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Effect of fining on the colour and pigment composition of young red wines

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ABSTRACT

This work aimed to evaluate the effect of four fining agents on the colour and pigment composition of red wines of Tannat. The wines were analysed 15 days after fining and immediately after separation of sediments and bottling. Colour was evaluated by spectrophotometry and polyphenols were analysed by spectrophotometry and HPLC–DAD. The colour intensity of wine was significantly decreased by bentonite and egg albumin. The most remarkable effects on wine phenolic composition were produced by bentonite and gelatin, which significantly decreased anthocyanin and tannin concentrations, respectively. Results show that each fining agent has very different impact on the wine attributes, and their effects depended as well on the composition of the clarified wine. The use of non-traditional agents of fining, as vegetable proteins, may have less impact on the colour and anthocyanin content of red wines.

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1. Introduction

The colour and limpidity are the first sensory attributes of wines that are appreciate by consumers, predisposing their acceptance or rejection. A suitable wine stabilisation and limpidity is progressively obtained after winemaking due to physical and chemical phenomena that determine the precipitation of unstable compounds and the sedimentation of the clouding particles. This process is often improve by using different agents that will interact with the components of wine because the natural clarification, in addition to being slow, may not be enough for proper clarity and stability of the wine. Clarification using fining agents can reach a better limpidity in less time and may improve the stability of the wines (Sims, Eastridge, & Bates, 1995). Additionally, protein based fining agents can determine some declines in astringency and bitterness of wine due to its interaction with tannins (Karamanidou, Kallithraka, & Hatzidimitrou, 2011; Oberholster, Carstens, & Du Toit, 2013; Tschiersch, Pour Nikfardjam, Schmidt, & Schwack, 2010).

Nevertheless, the interactions between protein based fining agents (like albumin and gelatin) and polyphenols can affect colour of young red wines due to the precipitation of pigments (Castillo-Sánchez, Mejuto, Garrido, & García-Falcón, 2006;

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Castillo-Sánchez et al., 2008) even though the impact reported in some cases was slight (Stankovic, Jovic, & Zivkovic, 2004). In the case of other fining agents as bentonite, important declines in the colour intensity of wines were reported (Patil, Kaur, & Sharma, 2012; Stankovic, Jovic, Zivkovic, & Pavlovic, 2012).

Polyphenols are the most important secondary metabolites and the major bioactive compounds synthesized in the berries. Several polyphenols extracted from grape skins and seeds along winemaking have an important role in sensory properties, as anthocyanins, pigments responsible for the colour of young red wines, and tannins, responsible for astringency and bitterness of wine (Cheynier et al., 2006; Fulcrand, Dueñas, Salas, & Cheynier, 2006).

The most commonly used fining agents perform their tasks by attracting the positively and negatively charged particles in the unclear wine since they also also are charge carriers. Examples include bentonite (negatively charged), gelatin (positively) and egg white (positively).

The emergence of bovine spongiform encephalopathy (BSE) has given considerable interest in the replacement of animal-derived protein in food processing and the alternative use of plant-derived proteins (Bindon & Smith, 2013; Chagas, Monteiro, & Ferreira, 2012). Additionally, food allergy and food intolerance based on hidden food ingredients increasingly raises public awareness (Tschiersch et al., 2010). Since 1999, many investigations have been carried out with wheat prolamins, commonly called gluten, as white musts and wines clarifying agents. Different experimental procedures were established to compare gluten efficaciousness





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with usual fining agents (Marchal, Marchal-Delhaut, Lallement, & Jeandet, 2002). At present, a wide variety of commercial preparations of plant-derived proteins from soy, gluten wheat, rice, potato, lupine or maize had been studied and proposed for oenological use with the name generically of "vegetable proteins" (Bindon & Smith, 2013; Chagas et al., 2012; Marchal et al., 2002; Tschiersch et al., 2010).

This work aimed to evaluate the effect of different fining agents on the colour and pigment composition of red wines of Tannat. In order to evaluate these effects there were four small-scale experiences, contrasting four treatments of clarification with a witness.

2. Materials and methods

2.1. Chemical and fining agents

Folin–Ciocalteu reagent and vainillin were purchased from Sigma–Aldrich (Switzerland). Sodium carbonate anhydrous was from Carlo Erba (Italy). Clorhidric acid was from J.T. Bakker (Mexico) and ethanol from Dorwil (Argentine).

Water for high-pressure liquid chromatography (HPLC) analyses was nanopure. The anthocyanin standard was malvidin glucoside chloride from Extrasynthèse (France). Formic acid and methanol HPLC grade were from Sigma–Aldrich (Switzerland).

The fining agents evaluated were bentonite, gelatine, vegetable protein and egg albumin. Bentonite (Bentogran) was from AEB (Italy) and Gelatin (Gelita Gold Strength/200 Bloom) was from Gelita (Spain). Egg albumin was added as fresh egg whites. Vegetable proteins used were gluten proteins.

Abastecimientos S.A. (Uruguay) sponsored gelatin and vegetable protein. The company did not provide additional information about these products.

2.2. Wine treatments

Four different wines were used in the clarification trials, including three wines of two months of age (wines 1-2 m, 2-2 m, 3-2 m) and one wine aged 14 months (wine 4-14 m). Each wine was clarified with the same four fining agents. The corresponding untreated wines were employed as a control (C) in each assay. The doses employed of each fining agent are the usually used in wineries. In each case, the doses were 50 g/HL for bentonite (B), 15 g/HL for gelatine (G), 15 g/HL for vegetable protein (VP), and 10 egg whites/HL for egg albumin (EA).

Wine 1–2 m was elaborated in 2010, wine 4–14 m was elaborated in 2011, and wines 2–2 m and 3–2 m were elaborated in 2012. All wines were produced employing Tannat grapes grown in the south of Uruguay. The harvest was made according to the relationship between sugars contents, total acidity and pH of musts.

The grapes employed to produce the wine 1–2 m had 20.8 Brix, 83.6 meq/L of total acidity and pH 3.44; for wine 2–2 m they had 21.2 Brix, 65.3 meq/L and pH 3.41; for wine 3–2 m they had 21.0 Brix, 73.4 meq/L and pH 3.30; and for wine 4–14 m they had 24.2 Brix, 89.8 meq/L and pH 3.35. These analyses were carried out using an Atago N1 refractometer (Atago, Japan) and a Hanna HI8521 pH metre (Hanna instruments, Italy), respectively.

At harvest, the clusters were transported in plastic boxes (20 kg each one) to the winery. The bunches of grapes were destemmed and crushed with an Alfa 60 R crusher (Italcom, Italy), and the barrelling was in stainless-steel tanks (100 L capacity each). Potassium metabisulfite (50 mg SO₂/100 kg of grapes) was added and dry active yeast (20 g/HL *Saccharomyces cerevisiae*, Natuferm 804; Oeno-BioTech, France) was inoculated in all the musts. The sulphur dioxide additions and yeast inoculations were realised immediately

after the crushing of grapes. Wines were made by classical fermentation on skins for 8 days. Two pumping over followed by punching the cap were carried out daily along the skin contact. The temperatures of fermentation were comprised between 23 and 26 °C.

At devatting, free-run juice was obtained and the marc was pressed with a stainless steel manual press. In all cases, free-run juices and press juices were mixed. The wines were maintained in the stainless-steel tanks, where the fermentations were completed, until racking. The wines were stabilized with dioxide additions (50 mg SO2/L) realised at the end of malolactic fermentation. Finally, the wines were kept in glass recipients of 10-litre capacity, closed with cork stoppers, until fining. Before clarification, we proceeded to standardize the total volume of wine that was used in each trial, then dividing it into 10-litre containers, which hosted the addition of clarifiers.

Fining was made in 2010 for wine 1–2 m and in 2012 for the others three wines. Fining treatments were performed in two replicates (two 10-litre containers of each wine for each fining agent). The wines were racked 15 days after fining and bottled in 750 mL green glass bottles, closed with cork stoppers. Immediately, the wines were analysed.

2.3. Analysis of turbidity of wines

Turbidity of wines was analysed before fining and 15 days after, following a separation of the sediment and bottling. Turbidity was measured by nephelometric analysis performed by an Oakton TN-100/T-100 turbidity metre (Oakton Instruments, USA). Measurements were realised according to the methodology proposed by the turbidity metre manufacturer. Briefly, a calibration was made using standard solutions of 0.02, 20, 80 and 800 NTU, provided by the manufacturer. After that, the turbidity index of the wines were measured by duplicate. Results are expressed in NTU (Nephelometric Turbidity Unit).

2.4. Spectrophotometric analysis of wines

Analyses of colour and polyphenol composition were realised 15 days after fining.

Polyphenol indexes and colour were analysed by spectrophotometric methods. The colour of the wines was evaluated with the indexes proposed by Glories (1984): colour intensity (CI) and hue. Also, the CIELAB parameters brightness (L*), chromaticity (C^{*}), redness (a^{*}) and yellowness (b^{*}) were determined, using the D65 illuminant and a 10° observer according to Ayala, Echávarri, and Negueruela (1997). Co-pigmentation indexes according to Boulton (2001) were measured and "colour due to anthocyanins" (CA), "colour due to co-pigmentation" (CC) and "colour due to polymers" (CP) were calculated. Total polyphenols were analysed with Folin-Ciocalteu reagent, according to the method proposed by Singleton and Rossi (1965), catechins according to Swain and Hillis (1959), and proanthocyanidin content was measured according to Ribéreau-Gayon and Stonestreet (1966). The DMACH index was measured and tannin polymerisation index was calculated as the relationship between the DMACH index and the proanthocyanidin content according to Vivas, Glories, Lagune, Saucier, and Augustin (1994).

The wines were centrifuged for 3 min at 3000 rpm before spectrophotometric analysis. The measurements were carried out using a Cole Parmer S2100-UV+ (Cole Parmer, USA) and a Shimadzu UV-1800 (Shimadzu, Japan) UV–VIS spectrophotometer, employing glass cells with a 1 mm path length for the colour analyses and glass cells with a 1 cm path length for the polyphenol analyses. All analyses were performed by duplicate.

386

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