Analysis of the impact of fining agents types, oenological tannins and mannoproteins and their concentrations on the phenolic composition of red wine

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A B S T R A C T

This paper aimed to evaluate and analyze the effect of five fining agents, commercial tannins and mannoproteins on the pigment, color and tannins composition of a Cabernet Sauvignon red wine. The wines were analyzed 2 d after treatment and immediately after separation of sedimentation. Color was evaluated by spectrophotometry and polyphenols were analyzed by spectrophotometry and HPLC-DAD. The results showed that all treatments affected the phenolic contents of the wine. The most remarkable effects on phenolic composition were produced by bentonite and Polyvinylpolypyrrolidone (PvPP) + potassium caseinate which significantly decreased anthocyanins and tannins concentrations, respectively. The use of vegetable protein and gelatin has a less impact on the color and phenolic contents of red wines. The antioxidant activity was little affected by treatments except the addition of tannins that increased it. Principal components analysis demonstrates the importance of a low concentration of agents for high total polyphenol levels.

Keywords:
Fining agents
Tannins
Mannoproteins
Anthocyanins
Antioxidant activity

1. Introduction

In winemaking, fining agents are used to ensure the physico-chemical stability by preventing the formation of hazes and deposits (El Rayess et al., 2011). Electrostatic interactions, chemical bond formation and absorption/adsorption are the three major mechanisms of action of fining agents. These mechanisms are responsible for elimination of some phenolic compounds of colloidal nature by fining agents. This can be perceived as improvement of wine characteristics or deterioration of wines if phenolic compounds are excessively removed (Ribérau-Gayon, Glories, Maujean, & Dubourdieu, 2006).

Phenolic compounds are one of the most important quality parameters in red wines and contribute to organoleptic characteristics of wines such as color, bitterness and astringency as well as other mouth-feel properties (Oberholster, Francis, Iland, & Waters, 2009). The phenolic composition of red wines is affected by the wine-making process. An important step in winemaking is the addition of fining agents, exogenous tannins and commercial mannoproteins.

Several fining agents (bentonite, casein, gelatin, isinglass, polyvinylpolypyrrolidone, etc) are used by winemakers and the choice depends on the compounds that need to be removed. They can be used separately and combined with each other in a defined dosage. Bentonite is mainly negatively-charged clay used to remove proteins, thus providing better clarity and stability during long term storage. However, it also attracts other positively charged compounds, such as anthocyanins and other phenolics. It is not reactive towards small phenolic compounds. In fact, it binds large phenolic compounds and may also bind phenolic compounds complexed

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with proteins (Threlfall, Morri, & Mauromoustakos, 1999). Egg albumin, casein, gelatine and PvPP (polyvinylpolypyrrolidone) reduce the phenolic content of wines and may decrease the intensity of the color of some wines (Castillo-Sanchez, Mejuto, Garrido, & Garcia-Falcon, 2006). These proteins are usually used to modulate the astringency, one of most important sensory characteristic of red wine. Astringency is mainly due to the interaction between salivary proteins and polyphenols such as condensed and ellagic tannins (Gambuti, Rinaldi, Pessina, & Moio, 2006).

Additionally, in response to winemaker's interest in finding alternatives to animal proteins for use as fining agents, a wide variety of commercial preparations of plant-derived proteins from soy, gluten wheat, rice, potato, lupine or maize had been proposed for oenological use with the name of vegetable proteins (Bindon & Smith, 2013). Moreover, some of these plant proteins may precipitate galloylated and condensed tannins depending on their origin and their molecular mass (Maury, Sarni-Manchado, Lefebvre, Cheynier, & Moutounet, 2003).

Mannoproteins are one of the major polysaccharide groups present in wine (Feuillat, 2003), and are increasingly being added in oenological products to wines with the intention of preventing tartaric and protein precipitation (Moine-ledoux & Dubourdieu, 2002). The interaction between mannoproteins and wine phenolic compounds is a subject of great interest. Studies showed the possible impact on color stability (Escot, Feuillat, Dulau & Charpentier, 2001), an improvement in the sensory characteristics, namely the reduction of red wine astringency (Guadalupe, Palacios, & Ayestaran, 2007) and improvement of wine aromatic profile (Chalier, Angot, Delteil, Doco, & Gunata, 2007). In order to prevent oxidation in must made from botrytized grapes, strengthen the wine structure and facilitate ageing, exogenous tannins can be added. The use of oenological tannins may contribute to improve wine color and its stability. Some of the positive effects of using enological tannins include wine color stabilization, improved wine structure, and the control of laccase activity and an elimination of reduction odors (Zamora, 2003). However, other studies showed (Bautista-Ortín, López-Roca, Martínez- Cutillas, & Gómez-Plaza, 2005) that the use of enological tannins should be treated with great care, because when used in inappropriate conditions, wines may lose their equilibrium. This effect was more accused when hydrolysable tannins (gallotannins and ellagitannins) were used.

In the literature, studies comparing the effect of the main fining agents and oenological additives and their concentrations on the phenolic composition of red wines are scarce. The ones dealing with the fining agents cover only a part of the fining agents or a part of the phenolic compounds. In this context, the aim of this study was to evaluate the effect of the most common fining agents used in wine industry (egg albumin, PVPP + casein, bentonite, gelatin and vegetable proteins) and two oenological additives (tannins and mannoproteins), as well as the effect of different concentrations on the chromatic characteristics, phenolic composition, and antioxidant activity of Cabernet Sauvignon red wine. This study contributes positively to the wine industry from scientific and technological points of view.

2. Materials and methods

2.1. Chemicals and fining agents

All chemicals used were of analytical reagent grade. All chromatographic solvents were high-performance liquid chromatography (HPLC) grade. Polyphenol standards were purchased from Extrasynthese (Genay, France). The fining agents (gelatin: GECOLL® Supra; PvPP + potassium caseinate: Polylact®; bentonite: Microcol alpha®; egg albumin: Ovoclaryl®; vegetable protein: Vegecoll®) and additives (Tannins: Tanin VR GRAPE®; Mannoproteins: Mannostab®) were purchased from Laffort Œnologie.

2.2. Wine treatments

Cabernet Sauvignon wine (pH 3.4, titratable acidity (TA) 3.53 g/L as sulphuric acid, residual sugar 1.8 g/L) from the 2014 vintage was provided from Lebanese winery (Clos St Thomas). This wine was made using classical commercial winemaking process and was obtained after the completion of malolactic fermentation. Fining procedures were conducted for 48 h in triplicate. For each experiment, 500 mL of wine were placed in closed graduated cylinders, at room temperature (20 °C, in the dark). After 48 h of adding the fining agents, a centrifugation step at 2500 rpm for 10 min allowed separating sediment from wine for further analyses. All fining agents were prepared according to the manufacturer's recommendations. The recommended minimum and maximum concentrations for all fining agents were used respectively as concentration 1 and 3. The concentration 2 was the mean concentration of the two others. Untreated wine was used as control. The specific concentrations of compounds used are given in Table 1.

2.3. Spectrophotometric analysis of polyphenols

The color intensity (CI) is defined as the sum of absorbance at 420 and 520 nm and 620 nm (Glories, 1984). Total polyphenols index (TPI) was determined following the method described by Ribérau-Gayon, Glories, Maujean, & Dubourdieu, 2006. Total phenolics were determined according to the Folin-Ciocalteu colorimetric method (Ribérau-Gayon, Glories, Maujean, & Dubourdieu, 2006) and the results are expressed as gallic acid equivalent (mg GAE/L). Total anthocyanins were calculated by measurement of the absorbance at 520 nm after bisulfite bleaching solution. Total anthocyanin concentration was expressed in mg/L as described by Ribéreau-Gayon and Stonestreet (1965). Total tannins were determined by absorbance measurement at 550 nm after acid hydrolysis of the samples and a blank. Total tannins concentration was expressed in mg/L as described by Ribéreau-Gayon and Stonestreet (1966). Antioxidant activity of wines was measured by the ABTS cation decolorization assay as described by Re et al. (1999). Vitamin C was used as a reference compound. The results were expressed as total polyphenols equivalent (mg GAE/L).

2.4. HPLC analysis of phenolic compounds

The HPLC analyses were performed using a Shimadzu chromatographic system equipped with a quaternary pump (LC-20AD), a UV-Vis diode-array detector (SPD-M20A), an automatic injector (SIL-20A) and Shimadzu LC solution software. Samples (20 μL injection volume) previously filtered through a 0.45 μm cellulose acetate membrane (Greyhound Chromatography and Allied Chemicals, England), were injected on a Shim-pack VP-ODSC18 column (250 × 4.6 mm, 5 μm particle size) protected with a guard

Table 1
The concentration of enological agents employed in this study.

Agents	Control	Conc. 1	Conc. 2	Conc. 3
Egg albumin (EA)	0	50 mg/L	100 mg/L	150 mg/L
PvPP + Casein (PvPP + Cas)	0	150 mg/L	525 mg/L	900 mg/L
Bentonite (B)	0	100 mg/L	450 mg/L	800 mg/L
Vegetable protein (VP)	0	10 mg/L	30 mg/L	50 mg/L
Gelatin (G)	0	0.4 mL/L	0.7 mL/L	1 mL/L
Tannins (T)	0	100 mg/L	250 mg/L	400 mg/L
Mannoproteins (M)	0	100 mg/L	250 mg/L	400 mg/L

column of the same material (10*4.6 mm, 5 μm particle size) maintained at 40 °C. All analyses were made in triplicate. The anthocyanin separation and identification method was performed using acetonitrile/acetic acid/water (3:10:87) as solvent A and acetonitrile/acetic acid/water (50:10:40) as solvent B at a flow rate of 0.6 mL/min. The elution profile was as follows: 0–10 min 90% A-10% B; 10–13 min 85% A-15% B; 13–20 min 75% A-25% B; 20–40 min 45% A-55% B; 40–43 min 100% B followed by washing and re-equilibration of the column. Quantification of flavan-3-ols and phenolic acids was performed using the following elution conditions: 0.6 mL/min flow rate, solvent A, acetonitrile/acetic acid (97:3); and solvent B acetic acid/water (3:97). The elution profile consists in 100% B for 0–25 min, 20% A-80%B for 25–45 min; 90% A-10%B for 45–55 min and then washing and re-equilibration of the column. Chromatograms were recorded at 520, 280 and 320 nm for anthocyanins, flavan-3-ols and phenolic acids respectively. Calibration curves were obtained for all phenols standards and the concentrations were expressed as mg/L.

2.5. Statistical data treatment

All experiments were carried out in triplicate. Analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test were used for mean separation, with a significant level of 95% ($p < 0.05$). These statistical analyses, together with PCA, were conducted using Xlstat software (2014).

3. Results and discussion

3.1. Spectroscopic analyses

3.1.1. Chromatic parameters and antioxidant activity

Table 2 shows the chromatic properties and the antioxidant activity of wines. The addition of fining agents and oenological additives decreased the color intensity and increased the hue of most of the treated wines compared to the control. The high concentration of bentonite had the highest impact on the color of wines by decreasing the intensity. Decreases in color intensity (0–5%), were accompanied by increases of hue (+1.9%–2.68%) in the wines clarified by this fining agent. So the bentonite affected ionized anthocyanins decreasing in this way the intensity of red color and consequently influences the hue of the wine (Stankovic, Jovic & Zivkovic & Palovic, 2004). Fining with PvPP + casein showed an equal importance to that of bentonite for the decreasing in color intensity (−1.56 to −4.3%), due to the effect of mixture of fining agents. Vegetable proteins had the less impact on color intensity comparing to the control. These observations are in accordance with those obtained by González-Neves, Favre, and Gil (2014). They found that bentonite affected color intensity while the plant proteins did not. The difference in the behavior between the agents used for the same type of wine determines a wide diversity of molecular masses, isoelectric points and surface charge densities that modify strongly their interactions with polyphenols and their effect on the color of wines (Maury et al., 2003).

TPI is largely affected by the fining treatments. The decrease of TPI is explained by the remove of some classes of polyphenols by the fining treatments especially by bentonite. The addition of tannins especially at high concentration leads to a significant increase in TPI compared to the control.

The antioxidant activity of wines was evaluated by the ABTS assay which is a simple and efficient method for the evaluation of antiradical activity. A little decrease in the antioxidant activity is observed when the wines are treated with fining agents comparing to control except for tannins. When tannins are added an increase in antioxidant activity is observed but it is independent from the concentration. It seems that the fact of adding tannins influence more the antioxidant activity than the concentration.

The correlation between the antioxidant activity and the total polyphenol has been justified by several authors (Di Majo, La Guardia, Giammanco, La Neve, & Giammanco, 2008; Ertan Anli & Vural, 2009). Di Majo et al. (2008) showed a linear correlation between antioxidant capacity and the content of total polyphenols. In our case, it seems that the antiradical activity is due to the flavan-3-ol fraction more than the anthocyanins because when observing

Table 2
The total polyphenol index, chromatic parameters (CI and Hue), and antioxidant activity of control and treated wines.

Agents concentrations	Treatments	TPI	CI	Hue	ABTS mg/ml (GAE)
Concentration 1	C	84.60 ± 2.62^{a}	2.93 ± 0.01^{a}	0.72 ± 0.01^{a}	2.91 ± 0.06^{b}
	EA	75.07 ± 0.46^{ab}	2.89 ± 0.03^{bcd}	0.73 ± 0.001^{a}	2.90 ± 0.00^{b}
	PvPP + Cas	80.97 ± 4.8^{a}	2.88 ± 0.03^{bcd}	0.74 ± 0.02^{a}	2.95 ± 0.00^{a}
	B	81.70 ± 2.35^{a}	2.95 ± 0.03^{a}	0.74 ± 0.00^{a}	2.91 ± 0.03^{b}
	VP	76.33 ± 0.84^{ab}	2.91 ± 0.006^{abc}	0.72 ± 0.00^{a}	2.91 ± 0.08^{b}
	G	75.73 ± 1.81^{ab}	2.93 ± 0.012^{a}	0.72 ± 0.00^{a}	2.91 ± 0.00^{b}
	T	86.67 ± 2.96^{a}	2.91 ± 0.02^{abc}	0.73 ± 0.00^{a}	1.40 ± 0.00^{c}
	M	84.47 ± 3.17^{a}	2.88 ± 0.005^{cd}	0.73 ± 0.00^{a}	2.97 ± 0.06^{a}
Concentration 2	C	84.6 ± 2.62^{a}	2.93 ± 0.005^{b}	0.72 ± 0.01^{b}	2.91 ± 0.06^{cd}
	EA	74.13 ± 1.88^{b}	2.89 ± 0.002^{c}	0.73 ± 0.00^{ab}	2.90 ± 0.06^{d}
	PvPP + Cas	77.43 ± 1.87^{b}	2.85 ± 0.005^{d}	0.73 ± 0.00^{ab}	3.10 ± 0.00^{b}
	B	78.53 ± 1.75^{ab}	2.88 ± 0.007^{c}	0.74 ± 0.00^{a}	2.90 ± 0.03^{d}
	VP	82.43 ± 1.82^{a}	2.93 ± 0.02^{b}	0.73 ± 0.00^{b}	2.90 ± 0.09^{d}
	G	80.63 ± 1.04^{a}	2.99 ± 0.06^{a}	0.74 ± 0.01^{a}	2.92 ± 0.00^{c}
	T	87.33 ± 2.28^{a}	2.88 ± 0.00^{c}	0.73 ± 0.01^{ab}	1.30 ± 0.00^{e}
	M	84.17 ± 1.99^{a}	2.89 ± 0.01^{c}	0.73 ± 0.00^{ab}	3.32 ± 0.03^{a}
Concentration 3	C	84.60 ± 2.62^{ab}	2.93 ± 0.01^{ab}	0.72 ± 0.01^{cd}	2.91 ± 0.06^{de}
	EA	74.77 ± 0.32^{b}	2.89 ± 0.01^{d}	0.73 ± 0.00^{bcd}	2.95 ± 0.00^{c}
	PvPP + Cas	78.33 ± 1.86^{b}	2.81 ± 0.00^{e}	0.73 ± 0.00^{bc}	3.30 ± 0.1^{b}
	B	74.17 ± 1.19^{b}	2.78 ± 0.00^{e}	0.75 ± 0.00^{a}	2.92 ± 0.05^{de}
	VP	78.90 ± 2.94^{b}	2.95 ± 0.02^{a}	0.73 ± 0.00^{bc}	2.90 ± 0.00^{e}
	G	80.83 ± 2.17^{b}	2.93 ± 0.01^{abc}	0.73 ± 0.01^{bc}	2.93 ± 0.08^{d}
	T	94.83 ± 0.64^{a}	2.91 ± 0.01^{bcd}	0.73 ± 0.00^{bc}	1.35 ± 0.00^{f}
	M	86.00 ± 1.63^{ab}	2.91 ± 0.03^{cd}	0.74 ± 0.01^{a}	3.33 ± 0.03^{a}

Mean value ± standard deviation. Different letters within the same row represents significant differences according to Tukey HSD test ($p < 0.05$).

the treatment with bentonite, which decreases hugely the anthocyanins content, no decreases in antioxidant activity is observed.

3.1.2. Total polyphenols, and total anthocyanins and total tannins

After fining, total polyphenols (Fig. 1A), total anthocyanins (Fig. 1B) and total tannins (Fig. 1C) of the wines were compared with those registered before treatments (the control). All treated wines showed a decrease in the content of total polyphenol except wines added by exogenous tannins; even though it is not significant except that for the maximum concentration (concentration 3). These results are principally due to the effect of different agents on anthocyanins (Fig. 1B) and tannins (Fig. 1C) contents of wines. PvPP + casein had the most important effect, with decreases of total polyphenols levels between 17.34% (150 mg/L) and 23.16% (900 mg/L) and total tannins around 7%. PvPP is a synthetic polymer that complexes with wine phenolic compounds by hydrogen bonds. Han et al. (2015) demonstrated that wines made from Cabernet Sauvignon cultivar treated with PvPP showed significant losses in polyphenol concentration as PvPP binds and removes phenolics. In addition to PvPP, casein fining can promote a decrease in polyphenol in monomeric and oligomeric flavanols as well as proanthocyanidins as shown by Braga, Cosme, Ricardo-da-Silva, and Laureano (2007).

Mannoproteins was the second agent that causes reduction of total polyphenols (20%) and total tannins (6%) contents when high concentrations are used. These results are in accordance with those obtained by Guadalupe and Ayestarán (2008) who showed that mannoproteins addition to wines coincided with substantial reduction in proanthocyanidin and pigments. They suggested a precipitation of the co-aggregates mannoproteins-tannins and mannoproteins-pigments. In contrary, Rodrigues, Ricardo-Da-Silva, Lucas, and Laureano (2012) showed that the addition of commercial mannoproteins to red wine did not have a significant effect on color and tannins while compared to untreated wine. The only effect shown in this study is a delay of tannins polymerization in red wines. Nguela, Poncet-Legrand, Sieczkowski, and Vernhet (2016) showed interactions between mannoproteins and wine tannins which led to stable colloidal aggregates with finite size. This was attributed to the glycosyl moiety of mannoproteins which may prevent multiple bridging between tannins and their protein part or may form a hydrophilic and negatively charged shell around aggregates that stop their growth. The remaining fining agents as bentonite, gelatin, egg albumin and vegetable proteins showed less effect on total polyphenol and total tannins contents.

Bentonite had the highest impact on the anthocyanins contents of wines. The concentration of bentonite has an important impact on the decrease of anthocyanins levels. Decreases of the levels of anthocyanins by bentonite, which is particularly emphasized with a dose of 800 mg/L, were 10%−19.6% in relation to their concentration in control wines. These proportions are less than the results reported by Stankovic, Jovic, Zivkovic, and Palovic (2012) and González-Neves et al. (2014) with other grape varieties, who found that the use of bentonite, significantly decreased the anthocyanin levels between 9.8% and 35%. The different behavior found in our study must relate to the wine age. The highest decrease in anthocyanins contents by bentonite were verified in older wines, so the impact of bentonite on the colloidal matter could explain the results (Ribérau-Gayon, Glories, Maujean, & Dubourdieu, 2006). Bentonite is a mainly negatively-charged clay of volcanic origin which indirectly binds phenols that have complexed with proteins and can also bind anthocyanins, with a resulting loss of color (Donovan, Mc Cauley, Tobelia, & Waterhouse, 1999, pp. 142−155). As a cation exchanger clay, bentonite can remove other positively charged molecules as anthocyanins (Chagas, Monteiro, & Ferreira, 2012).

The addition of oenological tannins exhibit antagonist effects. The addition increases the total polyphenol by 9% at higher concentration and total tannins by 8% at higher concentration while it decreases significantly the total anthocyanins. The oenological tannins are the second agent after the bentonite to lower the content of total anthocyanins between 10.29% and 13.46%. Several tannin products can be found on the market with different origins and chemical composition. The oenological tannins used in this study are condensed tannins which can combine with anthocyanins and generate colorless compounds and stabilize wine color. This can explain the decrease in anthocyanins contents. Bautista-Ortín, López-Roca, Martínez- Cutillas, & Gómez-Plaza (2005) showed that the addition of 400 mg/L of condensed tannins did not influence the anthocyanins content of Monastrell wines compared to the control. The same observations were made by Parker et al. (2007) while testing the addition of tannins at either prefermentation or postfermentation level. Harbertson, Parpinello, Heymann, and Downey (2012) studied the impact of adding of exogenous tannins at different concentrations on wine polyphenol content. They showed that the addition with the recommended concentrations had a little impact on wine polyphenol. The addition of tannins was found to retard the degradation of most anthocyanins in the process of winemaking (Liu, Liang, Wang, Pan, & Duan, 2013).

3.2. Determination of polyphenol classes by RP-HPLC

The individual anthocyanin composition of untreated and treated wines is represented in Table 3. In the control wine, malvidin-3-glucoside was the major individual anthocyanin followed by delphinidin-3-glucoside, peonidin-3-glucoside and cyaniding-3-glucoside. The petunidin-3-glucoside is not detected in the Cabernet Sauvignon wine used for this study. The levels of anthocyanin monomers composition were slightly diminished by most of the treatments except mannoproteins (Table 3). Although bentonite showed the highest decrease in total anthocyanins (Fig. 1B), this latter minimally correlated with the loss of glycosylated anthocyanins (Table 3), which suggests that bentonite eliminated other compounds of anthocyanins as acetyl and coumaroyl-glycosides. Results showed that the treatment with commercial mannoproteins can lead to a significant increase in monomeric anthocyanins especially malvidin-3-glucoside comparing to the control. Del Barrio-Galán, Pérez-Magariño, Ortega-Heras, Guadalupe & Ayestarán (2012) observed the same tendency when studying the effect of different commercial mannoproteins on the phenolics of red wine. They showed that 2 of the tested commercial mannoproteins increase the concentrations of monomeric anthocyanins. In fact, mannoproteins favored the formation of new anthocyanins pigments which are more stable and resistant to pH changes and oxidation reactions.

Table 4 represents the concentration of monomeric and dimeric flavanols as well as some phenolic acids and resveratrol. Monomeric flavanols were little affected by the fining agents except epigallocatechin. Epigallocatechin was the principal phenolic removed by bentonite fining agent (decreases of 41% by the maximum recommended concentration). Also, bentonite decreased significantly the concentrations of dimeric flavanols (procyanidin B1 and procyanidin B_2). Bentonite may indirectly binds phenols that have complexed with proteins (Donovan et al., 1999).

PvPP + casein showed to mainly remove catechin and epigallocatechin. Actually PvPP is a synthetic polymer that complexes with phenolic wine components by hydrogen bond formation. It has an affinity for low molecular weight phenols (catechin) and for compounds with a higher degree of hydroxylation

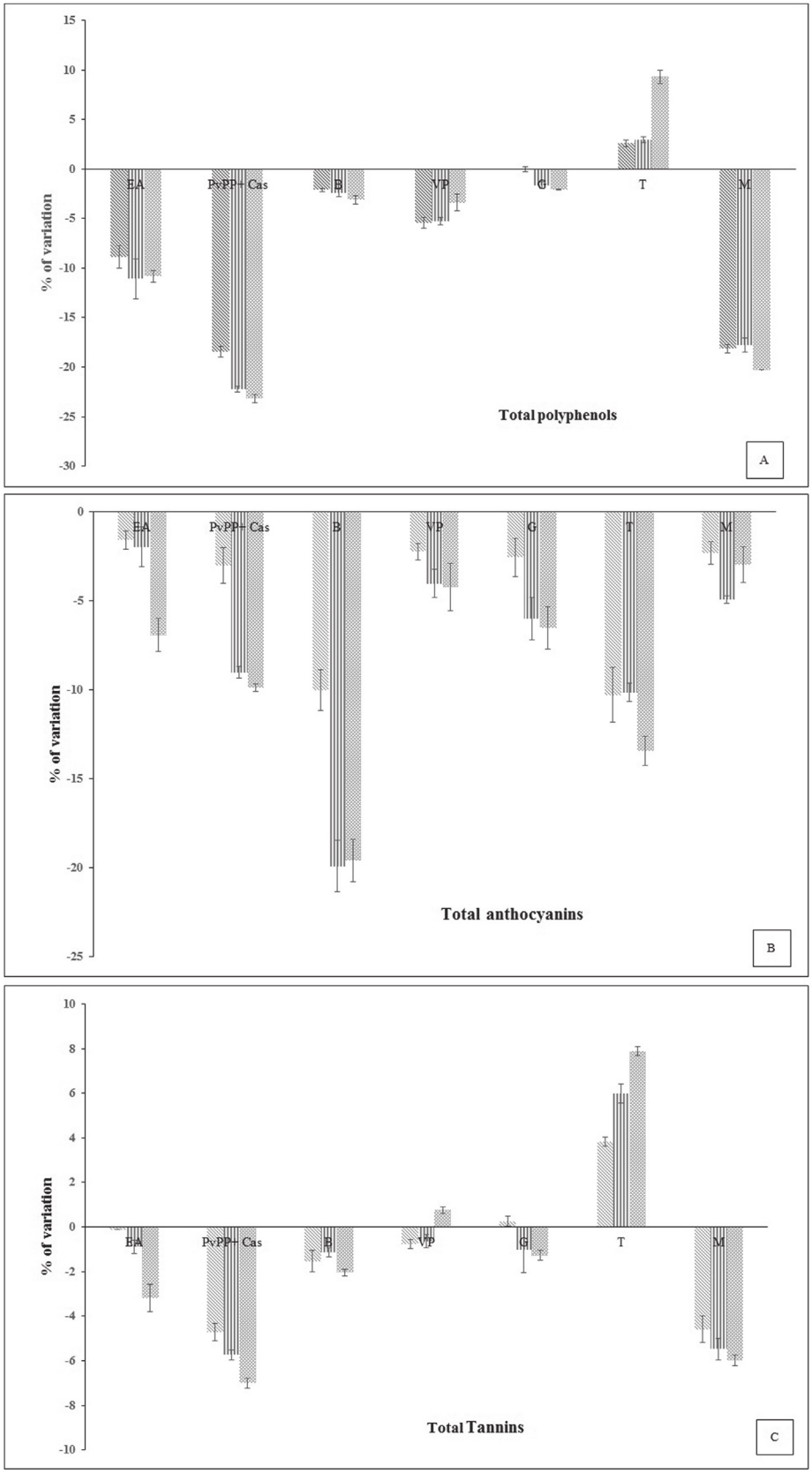

Fig. 1. The variation of total polyphenol (A), total anthocyanins (B) and total tannins (C) after treatment of wines with fining agents. Amounts of phenolic compounds were compared to wines before treatment (control) as external reference (0% of variation) (▨: concentration 1; ▥: concentration 2; ▩: concentration 3).

Table 3
Monomeric anthocyanins of control and treated wines.

Agents concentrations	Treatments	Delphinidin-3-glc (mg/l)	Cyanidin -3-glc (mg/l)	Peonidin-3-glc (mg/l)	Malvidin-3-glc (mg/l)
Conc. 1	C	24.96 ± 0.79^{c}	8.31 ± 0.11^{a}	9.41 ± 0.12^{a}	243.14 ± 2.66^{d}
	EA	25.26 ± 0.03^{bc}	5.47 ± 0.12^{cd}	5.77 ± 0.14^{c}	220.35 ± 1.37^{e}
	PvPP + Cas	27.45 ± 0.34^{a}	5.96 ± 0.41^{bc}	7.66 ± 0.26^{b}	288.27 ± 0.48^{b}
	B	27.42 ± 0.21^{a}	5.21 ± 0.09^{d}	6.89 ± 0.13^{bc}	248.27 ± 6.48^{c}
	VP	26.71 ± 0.75^{ab}	5.18 ± 0.26^{d}	5.84 ± 0.15^{c}	223.22 ± 1.48^{e}
	G	25.39 ± 0.26^{bc}	5.38 ± 0.13^{cd}	5.33 ± 0.05^{c}	219.64 ± 3.00^{f}
	T	25.68 ± 0.50^{bc}	6.40 ± 0.93^{bc}	6.42 ± 0.57^{bc}	224.90 ± 3.72^{e}
	M	26.17 ± 0.68^{abc}	6.82 ± 0.12^{b}	7.84 ± 0.29^{ab}	315.86 ± 5.02^{a}
Conc. 2	C	24.96 ± 0.79^{c}	8.31 ± 0.11^{a}	9.41 ± 0.12^{a}	243.14 ± 2.66^{bc}
	EA	25.01 ± 0.36^{c}	5.51 ± 0.26^{b}	5.80 ± 0.26^{c}	222.28 ± 2.11^{d}
	PvPP + Cas	26.53 ± 0.10^{b}	5.36 ± 0.45^{b}	6.42 ± 0.28^{c}	251.10 ± 0.68^{b}
	B	25.18 ± 0.15^{c}	4.98 ± 0.52^{b}	6.88 ± 1.41^{bc}	236.75 ± 2.44^{c}
	VP	27.17 ± 0.73^{ab}	5.30 ± 0.25^{b}	6.02 ± 0.18^{c}	223.30 ± 0.90^{d}
	G	27.88 ± 0.27^{a}	6.07 ± 1.21^{b}	5.71 ± 0.32^{c}	223.46 ± 2.53^{d}
	T	25.33 ± 0.16^{c}	5.76 ± 0.10^{b}	5.78 ± 0.25^{c}	226.10 ± 2.30^{d}
	M	26.83 ± 0.01^{ab}	5.44 ± 0.37^{b}	8.30 ± 0.17^{ab}	325.09 ± 4.29^{a}
Conc. 3	C	24.96 ± 0.79^{d}	8.31 ± 0.11^{a}	9.41 ± 0.12^{a}	243.14 ± 2.66^{b}
	EA	26.16 ± 0.66^{d}	5.26 ± 0.15^{cd}	5.53 ± 0.23^{e}	225.64 ± 0.74^{c}
	PvPP + Cas	25.43 ± 0.36^{d}	4.91 ± 0.13^{d}	6.62 ± 0.26^{c}	246.03 ± 0.57^{b}
	B	27.8 ± 0.63^{bc}	5.14 ± 0.14^{cd}	6.38 ± 0.51^{cd}	224.74 ± 3.11^{b}
	VP	27.85 ± 0.45^{b}	5.36 ± 0.27^{cd}	6.04 ± 0.21^{cde}	226.68 ± 1.84^{c}
	G	26.34 ± 0.25^{bcd}	5.08 ± 0.11^{cd}	5.93 ± 0.17^{cde}	226.78 ± 2.61^{c}
	T	26.10 ± 0.44^{cd}	5.57 ± 0.40^{c}	5.86 ± 0.15^{de}	228.25 ± 5.43^{c}
	M	30.24 ± 0.97^{a}	6.40 ± 0.28^{b}	8.59 ± 0.13^{b}	338.15 ± 1.30^{a}

Mean value ± standard deviation. Different letters within the same row represents significant differences according to Tukey HSD test ($p < 0.05$).

(epigallocatechin, with three hydroxyl radicals) (MCMur-rought, Madigan, & Smyth, 1995).

The mainly flavanols removed by gelatin and egg albumin were procyanidin B_1 and B_2. Procyanidin B_2 was decreased by 24.71%, followed by procyanidin B_1 (11.09%) for egg albumin while gelatin scored a decrease of 22.9% and 4% respectively. These results are in good agreement with the finding of Oberholster, Carstens & du Toit (2013), who showed that both egg albumin and gelatin significantly decreased the mean degree of polymerization (mDP) of the wine tannins by respectively 26.4% and 25.2%. Also, our results are in agreement with the findings of other researchers (Cosme, Ricardo-Da-Silva, & Laureano, 2009; Maury et al., 2003; Sarni-Manchado, Deleris, Avallone, Cheynier & Moutounet, 1999).

Vegetable proteins decreased procyanidin B_2 by 20.8% as efficiently as gelatin (22.9%). These results are in accordance with those obtained by Jauregi, Olatujoye, Cabezudo, Frazier, and Gordon (2016) who showed that whey proteins reduced astringency in wine as efficiently as gelatin, mainly via hydrophobic interactions and hydrogen bonding with tannins leading to their aggregation and precipitation. Other authors (González-Neves et al., 2014) showed that fining with vegetable proteins had no significant effect on proanthocyanidins contents of wines. Indeed, there is a wide variety of commercial preparations; the evaluation of it is use must refer to the characteristics of each particular product (Tschiersch, Pour Nikfardjam, Schmidt, & Schwack, 2010). The protein fining agents were found to bind more easily with condensed tannins more than monomeric tannins (Sarni-Manchado, Deleris, Avallone, Cheynier & Moutounet, 1999).

The addition of mannoproteins did not affect the monomeric flavanols as other author showed (Guadalupe & Ayestarán, 2008). Procyanidin B_2 was the only flavanols that decreased (−24.82%). Previous studies performed also observed an interaction of mannoproteins with procyanidins (Guadalupe & Ayestarán, 2008; Rodrigues et al., 2012).

The addition of tannins was shown to increase total polyphenols levels and total tannins levels. No significant effect was observed on the monomeric flavanols because the added tannins are condensed tannins which cannot release monomeric flavanols. Surprisingly, the addition of condensed tannins decreases the levels of procyanidin B_2 (−34.1%). This can be explained by the polymerization between added tannins and procyanidin B_2. The self-association of flavanols and their aggregation have been demonstrated in the literature (Pianet et al., 2008). It was demonstrated that the hydrophobic interactions are the major driving forces to the flavanols self-association.

All wine treatments didn't show any effect on the phenolic acids and resveratrol contents in the wines. This is suggest there is no interaction between small phenolic compounds and macromolecules or particles.

3.3. *Effect of treatment concentrations on the phenolic composition of wines*

In order to examine the effect of different agents concentrations on the phenolic composition of wines, principal component analysis was applied to a matrix of four variables (anthocyanins, total polyphenols, tannins and ABTS) explained by the first two principal components (PC1 and PC2) and representing 88.10% of the total variance (Fig. 2). Evaluating the positions of fining agents at different concentrations 5 groups formed. The first group was formed by egg albumin and mannoproteins, situated in the left upper part of the coordinate, which is opposite to total polyphenols, tannins and ABTS (relative to PC1), with the same direction of anthocyanins (relative to PC2). The second group was composed by control, vegetable protein and gelatin, located in the right upper part of the coordinate, positively correlated with total polyphenols and tannins and opposite to anthocyanins and ABTS. The third group included tannins located in the upper right part of the coordinate which was fitted with total polyphenols and tannins. The fourth group was constituted by bentonite situated in the right lower part of the coordinate opposite to anthocyanins and ABTS. The last one involved PvPP + casein located in the left lower part of the coordinate opposite to total polyphenols, tannins and anthocyanins. The best combination that fit the four variables without excess removing of different groups of phenolic compounds was the second group, confirming that vegetable protein and gelatin

Table 4
The monomeric and dimeric flavan-3-ols, phenolic acids and resveratrol of control and treated wines.

Agents concentrations	Treatments	Flavan-3-ols						Phenolic acids			Stilbenes
		Catechin	Epicatechin	Epigallo-catechin	Epicatechin gallate	Procyanidin B1	Procyanidin B2	Gallic acid	Caffeic acid	Ferulic acid	Resveratrol
Conc. 1	C	68.41 ± 0.38^{a}	121.24 ± 0.56^{a}	300.71 ± 3.73^{a}	41.05 ± 1.30^{a}	87.61 ± 1.47^{a}	139.98 ± 3.08^{a}	41.21 ± 0.54^{a}	2.06 ± 0.02^{a}	30.95 ± 0.72^{a}	3.87 ± 0.01^{a}
	EA	68.41 ± 0.78^{a}	122.80 ± 2.27^{a}	301.06 ± 1.08^{a}	41.36 ± 2.27^{a}	86.34 ± 2.23^{a}	108.17 ± 1.53^{c}	41.34 ± 0.55^{a}	2.05 ± 0.01^{a}	31.09 ± 1.58^{a}	3.95 ± 0.14^{a}
	PvPP + Cas	70.31 ± 1.15^{a}	121.66 ± 3.14^{a}	306.68 ± 5.57^{a}	42.58 ± 0.48^{a}	88.25 ± 1.70^{a}	115.51 ± 2.94^{bc}	41.73 ± 0.07 ^{a}	2.05 ± 0.01^{a}	31.27 ± 1.43^{a}	3.85 ± 0.03^{a}
	B	67.46 ± 3.59^{a}	115.54 ± 12.57^{a}	184.24 ± 9.18^{b}	41.22 ± 0.66^{a}	77.43 ± 0.40^{b}	123.55 ± 2.18^{b}	41.19 ± 0.54^{a}	2.04 ± 0.01^{a}	31.22 ± 0.84^{a}	3.92 ± 0.05^{a}
	VP	68.39 ± 1.13^{ab}	123.30 ± 2.52^{a}	302.86 ± 4.78^{a}	44.19 ± 2.58^{a}	85.71 ± 0.99^{ab}	111.34 ± 1.36^{c}	41.31 ± 0.59 ^{a}	2.05 ± 0.01^{a}	30.88 ± 1.79^{a}	3.99 ± 0.05^{a}
	G	67.68 ± 2.38^{a}	113.24 ± 3.16^{a}	294.97 ± 12.36^{a}	39.39 ± 1.37^{a}	87.42 ± 3.70^{a}	137.81 ± 1.14^{a}	41.74 ± 0.04^{a}	2.05 ± 0.01^{a}	30.34 ± 0.69^{a}	3.86 ± 0.05^{a}
	T	69.86 ± 0.94^{a}	121.02 ± 5.27^{a}	302.50 ± 9.30^{a}	40.35 ± 2.55^{a}	91.58 ± 2.43^{a}	92.20 ± 8.26^{d}	41.33 ± 0.56^{a}	2.05 ± 0.01^{a}	31.42 ± 1.31^{a}	3.88 ± 0.04^{a}
	M	69.63 ± 1.41^{a}	123.56 ± 3.6^{a}	301.25 ± 1.44^{a}	43.39 ± 2.69^{a}	86.54 ± 6.56^{a}	95.46 ± 1.71^{d}	41.57 ± 0.02^{a}	2.06 ± 0.01^{a}	31.05 ± 0.78^{a}	3.92 ± 0.04^{a}
Conc. 2	C	68.42 ± 0.38^{ab}	121.24 ± 0.56^{a}	300.71 ± 3.73^{a}	41.05 ± 1.30^{a}	87.61 ± 1.47^{ab}	139.98 ± 3.08^{a}	41.21 ± 0.54^{a}	2.06 ± 0.02^{a}	30.95 ± 0.72^{a}	3.87 ± 0.01^{a}
	EA	69.10 ± 1.30^{ab}	123.55 ± 5.38^{a}	301.66 ± 5.68^{a}	40.90 ± 0.40^{a}	85.52 ± 3.05^{ab}	110.03 ± 3.84^{b}	40.99 ± 0.60^{a}	2.05 ± 0.01^{a}	30.87 ± 1.12^{a}	3.93 ± 0.16^{a}
	PvPP + Cas	67.42 ± 1.65^{b}	118.14 ± 5.02^{a}	284.28 ± 0.89^{b}	44.55 ± 3.16^{a}	81.39 ± 3.62^{b}	110.20 ± 3.80^{b}	41.59 ± 1.00 ^{a}	2.05 ± 0.01^{a}	31.76 ± 0.36^{a}	3.83 ± 0.02^{a}
	B	69.82 ± 3.01^{ab}	123.91 ± 5.10^{a}	179.59 ± 5.92^{c}	44.81 ± 2.64^{a}	86.19 ± 2.14^{ab}	112.46 ± 1.19^{b}	41.59 ± 0.01^{a}	2.05 ± 0.01^{a}	31.26 ± 1.51^{a}	3.84 ± 0.09^{a}
	VP	69.37 ± 1.06^{ab}	124.30 ± 3.33^{a}	307.57 ± 4.41^{a}	44.42 ± 2.83^{a}	87.34 ± 2.28^{ab}	112.19 ± 1.81^{b}	41.61 ± 0.05^{a}	2.05 ± 0.01^{a}	30.88 ± 1.92^{a}	3.86 ± 0.05^{a}
	G	68.86 ± 0.58^{ab}	122.58 ± 0.92^{a}	297.69 ± 3.69^{a}	39.37 ± 1.64^{a}	85.86 ± 0.62^{ab}	108.01 ± 2.99^{b}	41.65 ± 0.07^{a}	2.06 ± 0.01^{a}	31.11 ± 0.89^{a}	3.85 ± 0.05^{a}
	T	72.41 ± 2.40^{a}	124.48 ± 5.91^{a}	296.71 ± 4.22^{ab}	42.31 ± 2.74^{a}	89.45 ± 2.33^{a}	100.71 ± 6.74^{b}	41.65 ± 0.04^{a}	2.05 ± 0.01^{a}	30.55 ± 1.25^{a}	3.79 ± 0.03^{a}
	M	70.04 ± 1.29^{ab}	123.49 ± 3.64^{a}	298.18 ± 6.95^{a}	43.72 ± 2.93^{a}	88.93 ± 2.35^{a}	105.24 ± 7.31^{b}	40.74 ± 0.64^{a}	2.05 ± 0.01^{a}	30.87 ± 1.98^{a}	3.83 ± 0.06^{a}
Conc. 3	C	68.41 ± 0.38^{b}	121.24 ± 0.56^{a}	300.71 ± 3.73^{a}	41.05 ± 1.30^{a}	87.61 ± 1.50^{a}	139.98 ± 3.08^{a}	41.21 ± 0.54^{a}	2.05 ± 0.02^{a}	30.95 ± 0.73^{a}	3.87 ± 0.01^{a}
	EA	68.27 ± 5.75^{b}	120.61 ± 7.64^{a}	295.93 ± 3.73^{ab}	39.94 ± 0.64^{a}	79.71 ± 1.00^{b}	105.35 ± 0.84^{c}	41.93 ± 0.65^{a}	2.05 ± 0.01^{a}	30.52 ± 1.63^{a}	3.96 ± 0.14^{a}
	PvPP + Cas	67.18 ± 0.72^{b}	119.49 ± 0.99^{a}	289.44 ± 1.76^{b}	41.12 ± 0.63^{a}	83.44 ± 1.01^{ab}	112.95 ± 2.63^{bc}	41.7 ± 0.05^{a}	2.05 ± 0.01^{a}	31.23 ± 1.18^{a}	3.93 ± 0.03^{a}
	B	69.65 ± 0.69^{b}	124.13 ± 2.55^{a}	177.24 ± 2.73^{c}	43.23 ± 1.48^{a}	86.18 ± 1.51^{a}	115.87 ± 2.85^{b}	41.66 ± 0.03^{a}	2.05 ± 0.02^{a}	31.13 ± 1.74^{a}	3.81 ± 0.06^{a}
	VP	68.26 ± 0.97^{b}	122.69 ± 4.1^{a}	304.13 ± 7.04^{a}	44.21 ± 3.43^{a}	84.74 ± 3.89^{ab}	110.85 ± 2.78^{bc}	41.65 ± 0.03^{a}	2.05 ± 0.01^{a}	31.07 ± 1.49^{a}	3.85 ± 0.04^{a}
	G	68.29 ± 1.15^{b}	120.39 ± 2.95^{a}	296.45 ± 3.88^{ab}	39.92 ± 0.01^{a}	84.16 ± 2.79^{ab}	107.92 ± 0.41^{c}	41.64 ± 0.01^{a}	2.05 ± 0.01^{a}	30.71 ± 1.48^{a}	3.87 ± 0.02^{a}
	T	76.91 ± 1.06^{a}	129.99 ± 3.70^{a}	304.33 ± 6.61^{a}	45.24 ± 2.90^{a}	89.53 ± 0.22^{a}	110.4 ± 3.23^{bc}	41.33 ± 0.57^{a}	2.05 ± 0.01^{a}	31.22 ± 1.28^{a}	3.89 ± 0.01^{a}
	M	70.15 ± 1.14^{b}	125.08 ± 4.22^{a}	302.01 ± 7.05^{a}	45.07 ± 2.83^{a}	86.59 ± 3.12^{a}	112.05 ± 3.9^{bc}	41.55 ± 0.22^{a}	2.05 ± 0.04^{a}	30.84 ± 2.05^{a}	3.85 ± 0.06^{a}

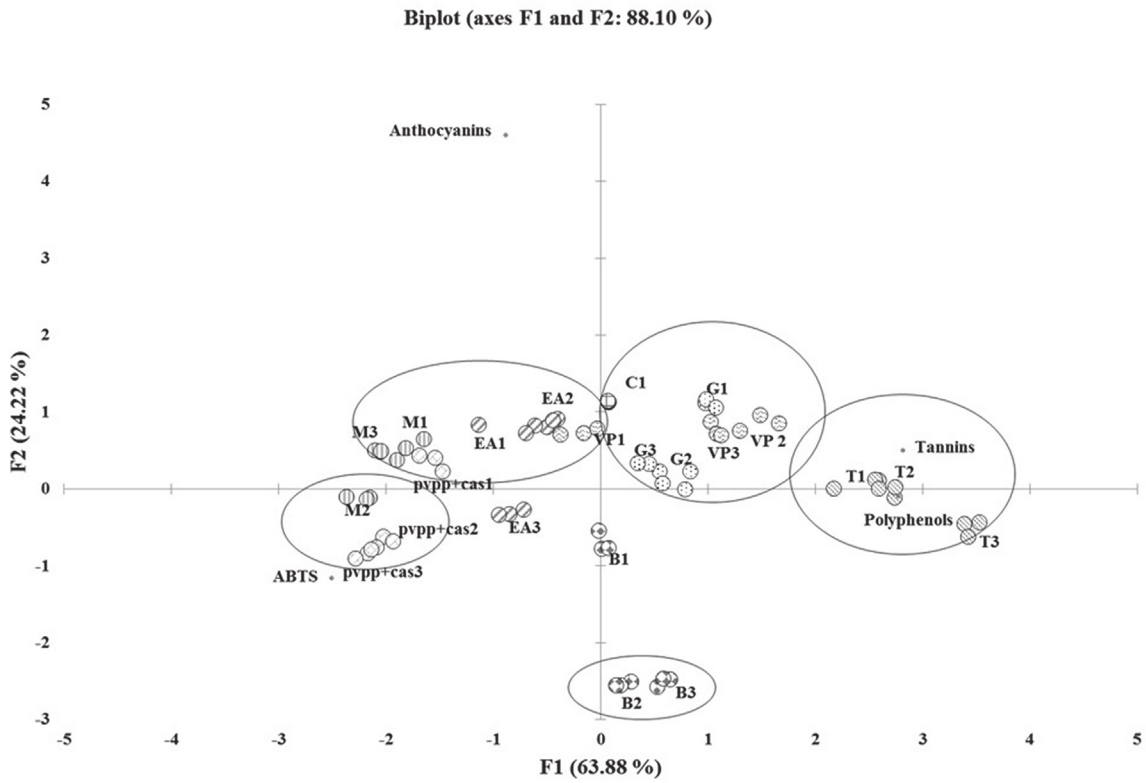

Fig. 2. PCA Biplot of the two first principal components of analyzed parameters: Anthocyanins (mg/L), total polyphenols (mg/L GAE), ABTS (mg/L GAE) and Tannins (mg/L) in samples treated with different fining agent (C, control; EA, egg albumin; PvPP + Cas, polyvinylpyrrolidone + Casein; B, bentonite; VP, vegetable proteins; G, gelatin; T, tannins; M, mannoproteins) at different concentrations (1, concentration 1; 2, concentration 2; 3, concentration 3).

fining agents had minimal effect on the phenolic composition of wines. The results of PCA showed the importance of using the recommended minimum amount of all fining agents for high phenolic compounds and antioxidant activity.

4. Conclusion

Using fining agents, adding tannins and commercial mannoproteins for red wines must be taken with care, since these agents determined a different impact on the sensory characteristics of wines according to their nature, the applied dose and the style of wine. The most remarkable effects were those obtained by bentonite which had a negative impact on the anthocyanins contents and wine color, in addition mannoprotein and PvPP + casein decreased significantly tannin levels, while vegetable protein and gelatin revealed the less impact on the wine phenolic composition. Antioxidant activity was positively affected by the addition of condensed tannins. After all, the results of principle components analyses showed the importance of a low concentration of fining agents for high antioxidant activity and high phenolic compounds.

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