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Influence of the structural features of commercial mannoproteins in white wine protein stabilization and chemical and sensory properties

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ABSTRACT

The cloudy aspect formed in white wines due to protein instability is a visual defect. Sodium bentonite is the most commonly used fining agent to treat this instability, but has usually a negative impact on the wine's physicochemical and sensory characteristics. Aiming to find suitable alternatives, eleven commercial mannoproteins were chemically characterized concerning their sugar composition and protein content, and their effectiveness on wine protein stabilization. Also, their effect on the amount and nature of phenolic compounds, browning potential, chromatic and sensory characteristic was evaluated. Protein stabilization effectiveness was related to their chemical composition, namely their high mannose to glucose ratio. Additionally, some mannoproteins decreased the browning potential. Thus, mannoproteins could be an effective alternative for protein stabilization, preserving or even improving wine quality.

1. Introduction

The appearance of haze or amorphous precipitate due to protein instability in commercial bottled white wines is considered a visual defect, and although not affecting the olfactory characteristics (Batista, Monteiro, Loureiro, Teixeira, & Ferreira, 2009), is unacceptable to consumers (Sauvage, Bach, Moutonet, & Vernhet, 2010).

Some white wine proteins that include thaumatin-like proteins and chitinases (Falconer et al., 2010) synthesized during ripening as a defence mechanism against fungal attacks (Waters et al., 2005) persist throughout the winemaking process (Linthorst, 1991), being responsible for this wine colloidal instability (Sauvage et al., 2010). The observed instability is dependent on proteins characteristics (isoelectric point – pI and molecular weight), and their concentration depends on a multitude of factors, such as grape variety, climatic conditions, soil type, grape maturity and winemaking process (Pashova, Guell & López, 2004; Sauvage et al., 2010). Protein fractions with low molecular weight (12.6–30 kDa) and low pI (4.1–5.8) are the major contributors to wine instability (Hsu & Heatherbell, 1987). Also the wine chemical properties, such as pH, ionic strength, ethanol content and also the storage temperature (Boulton, 1980), have an important effect in haze development. Changes in these parameters can lead to wine protein denaturation, aggregation and flocculation resulting in a cloudy suspension with the possible formation of an amorphous precipitate (Waters et al., 2005).

Protein instability problems are handled by the use of negatively charged fining agents, mainly sodium bentonite, leading to the flocculation and precipitation of unstable proteins. Bentonite, a montmorillonite clay, is the most commonly used fining agent in the wine industry to prevent protein instability in white wines, however, the efficiency of bentonite fining depends of the bentonite type, surface charge density, dose, as well as wine composition, pH and temperature (Lambri, Dordoni, Silva, & Faveri, 2012). Lambri, Dordoni, Silva, and Faveri (2010) claim that bentonite is not specific for proteins, and may also remove other charged compounds. Therefore, bentonite fining can affect the wine quality by removing colour, flavour and texture related compounds, changing wine sensory properties (Høj et al., 2001).

Consequently, alternative techniques to bentonite fining have been studied, such as ultrafiltration (Hsu & Heatherbell, 1987), addition of proteolytic enzymes (Dizy & Bisson, 1999), flash

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pasteurization (Pocock, Høj, Adams, Kwiatkowski, & Waters, 2003), alternative adsorbents (Sarmento, Oliveira, & Boulton, 2000), zirconium oxide treatment (Pashova et al., 2004), natural zeolites (Mercurio et al., 2010) and the use of mannoproteins (Gonzalez-Ramos, Cebollero, & Gonzalez, 2008). Some studies showed that mannoproteins could improve wine protein stability and also their sensorial quality, such as mouthfeel (Waters, Dupin, & Stockdale, 2000). Preliminary results from our laboratory showed that different commercial preparations of mannoproteins showed a different performance on white wine protein stabilization and this could be related to their chemical composition. However, as far as we know, there is a lack of information on the effect of the chemical composition of commercial mannoproteins (sugars composition and protein content) and their efficiency on wine protein stabilization. Therefore an improvement of this fining process could be achieved by a better knowledge of the mannoprotein chemical composition. Thus, the main purpose of this study was to evaluate the effectiveness of commercial mannoproteins, with different chemical sugar composition and protein content, on white wine protein stabilization, and their effect on phenolic compounds, as well as on the chromatic and sensorial characteristics of wine.

2. Materials and methods

2.1. Wine sample

A young white wine from Douro Valley 2011 vintage was used, and their main characteristics were as follows: alcohol content (% v/v) 14.2, specific gravity (20 °C) (g/mL) 0.9890, titratable acidity (g/L tartaric acid) 5.5, pH 3.33, volatile acidity (g/L acetic acid) 0.31, protein stability heat test 7.1 NTU (unstable > 2 NTU).

2.2. Analysis of conventional oenological parameters

Alcohol, specific gravity, pH, titratable acidity and volatile acidity were analysed using a FTIR Bacchus Micro (Microderm, France).

2.3. Bentonite and mannoprotein fining trials

Fining experiments were performed using five commercial bentonites B1 – sodium calcium (10–40 g/hL); B2 – activated sodium calcium (50–200 g/hL); B3 – natural sodium (40–120 g/hL); B4 – activated calcium (10–20 g/hL); B5 – natural calcium (40–100 g/hL), and eleven commercial mannoproteins all of them from yeast cell wall (M1 – 30 g/hL; M2 – 1–5 g/hL; M3 – 5–10 g/hL; M4 – 10–40 g/hL; M5 – 40 g/hL; M6 – 5–10 g/hL; M7 – 5–40 g/hL; M8 – 40 g/hL; M9 – 5–40 g/hL; M10 – 0.5–5 g/hL; M11 – 40 g/hL), according to manufacture. Preliminary tests were conducted with bentonite and mannoproteins in order to understand the doses from which the wine protein stability is achieve (data not shown). Based on this previous results, bentonite were tested at medium concentration (B1 – 25 g/hL; B2 – 125 g/hL; B3 – 80 g/hL; B4 – 15 g/hL; B5 – 70 g/hL) and mannoproteins at high concentration (M1 – 30 g/hL; M2 – 5 g/hL; M3 – 10 g/hL; M4 – 40 g/hL; M5 – 40 g/hL; M6 – 10 g/hL; M7 – 40 g/hL; M8 – 40 g/hL; M9 – 40 g/hL; M10 – 5 g/hL; M11 – 40 g/hL). All the oenological products were prepared to the manufacture's specifications. Wine without any treatment was used as a control.

The products were thoroughly mixed, added to each treatment and allowed to stand in contact with the wine in 375 mL flasks at 20 °C for 7 days. Before analysis, the samples were centrifuged at 537.6*g* for 10 min. All experiments were performed in duplicate.

2.4. Commercial mannoprotein characterization

2.4.1. Sugar composition and content

Commercial mannoproteins were characterized for their sugar composition and content by anion-exchange chromatography with pulsed amperometric detection, after acid hydrolysis.

Two parallel acid hydrolyses were performed, with and without Saeman hydrolysis, in order to obtain the amount of insoluble polysaccharide present in commercial mannoproteins. For Saeman hydrolysis, each sample (5 mg) was treated for 3 h at room temperature, with 400 μL of H_2SO_4 (72%) (vortexed every 15 min). After this time 4.4 mL of water was added and the material was hydrolysed for 2.5 h at 100 °C. After cooling, 500 μL of 2-desoxiglucose (0.5 mg/mL, internal standard) was added. The second hydrolysis was performed in the same way without the Saeman hydrolysis. The use of a previous Saeman hydrolysis followed by the acid hydrolysis allows us to solubilize the insoluble polysaccharides present in the mannoproteins and to obtain the total amount of polysaccharides present in these additives. On the other hand, without Saeman hydrolysis step, only the more soluble polysaccharides were quantified in the additive.

For chromatographic analysis 400 μL of each sample were diluted into vials with 4600 μL of water. Quantification was performed by the internal standard method using calibration curves of fucose, rhamnose, arabinose, galactose, glucose, mannose, xylose, galacturonic and glucoronic acid standards (0.25–2.5 mg of sugar/0.5 mg of internal standard).

Sugar separation was performed with a CarboPac PA-20 column (150 mm × 3 mm) with a CarboPac PA20 pre-column (Dionex) using eluent A – 1.25 mM NaOH solution containing 2 mM $Ba(OH)_2$, eluent B – 400 mM sodium acetate containing 2 mM $Ba(OH)_2$ and eluent C – 500 mM NaOH containing 2 mM $Ba(OH)_2$. The eluent was kept under nitrogen all times to reduce carbonate build up and biological contamination. The injection volume was 5 μL, the flow rate was 0.3 mL/min and the column temperature was maintained at 35 °C during the run. The following elution program was used: 0–19 min, 100% A, increase to 50% B until 27 min and maintained until 37 min; increase to 40% C and decreasing to 0% B until 47 min and maintained until 57 min. The column was conditioned with 100% A during 15 min before injection. The sugar detection was performed with an electrochemical detector containing an Au working electrode, Ag/AgCl reference electrode, and Ti counter electrode. The ED cell waveform was +0.1 V from 0.00 to 0.40 s, then −2.0 V from 0.41 to 0.42 s, and a ramp −2.0 to +0.6 V from 0.42 to 0.43 s, followed by −0.1 V from 0.44 to 0.50 s (end of cycle). The integration region was from 0.2 to 0.4 s. All analyses were performed in duplicate.

2.4.2. Protein concentration

Total nitrogen was determined by the Kjeldahl method and total protein content was estimated as Kjeldahl nitrogen multiplied by a factor 6.25 (OIV, 2006a).

2.5. Protein stability tests

2.5.1. Heat test

Heat test was performed according Pocock and Rankine (1973). If the difference (ΔNTU) in nephelometric turbidity unit (NTU), between the heated and unheated samples was higher than 2 NTU units, means that the wine sample is unstable (Dubourdieu, Serrano, Vannier, & Ribéreau-Gayon, 1988). All analyses were performed in duplicate.

2.5.2. Tricloroacetic acid test (TCA)

Tricloroacetic acid test was performed according to Berg and Akihoshy (1961). Turbidity was measured in nephelometric