scattered light to transmitted light using a Hach 2100P turbidimeter (Loveland, Colorado, USA). Direct measurements of wine absorbance at 420, 520 and 620 nm were carried out using a Hitachi U-2000 spectrophotometer (Tokyo, Japan). Color intensity was calculated as the sum of absorbances at 420, 520, and 620 nm (1 mm path length) and hue was calculated as the ratio between absorbance at 420 and 520 nm. Total anthocyanin content and ionization degree (%), as well as gelatin and HCl index were determined according to Ribéreau-Gayon *et al.* (1998a). The Glories method (Glories, 1978) was used for the determination of tannin concentration. Total phenol content was determined by the Folin-Ciocalteu assay using the method of Singleton and Rossi (1965). Results are expressed as gallic acid equivalents (GAE). The radical scavenging activity of the wines was determined according to the method of Arnous *et al.* (2001). All samples were diluted (1:10 v/v) with methanol prior to analysis. Results are

Table 1 - Fining agents used in the study.

Code	G1	G2	G3	EA
Fining agent	Gelatin	Gelatin	Gelatin	Egg albumin
Concentration (g/L)	50	250	200	250
Surface charge at pH 3,2 (µeq/g)	895	354	120	
Degree of hydrolysis	Very low	Medium	Very high	-
MW (Dalton)	64 000	20 000	5 000-10 000	

expressed as DPPH radical scavenging (%RSA) = [[$abs_{t=0}$] - [$abs_{t=30}$]/[$abs_{t=0}$]] x 100, where $abs_{t=0}$ is the absorbance of the 60 µM DPPH• methanolic solution at t = 0 min and abst = 30 is the absorbance of the mixture at t = 30 min. Total polysaccharides, expressed as mg D-galactose/L, were determined according to Segarra *et al.* (1995). All measurements were performed in triplicate.

6. HPLC determination of individual phenolics

The concentration of individual polyphenols was determined by HPLC, employing a direct-injection method according to Makris *et al.* (2003). Wines were filtered through 0.45 µm syringe filters prior to analysis. The equipment used was an HP 1050 M Series II liquid chromatograph coupled with a diode array detector and controlled by Agilent ChemStation software. The column was a LiChrospher RP-18, 5 µm, 250x4 mm (Merck), protected by a guard column packed with the same material. Both columns were maintained at 40°C. Eluent (A) was 9 mm aqueous orthophosphoric acid (pH 2.5), eluent (B) was MeCN : water (4:6) containing 9 mm orthophosphoric acid, and the flow rate was 1 ml/min. The elution was as follows : 100 % A for 20 min, from 100 % A to 60 % A in 80 min, isocratic for 10 min, from 60 % A to 30 % A in 20 min, and then isocratic for another 10 min (total run time 140 min).

Peaks were identified by comparison of retention times and ultraviolet (UV) spectra with commercial standards : gallic acid, protocatechuic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, (+)-catechin, (-)-epicatechin, myricetin, rutin (all from Sigma), and procyanidins B1 and B2 (Extrasynthese, France). Procyanidins were expressed as mg/L (+)-catechin, whereas the rest of the compounds were expressed against their own calibration curves. Caftaric and coutaric acids were expressed as mg/L caffeic acid and p-coumaric acid, respectively. Detection was performed at 280, 320 and 360 nm for flavanols, hydroxycinnamates and flavonols, respectively. All analyses were performed in duplicate.

7. Sensory evaluation

Wine samples were rated on a scale of 0-8 by a panel of seven wine experts for different sensory characteristics : appearance [limpidity (clarity), brilliance, color intensity, color hue], taste (acidity, astringency, bitterness, mellow sensation, « body », balance), aftertaste and harmony/overall impression. The panelists first tasted and evaluated the two control wines, and then the fined

Table 2 - Analytical parameters of the wines used in the study.

	Agiorgitiko	Xinomavro
Ethanol (% v/v)	12.5	12.2
Specific gravity (g/mL)	0.9935	0.9935
Titratable acidity (g tartaric acid/L)	5.2	5.7
Volatile acidity (g acetic acid/L)	0.54	0.59
pH	3.56	3.46
Reducing sugars (g/L)	2.3	2.2
Tartaric acid (g/L)	2.5	2.8
Potassium (mg/L)	1052	823
Anthocyanins (mg/L)	328	108
Color intensity	6.35	3.04
Hue	0.759	0.926
Tannins (g/L)	2.3	3.0
Total phenols (ppm gallic acid equivalents)	2781	3936
Ionization degree	19.7	24.8
HCl index	34	25
Gelatin index	62	73
Polysaccharides (mg D-galactose/L)	764	689
Turbidity (NTU)	16.0	5.4

samples. Two sessions were performed. Results are expressed as the mean ratings of the two sensory analyses.

8. Statistical analysis

Statistical comparisons of the mean values of all the analytical parameters of wines were performed by one-way analysis of variance (One-way ANOVA), followed by the multiple Duncan test ($p < 0.05$ confidence level). All statistical analyses were performed using SPSS 14.0 program for Microsoft Windows (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

1. Analytical parameters of wines

The results of the analyses of the experimental wines are presented in table 2. The two wines were very similar in what is called the « conventional enological parameters » (ethanol content, specific gravity, titratable and volatile acidity, pH, reducing sugars), but they differed considerably in their phenolic profile. As far as color is concerned, the Agiorgitiko wine was richer in total anthocyanins, which were less ionized, and it had twice the color intensity and lower hue compared with the Xinomavro one. Regarding phenolic content, it contained less total phenols and less but more polymerized tannins (higher HCl index). It was also characterized by a higher polysaccharide content. The Xinomavro wine was typical of this grape variety characterized by the highly ionized anthocyanins and the more astringent tannins (higher gelatin index).

2. Effect of protein fining on wine composition

a) Color parameters

Protein fining treatment reduced the anthocyanin content in nearly all Agiorgitiko and Xinomavro samples by up to 18.5 % and 22 %, respectively (Table 3). This effect was statistically significant in the Agiorgitiko samples depending on fining agent, dosage and hydrolysis degree of the gelatins. In fact, the more (G3) and the less hydrolyzed gelatin (G1) resulted in the highest and lowest anthocyanin reduction, respectively, while treatments with EA showed the most pronounced effect on these compounds. In the Xinomavro wine, no significant effect was observed with gelatin G1, while as in the Agiorgitiko sample, the lowest significant anthocyanin content was noted after the addition of EA, followed by gelatin G2 and then gelatin G3. These findings are in agreement with Ricardo-da-Silva *et al.* (1991), who applied the same dosages (0.1 and 0.2 g/L) of four different gelatins in a Mourvèdre red wine and observed a reduction in the anthocyanin content between 8 and 28 %. In addition, Stankoviç *et al.* (2004) reported that gelatin addition (0.05 and 0.1 g/L) resulted in a decrease (2-10 % and 6-16 %, respectively) in the free, colorless, ionized and polymerized forms of anthocyanins in Vranac, Pinot noir and Gamay noir wines. On the other hand, pre-fermentation addition of bentonite and pvpp resulted in higher anthocyanin content in the fined wines compared to the untreated ones, probably due to the elimination of solids and yeast lees to which pigments could be otherwise fixed (Gómez-Plaza *et al.* 2000b, 2002). However, when the same products were added to the wines at a post-fermentation stage, a reduced anthocyanin content was observed after fining (Gómez-Plaza *et al.*, 2000a). Variations in the anthocyanin content after the use of gelatin, egg albumin, pvpp and casein were also reported in the work of Castillo-Sanchez *et al.* (2006, 2008).

Under the present experimental conditions, treatments with gelatins and EA had either no effect on wine color intensity and hue i. e. in the Xinomavro wine or resulted in a small decrease (up to 13 % for intensity and up to 4 % for hue with 0.2 g/L of gelatin G1) in the Agiorgitiko wine. Similar results were observed by other researchers as well (Ricardo-da-Silva *et al.*, 1991 ; Versari *et al.*, 1998 ; Stankoviç *et al.*, 2004).

Anthocyanin ionization degree reflects the percentage of anthocyanins present in wine as red flavylium form (A^+). Values vary between 10 and 30 % in young wines to between 80 and 90 % in aged ones (Ribéreau-Gayon *et al.*, 1998a). High ionization degree values are characteristic of young Xinomavro wines compared to wines from other Hellenic grape varieties (ENOAVE, 2005) (Table 2). For the Xinomavro samples, statistical analysis revealed no significant differences in the values of this analytical parameter after fining with gelatins. However, a small but significant increase was observed after the addition of 0.2 g/L EA. As regard to the Agiorgitiko wine, G3 and G2 had no influence on the ionization degree of anthocyanins, while G1 significantly reduced it (up to 12 % after the addition of 0.2 g/L). However, as observed in the Xinomavro wine, the highest concentration of EA significantly increased the ionization degree. A small reduction of ionized anthocyanins in red wines after the combined use of bentonite, gelatin and/or pvpp was also previously reported (Gil-Muñoz *et al.*, 1997 ; Gómez-Plaza *et al.*, 2000b ; Stankoviç *et al.*, 2004).

b) Total phenols and radical scavenging activity

Total phenolic content showed a significant yet not dramatic decrease in the treated samples of both grape varieties (Table 3). The % reduction varied from 0.9 (G2, 0.05 g/L) to 17 % (G1, 0.2 g/L) and from 4.1 (EA, 0.05 g/L) to 28.4 % (G1, 0.2 g/L) in the Agiorgitiko and Xinomavro samples, respectively. Fining agent, degree of hydrolysis of gelatins and dosage played a crucial role

Table 3 - Effects of fining on the anthocyanin content and ionization degree, color characteristics, total phenol content and radical scavenging activity towards the DPPH radical (%RSA) of the Agiorgitiko and Xinomavro wines.

Variety	Fining agent	Dosage (g/L)	Anthocyanins (mg/L)	Color intensity	Hue	Ionization degree (%)	Total phenols (ppm GAE)	RSA (%)
Agiorgitiko	G1	-	363 ± 18 ^{a}	6.35 ± 0.03 ^{a}	0.759 ± 0.00 ^{a}	19.7 ± 1.2 ^{a}	2781 ± 82 ^{a}	65.7 ± 2.3 ^{a}
		0.05	358 ± 11 ^{a,A}	6.03 ± 0.15 ^{b,B}	0.736 ± 0.01 ^{ab,A}	17.7 ± 1.4 ^{b,A}	2598 ± 36 ^{b,B}	62.6 ± 2.1 ^{b,BC}
		0.1	342 ± 10 ^{a,A}	5.64 ± 0.02 ^{c,B}	0.736 ± 0.01 ^{ab,A}	17.6 ± 1.2 ^{b,A}	2380 ± 30 ^{c,C}	58.4 ± 2.7 ^{c,C}
		0.2	332 ± 4 ^{b,B}	5.52 ± 0.03 ^{c,B}	0.729 ± 0.01 ^{b,A}	17.3 ± 1.6 ^{b,B}	2312 ±44 ^{d,D}	58.6 ± 1.3 ^{c,B}
	G2	-	363 ± 18 ^{a}	6.35 ± 0.03 ^{a}	0.759 ± 0.00 ^{a}	19.7 ± 1.2 ^{ab}	2781 ± 82 ^{a}	65.7 ± 2.3 ^{a}
		0.05	354 ± 2 ^{ab,A}	6.21 ± 0.03 ^{a,AB}	0.736 ± 0.02 ^{a,A}	19.8 ± 1.6 ^{ab,A}	2757 ± 103 ^{a,A}	67.1 ± 3.3 $^{a, AB}$
		0.1	344 ± 5 ^{b,A}	6.20 ± 0.02 ^{a,A}	0.724 ± 0.03 ^{a,A}	18.3 ± 1.1 ^{b,A}	2714 ± 67 ^{ab,A}	64.6 ± 2.7 ^{a,B}
		0.2	353 ± 1 ^{ab,A}	6.21 ± 0.43 ^{a,A}	0.721 ± 0.03 ^{a,A}	20.7 ± 1.2 ^{a,A}	2632 ± 76 ^{b,A}	57.0 ± 2.7 ^{b,B}
	G3	-	363 ± 18 ^{a}	6.35 ± 0.03 ^{a}	0.759 ± 0.00 ^{a}	19.7 ± 1.2 ^{a}	2781 ± 82 ^{a}	65.7 ± 2.3 ^{a}
		0.05	317 ± 8 ^{b,B}	6.37 ± 0.29 ^{a,A}	0.733 ± 0.03 ^{a,A}	19.0 ± 2.6 ^{a,A}	2610 ± 87 ^{b,B}	61.7 ± 4.6 ^{b,C}
		0.1	324 ± 7 ^{b,B}	6.25 ± 0.22 ^{a,A}	0.729 ± 0.02 ^{a,A}	19.2 ± 1.4 ^{a,A}	2595 ± 66 ^{b,B}	58.2 ± 1.5 ^{c,C}
		0.2	313 ± 4 ^{b,C}	5.81 ± 0.18 ^{b,AB}	0.726 ± 0.02 ^{a,A}	18.9 ± 2.6 ^{a,AB}	2549 ± 45 ^{b,B}	55.5 ± 1.8 ^{c,C}
	EA	-	363 ± 18 ^{a}	6.35 ± 0.03 ^{a}	0.759 ± 0.00 ^{a}	19.7 ± 1.2 ^{ab}	2781 ± 82 ^{a}	65.7 ± 2.3 ^{a}
		0.05	323 ± 3 ^{b,B}	6.38 ± 0.01 ^{a,A}	0.733 ± 0.03 ^{a,A}	18.1 ± 2.4 ^{b,A}	2649 ± 71 ^{b,B}	69.5 ± 7.8 ^{a,A}
		0.1	307 ± 5 ^{bc,C}	6.37 ± 0.34 ^{a,A}	0.733 ± 0.03 ^{a,A}	19.7 ± 1.3 ^{ab,A}	2709 ± 95 ^{bc,A}	67.7 ± 4.2 ^{a,A}
		0.2	296 ± 4 ^{c,D}	6.09 ± 0.14 ^{b,A}	0.733 ± 0.02 ^{a,A}	20.3 ± 0.9 ^{a,AB}	2402 ± 62 ^{c,C}	67.6 ± 2.1 ^{a,A}
Xinomavro	G1	-	108 ± 13 ^{a}	3.04 ± 0.12 ^{a}	0.926 ± 0.02 ^{ab}	24.8 ± 3.2 ^{a}	3737 ± 37 ^{a}	78.2 ± 1.6 ^{a}
		0.05	109 ± 10 ^{a,A}	2.94 ± 0.16 ^{a,AB}	0.910 ± 0.02 ^{a,A}	23.8 ± 3.8 ^{a,A}	3360 ± 55 ^{b,B}	64.4 ± 2.1 ^{b,B}
		0.1	107 ± 13 ^{a,A}	2.77 ± 0.17 ^{a,C}	0.901 ± 0.02 ^{a,A}	24.7 ± 3.5 ^{a,A}	2754 ± 89 ^{c,C}	63.3 ± 2.5 ^{b,B}
		0.2	109 ± 13 ^{a,A}	2.73 ± 0.18 ^{a,A}	0.893 ± 0.02 ^{a,B}	25.1 ± 4.0 ^{a,A}	2677 ± 58 ^{d,D}	58.4 ± 0.9 ^{c,B}
	G2	-	108 ± 13 ^{a}	3.04 ± 0.12 ^{a}	0.926 ± 0.02 ^{ab}	24.8 ± 3.2 ^{a}	3737 ± 37 ^{a}	78.2 ± 1.6 ^{a}
		0.05	86 ± 22 ^{a,B}	2.90 ± 0.05 ^{a,B}	0.907 ± 0.03 ^{ab,A}	22.9 ± 2.8 ^{a,A}	3164 ± 73 ^{b,D}	74.8 ± 2.4 ^{a,A}
		0.1	85 ± 22 ^{a,B}	2.84 ± 0.03 ^{a,BC}	0.902 ± 0.02 ^{b,A}	24.9 ± 2.6 ^{a,A}	3140 ± 58 ^{b,B}	71.9 ± 4.5 ^{ab,A}
		0.2	88 ± 24 ^{a,A}	3.17 ± 0.42 ^{a,A}	0.974 ± 0.04 ^{a,A}	24.2 ± 1.6 ^{a,A}	3080 ± 20 ^{b,B}	69.9 ± 4.6 ^{bc,A}
	G3	-	108 ± 13 ^{a}	3.04 ± 0.12 ^{a}	0.926 ± 0.02 ^{ab}	24.8 ± 3.2 ^{a}	3737 ± 37 ^{a}	78.2 ± 1.6 ^{a}
		0.05	97 ± 10 ^{a,AB}	3.12 ± 0.12 ^{a,A}	0.918 ± 0.01 ^{a,A}	23.4 ± 4.0 ^{a,A}	3256 ± 39 ^{b,C}	74.6 ± 2.5 ^{a,A}
		0.1	99 ± 9 ^{a,AB}	3.21 ± 0.18 ^{a,A}	0.917 ± 0.01 ^{a,A}	24.5 ± 5.7 ^{a,A}	3161 ± 38 ^{bc,B}	73.1 ± 2.3 ^{ab,A}
		0.2	95 ± 11 ^{a,A}	3.00 ± 0.11 ^{a,A}	0.914 ± 0.01 ^{a,B}	24.4 ± 3.8 ^{a,A}	2974 ± 50 ^{c,C}	70.9 ± 2.9 ^{bc,A}
	EA	-	108 ± 13 ^{a}	3.04 ± 0.12 ^{a}	0.926 ± 0.02 ^{a}	24.8 ± 3.2 ^{b}	3737 ± 37 ^{a}	78.2 ± 1.6 ^{a}
		0.05	88 ± 17 ^{b,B}	3.15 ± 0.05 ^{a,A}	0.935 ± 0.00 ^{a,A}	26.9 ± 2.4 ^{a,A}	3585 ± 55 ^{b,A}	73.0 ± 2.5 ^{a,A}
		0.1	84 ± 14 ^{b,B}	3.04 ± 0.06 ^{a,AB}	0.934 ± 0.00 ^{a,A}	28.8 ± 0.6 ^{ab,A}	3427 ± 29 ^{bc,A}	70.8 ± 1.4 ^{ab,A}
		0.2	87 ± 17 ^{b,A}	3.01 ± 0.05 ^{a,A}	0.919 ± 0.00 $^{a, AB}$	28.8 ± 2.5 ^{a,A}	3246 ± 56 ^{c,A}	69.0 ± 2.9 ^{bc,A}

G1,G2,G3: Gelatin 1,2,3; EA: Egg Albumin; $^{a-c}$ Within the same fining agent and the same analytical parameter, different lowercase letters are significantly different at $p < 0.05$; $^{A–D}$ Regarding the same dosage and the same analytical parameter, different uppercase letters are significantly different at $p < 0.05$; $n = 6$.

in the variation of the results, in accordance with other authors (Ricardo-da-Silva *et al.*, 1991 ; Sims *et al.*, 1995 ; Versari *et al.*, 1998 ; Gómez-Plaza *et al.*, 2000a ; Gómez-Plaza *et al.*, 2002 ; Stankoviç *et al.*, 2004 ; Villaño *et al.*, 2006).

It is well known that wine total phenolic content is linearly related with its radical scavenging activity (Paixão *et al.*, 2007 ; Li *et al.*, 2009 ; Alén-Ruiz *et al.*, 2009). Since fining affects mainly wine phenolics, it was of interest to examine how antiradical activity is influenced by this treatment. Results (%RSA) are given in Table 3. The wine samples were diluted 1:10 v/v prior to analysis in order to achieve %RSA values between 60 and 80 %, where differences are better expressed (Nenadis and Tsimidou, 2002).

In general, the untreated Xinomavro wine exhibited a higher %RSA value (78.2 %) than the untreated Agiorgitiko wine (65.7 %). Fining reduced %RSA in all the samples analyzed, in accordance with other authors (Villaño *et al.*, 2006). As expected, results varied with regard to grape variety, fining agent and dosage. The use of the less hydrolyzed gelatin G1 in Agiorgitiko wine significantly decreased %RSA (up to 10.8 % with the addition of 0.2 g/L), while that of gelatin G2 and EA had little and no effect, respectively. A 15.5 % reduction was also observed with the use of the more hydrolyzed gelatin G3. Regarding Xinomavro wine, gelatin G3 induced the lowest reduction in the %RSA value (7 %) and gelatin G1 induced the greatest one (25 %).

c) Tannins, gelatin index, and HCl index

Tannin-protein interaction results in the precipitation of the complexes formed. The most significant fining effect leading to a 26.1 % reduction of tannin concentration in the Agiorgitiko wine was noted after the addition of increasing concentrations of gelatin G2. The less hydrolyzed gelatin G1 resulted in a 17.4 % reduction followed by the more hydrolyzed gelatin G3 and EA (Table 4). On the contrary, in the Xinomavro wine, only EA had a notable effect (removed 13.3 % of tannins). Tannin as well as protein characteristics such as molecular size and weight (Hagerman and Butler, 1981 ; Yokotsuka and Singleton, 1987), relative concentrations (Hagerman and Butler, 1981), « proline richness » of the proteins (Hagerman and Butler, 1981 ; Yokotsuka and Singleton, 1987 ; Ricardo-da-Silva *et al.*, 1991), and structure and charge of the tannins (Ribéreau-Gayon *et al.*, 1998a) are usually responsible for this type of interactions. Larger protein molecules (lower hydrolysis degree) precipitate lower amount of tannins (Yokotsuka and Singleton, 1987 ; Sarni-Manchado *et al.*, 1999 ; Maury *et al.*, 2001 ; Maury *et al.* 2003) as large protein fragments might impede accessibility to polyphenol binding sites or might allow binding but not precipitation. In contrast, hydrolysis, which induces protein conformational changes, ameliorates binding site accessibility and favors protein-tannin interactions leading to precipitation (Sarni-Manchado *et al.*, 1999). However, under the present experimental conditions, a more significant decrease of tannin (and total phenol) content was observed after the addition of the less hydrolyzed gelatin G1, which in return presented a significantly higher surface charge (Table 1). Hence, the outcomes of the present study could be explained based mainly on the surface charge rather than on the hydrolysis degree of the fining agent. Although gelatin surface charge is not usually taken into consideration in the interpretation of the fining results, scientific data including ours suggest that it might be an important parameter (Scotti and Poinsaut, 1997 ; Versari *et al.*, 1998, 1999).

The reduction of astringency and hence the perception of balance is the principal aim of protein fining. An effective fining product is capable of precipitating the astringent tannins and thus lowers the value of « gelatin index ». Since the principle of the « gelatin index » method is based on protein-tannin interactions, its determination offers information regarding the percentage of the wine tannins that are capable to react with salivary proteins and thus to enhance astringency. The measured values vary from 25 to 80 %. A value higher than 60 is an indication of the presence of astringent tannins in the wine (Ribéreau-Gayon *et al.*, 1998a ; Arriagada-Carrazana *et al.*, 2005).

The Agiorgitiko wine exhibited a lower gelatin index value (62 %), indicating a lower initial astringency, compared to the Xinomavro wine (73 %), together with a lower level of total phenols and tannins (Tables 3 and 4). Fining with the more hydrolyzed gelatin G3 induced the most pronounced reduction of the gelatin index for both the Agiorgitiko and Xinomavro wines (34 % and 22 %, respectively). These results are in accordance with Scotti and Poinsaut (1997), who mentioned that the use of a gelatin with a high hydrolysis degree has better results on astringency reduction than a gelatin with a low hydrolysis degree, although under our experimental conditions these differences were not statistically significant. On the contrary, significant variations within each fining agent were observed with the dosage applied.

HCl index reflects tannins' polymerization degree. It is correlated with aging potential and it is found to decrease after some years of bottle aging. Fining is believed to have a reducing effect on this index (Ribéreau-Gayon *et al.*, 1998a). Agiorgitiko wine (HCl index equal to 34 %) is characterized by more polymerized tannins compared to Xinomavro wine (HCl index equal to 25 %) (Table 2). Both values decreased significantly after fining, depending on grape variety, fining agent, hydrolysis degree of gelatins

Table 4 - Effects of fining on the tannin content, gelatin and HCl index, and polysaccharide content of the Agiorgitiko and Xinomavro wines.

Variety	Fining agent	Dosage (g/L)	Tannins (g/L)	Gelatin index (%)	HCl index (%)	Polysaccharides (mg D-galactose/L)
Agiorgitiko	G1	-	2.3 ± 0.1 ^{a}	62 ± 5 ^{a}	34 ± 4 ^{a}	764 ± 9 ^{a}
		0.05	2.1 ± 0.1 ^{b,A}	52 ± 4 ^{b,A}	32 ± 1 ^{a,A}	725 ± 5 ^{b,A}
		0.1	2.0 ± 0.1 ^{c,A}	51 ± 5 ^{b,AB}	18 ± 1 ^{b,BC}	696 ± 3 ^{c,B}
		0.2	1.9 ± 0.1 ^{c,AB}	47 ± 4 ^{b,A}	9 ± 2 ^{c,C}	624 ± 5 ^{d,BC}
	G2	-	2,3 ± 0.1 ^{a}	62 ± 5 ^{a}	34 ± 4 ^{a}	764 ± 9 ^{a}
		0.05	2.1 ± 0.3 ^{a,A}	52 ± 3 ^{b,A}	32 ± 1 ^{a,A}	745 ± 5 ^{b,A}
		0.1	2.0 ± 0.3 ^{ab,A}	52 ± 3 ^{b,A}	33 ± 1 ^{a,A}	727 ± 2 ^{c,A}
		0.2	1.7 ± 0.1 ^{b,B}	48 ± 3 ^{b,A}	32 ± 5 ^{a,A}	648 ± 9 ^{d,A}
	G3	-	2.3 ± 0.1 ^{a}	62 ± 5 ^{a}	34 ± 4 ^{a}	764 ± 9 ^{a}
		0.05	2.0 ± 0.0 ^{b,A}	54 ± 3 ^{a,A}	14 ± 1 ^{b,C}	732 ± 13 ^{b,A}
		0.1	2.0 ± 0.1 ^{b,A}	43 ± 9 ^{b,B}	17 ± 1 ^{b,C}	696 ± 20 ^{c,B}
		0.2	2.0 ± 0.2 ^{b,A}	41 ± 9 ^{b,A}	16 ± 1 ^{b,B}	643 ± 1 ^{d,AB}
	EA	-	2.3 ± 0.1 ^{a}	62 ± 5 ^{a}	34 ± 4 ^{a}	764 ± 9 ^{a}
		0.05	1.9 ± 0.2 ^{b,A}	53 ± 3 ^{b,A}	20 ± 4 ^{b,B}	724 ± 23 ^{b,A}
		0.1	1.9 ± 0.2 ^{b,A}	49 ± 4 ^{bc,AB}	21 ± 4 ^{b,B}	701 ± 14 ^{b,B}
		0.2	2.0 ± 0.2 ^{b,A}	44 ± 5 ^{c,A}	7 ± 1 ^{c,C}	612 ± 20 ^{c,C}
Xinomavro	G1	-	3.0 ± 0.3 ^{a}	73 ± 4 ^{a}	25 ± 2 ^{a}	689 ± 60 ^{a}
		0.05	2.9 ± 0.4 ^{a,A}	64 ± 6 ^{b,AB}	18 ± 1 ^{b,A}	584 ± 62 ^{b,A}
		0.1	2.9 ± 0.3 ^{a,A}	61 ± 7 ^{b,AB}	17 ± 2 ^{b,AB}	538 ± 87 ^{bc,A}
		0.2	2.7 ± 0.3 ^{a,A}	58 ± 7 ^{b,B}	14 ± 2 ^{c,A}	449 ± 94 ^{c,A}
	G2	-	3.0 ± 0.3 ^{a}	73 ± 4 ^{a}	25 ± 2 ^{a}	689 ± 60 ^{a}
		0.05	3.0 ± 0.4 ^{a,A}	66 ± 2 ^{b,AB}	20 ± 4 ^{a,A}	519 ± 8 ^{b,A}
		0.1	3.0 ± 0.4 ^{a,A}	64 ± 3 ^{bc,AB}	20 ± 4 ^{a,A}	462 ± 22 ^{c,A}
		0.2	2.8 ± 0.4 ^{a,A}	60 ± 5 ^{c,AB}	14 ± 7 ^{b,A}	373 ± 40 ^{d,A}
	G3	-	3.0 ± 0.3 ^{a}	73 ± 4 ^{a}	25 ± 2 ^{a}	689 ± 60 ^{a}
		0.05	3.0 ± 0.3 ^{a,A}	61 ± 3 ^{b,B}	17 ± 1 ^{b,A}	581 ± 41 ^{b,A}
		0.1	3.1 ± 0.2 ^{a,A}	59 ± 5 ^{b,B}	16 ± 3 ^{b,B}	512 ± 75 ^{bc,A}
		0.2	3.0 ± 0.3 ^{a,A}	57 ± 3 ^{b,B}	11 ± 3 ^{c,A}	456 ± 106 ^{c,A}
	EA	-	3.0 ± 0,3 ^{a}	73 ± 4 ^{a}	25 ± 2 ^{a}	689 ± 60 ^{a}
		0.05	2.7 ± 0.1 ^{b,A}	68 ± 2 ^{b,A}	13 ± 2 ^{b,B}	573 ± 72 ^{b,A}
		0.1	2.7± 0.1 ^{b,A}	66 ± 2 ^{bc,A}	14 ± 3 ^{b,B}	514 ± 36 ^{bc,A}
		0.2	2.6 ± 0.1 ^{b,A}	65 ± 0 ^{c,A}	11 ± 3 ^{b,A}	458 ± 30 ^{c,A}

G1,G2,G3: Gelatin 1,2,3; EA: Egg Albumin; $^{a-c}$ Within the same fining agent and the same analytical parameter, different lowercase letters are significantly different at $p < 0.05$; $^{A–D}$ Regarding the same dosage and the same analytical parameter, different uppercase letters are significantly different at $p < 0.05$; $n = 6$.

and dosage (table 4). Indeed, the reduction was more pronounced in the Agiorgitiko wine compared to Xinomavro, and this was probably due to their different phenolic profiles. More specifically, in the Agiorgitiko samples, EA induced the greatest decrease of HCl index (79 % with 0.2 g/L), while among gelatins, the less hydrolyzed gelatin G1 had the most pronounced effect (74 % reduction with 0.2 g/L), followed by G3 and G2, which had almost no effect. In the Xinomavro wine, EA and the more hydrolyzed gelatin G3 induced the greatest reduction, while gelatins G1 and G2 had a lower influence.

It is known that proline-rich proteins such as gelatin are characterized by higher affinity and selectivity for tannin molecules (especially high molecular weight galloylated procyanidins) (Ricardo-da-Silva *et al.*, 1991 ; Sarni-Manchado *et al.*, 1999 ; Maury *et al.*, 2001). The more pronounced fining-induced reduction of HCl index observed in Agiorgitiko samples could be attributed to its initial higher content of polymerized tannins compared to Xinomavro samples.

d) Individual phenols

HPLC analysis of the wine samples before and after fining provided some interesting information. A characteristic chromatogram recorded at 320 nm is presented in figure 1. Agiorgitiko wine was rich in benzoates (sum of gallic, protocatechuic, syringic and vanillic acid), gallic acid being the prevalent one (77 %), while Xinomavro wine was rich in hydroxycinnamates (sum of ferulic, caftaric, coutaric, caffeic and p-coumaric), caftaric acid being the prevalent acid (80 %) (figure 2). In the Xinomavro wine, catechin, epicatechin, procyanidin B1, B2 and C1 concentrations were higher, while those of myricetin, rutin and resveratrol were lower than in the Agiorgitiko wine (figure 3). Other authors who analyzed these specific varieties reported similar results (Arnous *et al.*, 2001 ; Kallithraka *et al.*, 2001 ; Makris *et al.*, 2006). Under our experimental conditions, protein fining, in accordance with certain authors (Castellari *et al.*, 1998 ; Cosme *et al.*, 2008) and in contrast with others (Yokotsuka and Singleton, 1987 ; Ricardo-da-Silva *et al.*, 1991 ; Sarni-Manchado *et al.*, 1999 ; Maury *et al.*, 2001 ; Villaño *et al.*, 2006 ; Braga *et al.*, 2007), reduced the content of all the phenolic compounds analyzed [phenolic acids, monomeric flavan-3-ols (catechin, epicatechin), dimeric and trimeric procyanidins, myricetin, rutin and resveratrol], with differences being more or less significant depending on grape variety, fining agent used and dosage applied (figures 2 and 3). Treatment with egg albumin particularly affected procyanidins B1-B2 and procyanidin B2 in the Agiorgitiko and Xinomavro wines, respectively. The less hydrolyzed gelatin G1 had practically no effect on phenolic acids in the Agiorgitiko wine, and it slightly reduced caftaric and coutaric acid content in the Xinomavro wine as well as the procyanidin C1 levels in both wines. The more hydrolyzed gelatin G3 decreased the concentration of all individual phenolics in both wines ;

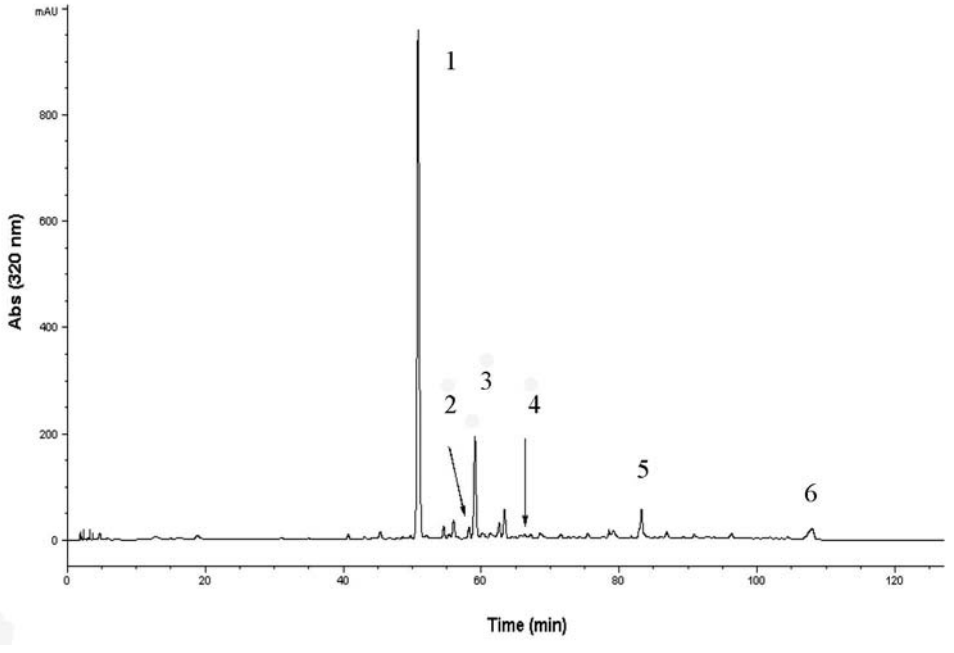

Figure 1 - Characteristic chromatogram showing major hydroxycinnamates and resveratrol determined at 320 nm. Peak assignement:1: caftaric acid, 2: caffeic acid, 3: coutaric acid, 4: p-coumaric acid, 5: ferulic acid, 6: resveratrol

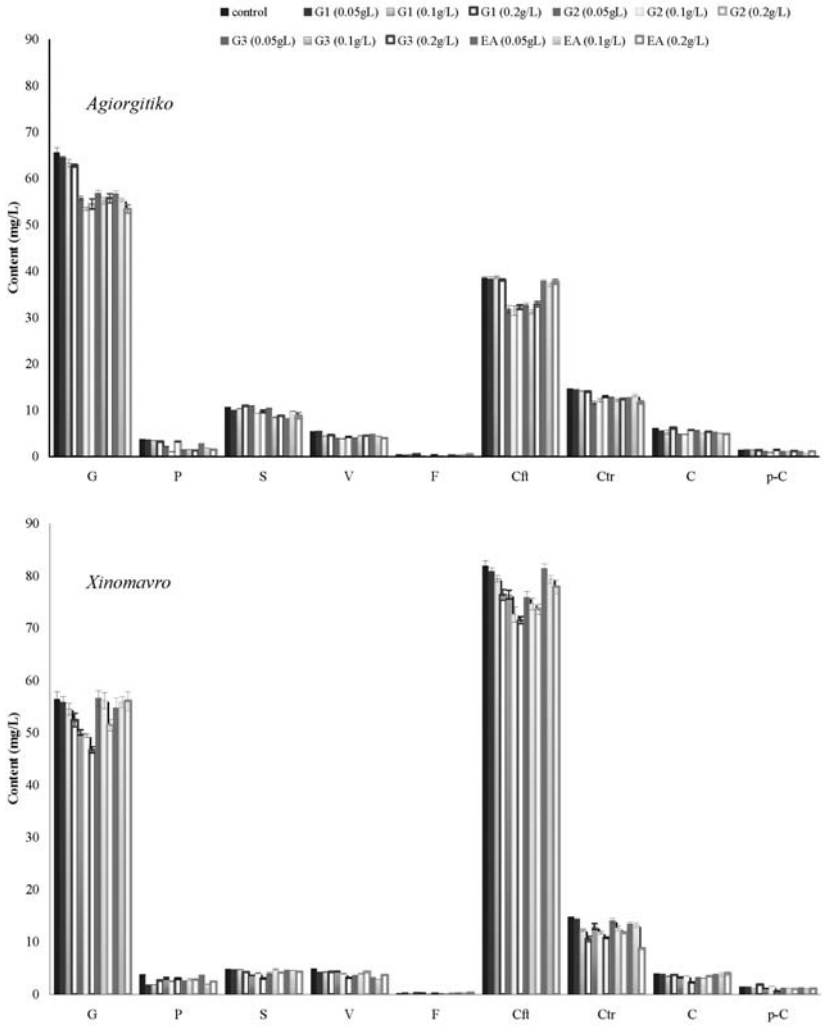

Figure 2 - Effect of fining on the benzoic and hydroxycinnamic acids content (mg/L), analyzed by HPLC, of the Agiorgitiko and Xinomavro wines.

(G1, gelatin G1; G2, gelatin G2; G3, gelatin G3; EA, egg albumin; G, gallic; P, protocatechuic; S, syringic; F, ferulic; Cft, caftaric; Ctr, coutaric; C, caffeic; V, vanillic; p-C, p-coumaric)

flavan-3-ols, procyanidins and resveratrol were the most affected (reduction up to 60 %). These results are probably related to the reported differences in the interactions of phenolic monomers, dimers, and tannins with different molecular weight fractions of gelatin studied in model-wine solutions (Yokotsuka and Singleton, 1987) and are in agreement with similar observations made in white wines (Cosme *et al.*, 2008).

e) Polysaccharides

The results regarding the influence of protein fining on the polysaccharide content (mg D-galactose/L) of the two varietal wines are presented in Table 4. A reduction of the polysaccharide content was observed in both wines. The effect of grape variety, fining agent and dosage was significant. In the Agiorgitiko wine, EA and gelatin G3 caused the highest and lowest decrease, equal to 20 % and 15 %, respectively. In the case of Xinomavro, a reduction equal to 46 % was noted with the use of gelatin G2, while EA seemed to precipitate polysaccharides to a lesser extent (33.5 % reduction). These results could probably be explained by the formation of either soluble or insoluble complexes between the wine polysaccharides and the proteins used in the fining process. It has been shown that the main wine polysaccharides are negatively charged at wine pH so they may establish ionic or electrostatic interactions with positively charged compounds such as gelatin (Siebert *et al.*, 1996; Vernhet *et al.*, 1996).

3. Sensory evaluation

The sensory evaluation of a wine is undoubtedly essential for the final appreciation of its quality. Although chemical determinations of the various wine constituents contribute to a better knowledge of the qualitative and quantitative wine composition, they cannot provide

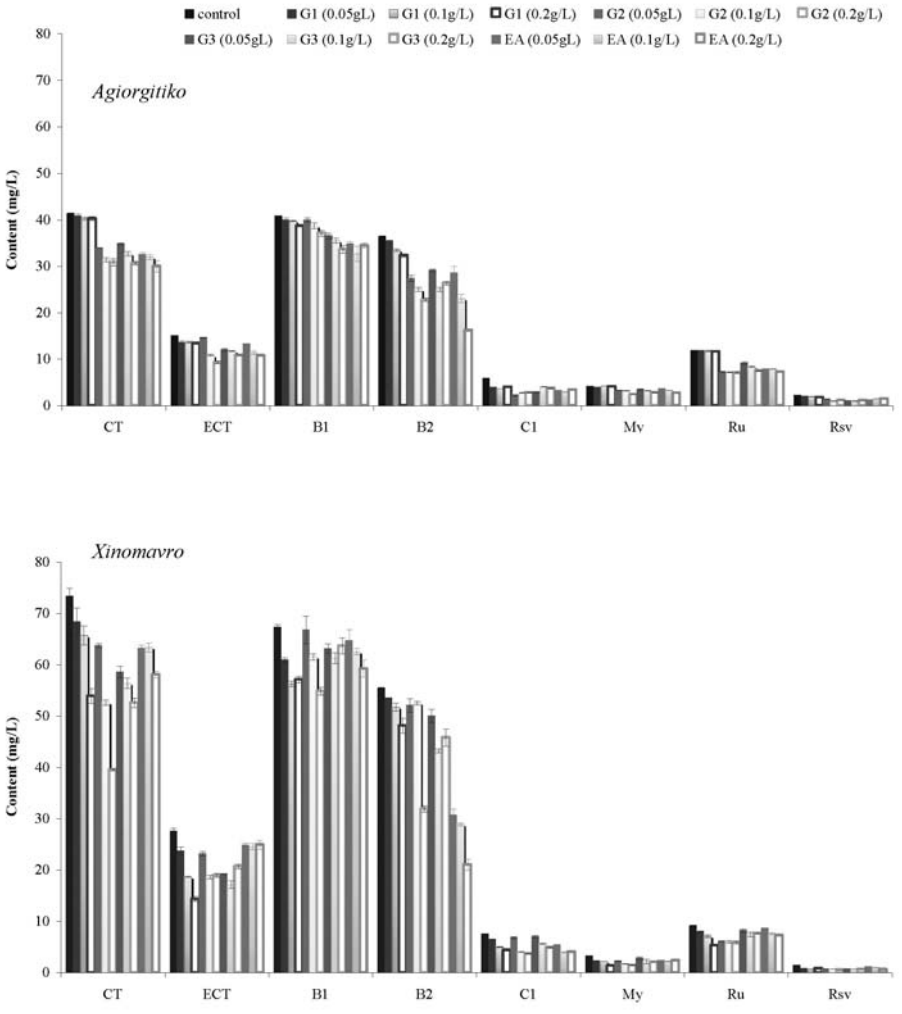

Figure 3 - Effect of fining on the flavan-3-ols, dimeric and trimeric procyanidins, myricetin, rutin and resveratrol content, analyzed by HPLC, of the Agiorgitiko and Xinomavro wines.

(G1, gelatin G1; G2, gelatin G2; G3, gelatin G3; EA, egg albumin; CT, (+)-catechin; ECT, (-)-epicatechin; B1, procyanidin B1; B2, procyanidin B2; C1, procyanidin C1; My, myricetin; Ru, rutin, Rsv, resveratrol)

information regarding the wine quality and the overall appreciation.

Since significant differences regarding wine chemical composition were observed in most of the treated wines, all samples were submitted to sensory analysis. In general, the fined wines were more appreciated and had slightly better scores than the control ones. However, only six parameters (color intensity and hue, acidity, balance, aftertaste and overall impression) varied significantly ($p < 0.05$) among the treated samples of the Agiorgitiko grape variety, while no significant organoleptic differences were obtained with the Xinomavro samples. Schematic representation of these results is given in figures 4 and 5. The panelists did not perceive any significant differences concerning limpidity in the fined wines (figures 4 and 5) probably due to the initially low turbidity values in the control wines (16.0 and 5.4 NTU in the Agiorgitiko and Xinomavro wines, respectively ; Table 2). Astringency was another parameter expected to be affected by the fining process, since a reduction in the gelatin index was observed after the addition of the fining agents. However, no significant differences were observed between the perceived astringency values of the treated and control wines tested.

CONCLUSION

In conclusion, the use of three gelatins and egg albumin differently affected the phenolic composition of the Hellenic *Vitis vinifera* L. cv Agiorgitiko and Xinomavro varietal red wines. In general, more pronounced effects were observed in Agiorgitiko samples, probably due to the different characteristic phenolic profile of this wine. However, although most of the changes in the chemical parameters were statistically significant, they were not detected at the sensory level.

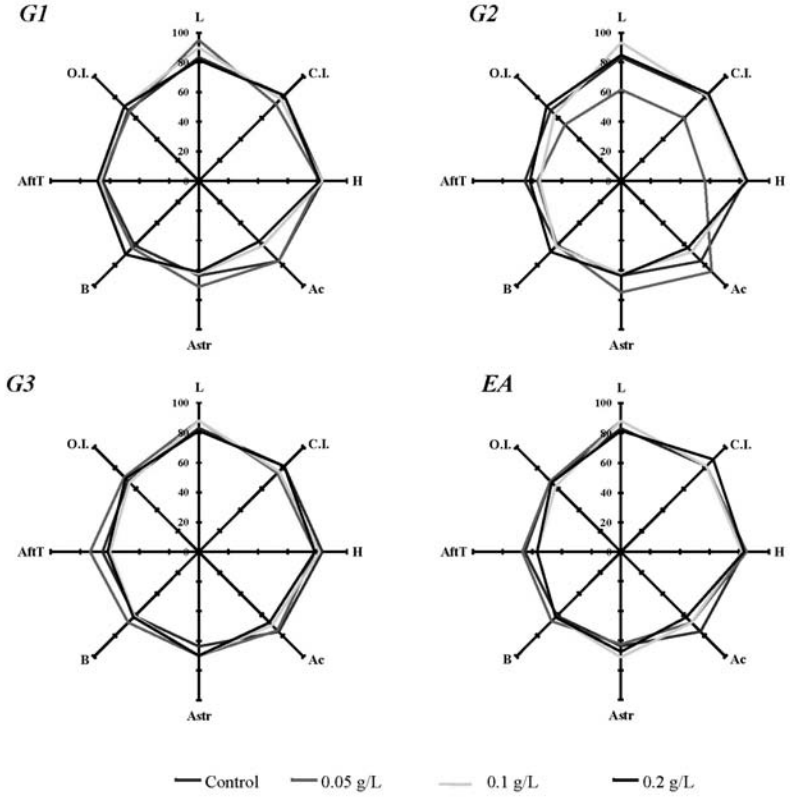

Figure 4 - Impact of fining on the sensory characteristics of the Agiorgitiko wine.
(G1, gelatin G1; G2, gelatin G2; G3, gelatin G3; EA, egg albumin; L, limpidity; C.I., color intensity; H, hue; Ac, acidity; Astr, astringency; B, balance; Aft T, after taste; O.I., overall impression)

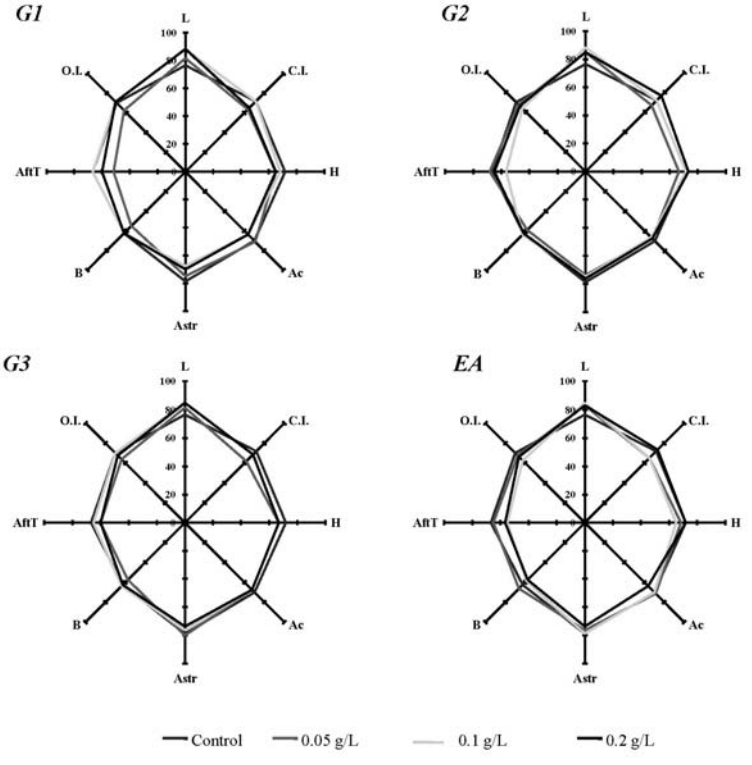

Figure 5 - Impact of fining on the sensory characteristics of the Xinomavro wine.
(G1, gelatin G1; G2, gelatin G2; G3, gelatin G3; EA, egg albumin; L, limpidity; C.I., color intensity; H, hue; Ac, acidity; Astr, astringency; B, balance; Aft T, after taste; O.I., overall impression)

In both wines, the less hydrolyzed gelatin G1 had the highest effect on total phenol reduction, while the more hydrolyzed G3 had the highest effect on gelatin index reduction and on the individual phenolic removal. In addition, in both wines, EA mostly affected anthocyanin concentration and phenol polymerization degree (HCl index). Regarding color parameters such as hue and intensity, in Xinomavro wines they remained practically unaffected by fining, whereas in Agiorgitiko samples they were most affected by G1. Anthocyanin ionization degree was only affected slightly by G1 in Agiorgitiko samples and by EA in Xinomavro wines.

Moreover, G2 exhibited the most pronounced effect on tannin reduction in Agiorgitiko samples, while the highest tannin reduction was obtained with EA in the Xinomavro ones. Regarding polysaccharide content, EA and G2 had the highest effect in Agiorgitiko and Xinomavro wines, respectively. Regarding antioxidant activity reduction, G3 was the most efficient in Agiorgitiko samples, while G1 was the most efficient in Xinomavro ones. Particularly encouraging was the observation that, under the present experimental conditions, the reduction in the DPPH radical scavenging activity due to protein fining was not dramatic.

Since the use of fining agents is essential in red winemaking, more in-depth research is needed in order to optimize production technology by selecting the appropriate fining agent according to the specific phenolic profile of a particular wine.

Acknowledgements: The authors are grateful to the Agricultural Viticultural Winemaking Cooperatives of Naoussa and Nemea for providing the wine samples and to Ampelooiniki Ltd. for the use of equipment.

REFERENCES

Alén-Ruiz F., García-Falcón M.S., Pérez-Lamela M.C., Martínez-Carballo E. and Simal-Gándara J., 2009. Influence of major polyphenols on antioxidant activity in Mencía and Brancellao red wines. *Food Chem.*, **113**, 53-60.

Arnous A., Makris D.P. and Kefalas P., 2001. Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red wines. *J. Agric. Food Chem.*, **49**, 5736-3742.

Arriagada-Carrazana J.P., Sáez-Navarrate C. and Bordeu E., 2005. Membrane filtration effects on aromatic and phenolic quality of Cabernet-Sauvignon wines. *J. Food Engin.*, **68**, 363-368.

Boulton R.B., Singleton V.L., Bisson L.F. and Kunkee R.E., 1996. The fining and clarification of wines, 279-319. *In : Principles and Practices of Winemaking*. Chapman & Hall, New York, 604 p.

Braga A., Cosme F., Ricardo-da-Silva J.M. and Laureano O., 2007. Gelatine, casein and potassium caseinate as distinct wine fining agents : Different effects on colour, phenolic compounds and sensory characteristics. *J. Int. Sci. Vigne Vin*, **41**, 203-214.

Cabello-Pasini A., Victoria-Cota N., Marcias-Carranza V., Hernandez-Garibay E. and Muñiz-Salazar R., 2005. Clarification of wines using polysaccharides extracted from seaweeds. *Am. J. Enol. Vitic.*, **56**, 52-59.

Calderon P., Van Buren J.P. and Robinson W.B., 1968. Factors influencing the formation of precipitates and hazes by gelatin and condensed and hydrolysable tannins. *J. Agric. Food Chem.*, **16**, 479-482.

Castellari M., Spinabelli U., Riponi C. and Amanti A., 1998. Influence of some technological practices on the quantity of resveratrol in wine. *Z. Lebensm. Unters. Forsch. A.*, **206**, 151-155.

Castillo-Sánchez J.J., Mejuto J.C., Garrido J. and García-Falcón S., 2006. Influence of wine-making protocol and fining agents on the evolution of the anthocyanin content, colour and general organoleptic quality of Vinhão wines. *Food Chem.*, **97**, 130-136.

Castillo-Sánchez J.X., García-Falcón M.S., Garrido J., Martínez-Carballo E., Martins-Dias L.R. and Mejuto X.C., 2008. Phenolic compounds and colour stability of Vinhão wines : Influence of wine-making protocol and fining agents. *Food Chem.*, **106**, 18-26.

Chalier P., Angot B., Delteil D., Doco T. and Gunata Z., 2007. Interactions between aroma compounds and whole mannoprotein isolated from *Saccharomyces cerevisiae* strains. *Food Chem.*, **100**, 22-30.

Cosme F., Ricardo-da-Silva J.M. and Laureano O., 2008. Interactions between protein fining agents and proanthocyanidins in white wine. *Food Chem.*, **106**, 536-544.

ENOAVE (Wine Producers Association of the Northern Greece Vineyard). Study : Re-evaluation of the V.Q.P.R.D. appellation regions of Naoussa and Goumenissa (in Greek), 2005 http://www.wineroads.gr/Meleti_Naoussa_Goumenissa_2005.pdf

Gil-Muñoz R., Gómez-Plaza E., Martínez A. and López-Roca J.M., 1997. Evolution of the CIELAB and other spectrophotometric parameters during wine fermentation. Influence of some pre and postfermentative factors. *Food Res. Int.*, **30**, 699-705.

Glories Y., 1978. Recherches sur la matière colorante des vins rouges. *Thèse de Doctorat*, Université de Bordeaux II.

Gómez-Plaza E., Gil-Muñoz R., López-Roca J.M. and Martínez A., 2000a. Color and phenolic compounds of a young red wine. Influence of wine-making techniques, storage temperature, and length of storage time. *J. Agric. Food Chem.*, 48, 736-741.

Gómez-Plaza E., Gil-Muñoz R., López-Roca J.M., De la Hera-Orts M.L. and Martínez-Cutillas, A., 2000b. Effect of the addition of bentonite and polyvinylpolypyrrolidone on the colour and long-term stability of red wine. *J. Wine Res.*, **11**, 223-231.

Gómez-Plaza E., Gil-Muñoz R., López-Roca J.M., Martínez-Cutillas A. and Fernández- Fernández J.I., 2002. Maintenance of color of a red wine during storage. Influence of prefermentative practices, maceration time and storage. *Lebensm. Wiss u. Technol.*, **35**, 46-53.

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

Kallithraka S., Arvanitoyannis I.S., Kefalas P., El-Zajouli A., Soufleros E. and Psarra E., 2001. Instrumental and sensory analysis of Greek wines ; implementation of principal component analysis (PCA) for classification according to geographical origin. *Food Chem.*, **73**, 501-514.

Kallithraka S., Mohdaly A.A.-A., Makris D.P. and Kefalas P., 2005. Determination of major anthocyanin pigments in Hellenic native grape varieties (*Vitis vinifera* sp.): Association with antiradical activity. *J. Food Compos. Anal*., **18**, 375-386.

Lagune L. and Glories Y., 1996a. Les gélatines œnologiques: matière première, fabrication. *Rev. Fr. Œnol.*, **157**, 35-38.

Lagune L. and Glories Y., 1996b. Les gélatines œnologiques: caractéristiques, propriétés. *Rev. Fr. Œnol*., **158**, 19-25.

Ledoux-Moine V., 1996. Recherches sur les mannoprotéines de levures vis-à-vis de la stabilisation protéique et tartrique des vins. *Thèse de Doctorat,* Université de Bordeaux II.

Li H., Wang X., Li Y., Li P. and Wang H., 2009. Polyphenolic compounds and antioxidant properties of selected China wines. *Food Chem.*, **112**, 454-460.

Makris D.P., Psarra E., Kallithraka S. and Kefalas P., 2003. The effect of polyphenolic composition as related to antioxidant capacity in white wines. *Food Res. Intern*., **36**, 805-814.

Makris D.P., Kallithraka S. and Mamalos A., 2006. Differentiation of young red wines based on cultivar and geographical origin with application of chemometrics of principal polyphenolic constituents. *Talanta*, **70**, 1143-1152.

Marchal R., Marchal-Delahaut L., Lallement A. and Jeandet P., 2002. Wheat gluten used as a clarifying agent of red wines. *J. Agric. Food Chem.*, **50**, 177-184.

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

Maury C., Sarni-Manchado P., Lefebvre S., Cheynier V. and Moutounet M., 2003. Influence of fining with plant proteins on proanthocyanidin composition of red wines. *Am. J. Enol. Vitic.*, **54**, 105-111.

Nenadis N. and Tsimidou M., 2002. Observations on the estimation of scavenging activity of phenolic compounds using rapid 1,1-diphenyl-2-picrylhydrazyl (DPPH) tests. *J. Am. Oil Chem. Soc.*, **79**, 1191-1195.

Paixão N., Perestrelo R., Marques C. and Câmara J., 2007. *Relationship between antioxidant capacity and total phenolic content of red, rosé and white wines*. *Food Chem*., **105**, 204-214.

Poppe J., 1999. Gelatin, 144-168. *In: Thickening and gelling agents for food*. Ed. A. Imeson, Aspen Publishers Inc., Maryland, 320 p.

Ribéreau-Gayon P., Glories Y., Maujean A. and Dubourdieu D., 1998a. Les composés phénoliques, 163-237. *In: Chimie du vin. Stabilisation et traitements. Traité d'Œnologie, vol. 2.* Dunod ed., Paris, 519 p.

Ribéreau-Gayon P., Glories Y., Maujean A. and Dubourdieu D., 1998b. Les traitements de clarification et de stabilisation. Le collage des vins, 343-381. *In: Chimie du vin. Stabilisation et traitements. Traité d'œnologie , vol. 2.* Dunod ed., Paris, 519 p.

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

Riou V., Vernhet A., Doco T. and Moutounet M., 2002. Aggregation of grape seed tannins in model wine - effect of wine polysaccharides. *Food Hydrocolloids*, **16**, 17-23.

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

Scotti B. and Poinsaut P., 1997. Le collage à la gélatine: Entre science et traditions. *Rev. Œnol.*, **85s**, 1-7.

Segarra I., Lao C., López-Tamames E. and De La Torre-Boronat M.C., 1995. Spectrophotometric methods for the analysis of polysaccharide levels in winemaking products. *Am. J. Enol. Vitic*., **46**, 564-570.

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

Sims C.A., Eastridge J.S. and Bates R.P., 1995. Changes in phenols, color, and sensory characteristics of Muscadine wines by pre- and post-fermentation additions of PVPP, casein and gelatin. *Am. J. Enol. Vitic*., **46**, 155-158.

Singleton V.L., 1967. Adsorption of natural phenols from beer and wine. *Tech. Q. Master Brew. Assoc. Am*., **4**, 245-253.

Singleton V.L. and Rossi J.A., 1965. Colorimetry of total phenolics with phosphomolybdic and phosphotungstic acid reagents. *Am. J. Enol. Vitic*., **16**, 144-158.

Soleas G.J., Diamandis E.P. and Goldberg D.M., 1997. Wine as a Biological Fluid: History, Production, and Role in Disease Prevention. *J. Clin. Lab. Anal.*, **11**, 287-313.

Spinthiropoulou H., 2000. *Wine – making grape varieties of the Hellenic vineyard (in Greek)*. Ed. Olive Press, Athens.

Stankoviç S., Joviç S. and Îivcoviç J., 2004. Bentonite and gelatine impact on the young red wine coloured matter. *Food Technol. Biotechnol.*, **42**, 183-188.

Vernhet A., Pellerin P., Prieur C., Osmiansky J. and Moutounet M., 1996. Charge properties of some grape and wine polysaccharide and polyphenolic fractions. *Am. J. Enol. Vitic*., **47**, 25-30.

Versari A., Barbanti D., Potentini G., Mannazu I., Salvucci A. and Galassi S., 1998. Physico-chemical characteristics of some oenological gelatins and their action on selected red wine components. *J. Sci. Food Agric*., **78**, 245-250.

Versari A., Barbanti D., Potentini G., Parpinello G.P. and Galassi S., 1999. Preliminary study on the interaction of gelatin-red wine components. *Ital. J. Food Sci.*, **11**, 231-239.

Vidal S., Francis L., Williams P., Kwiatkowski M., Gawel R., Cheynier V. and Waters E., 2004. The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium. *Food Chem*., **85**, 519-525.

Villaño D., Fernández-Pachón M.S., Troncoso A.M. and García-Parrilla M.C., 2006. Influence of enological practices on the antioxidant activity of wines. *Food Chem.*, **95**, 394-404.

Yokotsuka K. and Singleton V.L., 1987. Interactive precipitation between graded peptides from gelatin and specific grape tannin fractions in wine-like model solutions. *Am. J. Enol. Vitic*., **38**, 199-206.

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