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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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ABSTRACT

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.

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