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Interactions of grape tannins and wine polyphenols with a yeast protein extract, mannoproteins and β-glucan

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ABSTRACT

At present, there is a great interest in enology for yeast derived products to replace aging on lees in winemaking or as an alternative for wine fining. These are yeast protein extracts (YPE), cell walls and mannoproteins. Our aim was to further understand the mechanisms that drive interactions between these components and red wine polyphenols. To this end, interactions between grape skin tannins or wine polyphenols or tannins and a YPE, a mannoprotein fraction and a β-glucan were monitored by binding experiments, ITC and DLS. Depending on the tannin structure, a different affinity between the polyphenols and the YPE was observed, as well as differences in the stability of the aggregates. This was attributed to the mean degree of polymerization of tannins in the polyphenol fractions and to chemical changes that occur during winemaking. Much lower affinities were found between polyphenols and polysaccharides, with different behaviors between mannoproteins and β-glucans.

1. Introduction

In recent years, a large variety of commercial products obtained from the yeast cells have been developed to promote red wine sensory characteristics, such as astringency, texture, color and mouthfeel (Alcalde-Eon, García-Estévez, Puente, Rivas-Gonzalo, & Escribano-Bailón, 2014; Del Barrio-Galán, Pérez-Magariño, Ortega Heras, & Guadalupe, 2012; Escot, Feuillat, Dulau, & Charpentier, 2001; Palomero et al., 2009). These products are inactivated yeasts (IY), cell walls (CW) and mannoproteins (MPs) in the context of wine aging and yeast protein extracts (YPEs) for wine fining (Charpentier, Caillet, & Feuillat, 2006; Iturmendi, Durán, Marín-Arroyo, & Marin-Arroyo, 2010; Lochbühler et al., 2014). Their impact is attributed to interactions between red wine polyphenols and these yeast derived products (YDPs).

In the case of IY, CW and MPs, this impact has been attributed to the release of yeast mannoproteins (Guadalupe, Martínez, & Ayestarán, 2010; Loira et al., 2013; Pozo-Bayón, Andújar-Ortiz, & Moreno-Arribas, 2009; Rodrigues, Ricardo-da-silva, Lucas, & Laureano, 2012) in wine or to polyphenol adsorption on yeast cell walls, and especially on the outer mannoprotein layer (Mazauric & Salmon, 2005; Morata et al., 2003). Thus, physico-chemical interactions between MPs and polyphenols are regarded as a key mechanism. Although β-glucans are also major components of cell walls, their potential role has never been considered. When dealing with YPEs and the fining of red wines, protein-polyphenol interactions are expected to be decisive. Fining is used to soften tannic agressivity/intensity, to improve mouthfeel or to improve filterability or stabilization with regards to the precipitation of colloidal coloring matter. The effect of fining proteins is mostly related to their ability to interact with wine polyphenols and especially tannins (Granato et al., 2010).

In spite of a growing interest for the use of these YDPs in enology, the exact mechanisms involved in their potential effect on wine quality (clarification, sensory characteristics and wine stability) remain poorly understood. This is related: i) to the diversity of the products proposed and to their complex composition and ii) to the variability of red wine composition. In our previous works, we focused on studying polyphenols interactions with different YDPs (dead and inactivated yeast, yeast cell walls) obtained from the same enological *Saccharomyces cerevisiae* strain. The adsorption of grape tannins and wine polyphenols was studied by means of adsorption isotherms (Mekoue Nguela, Sieczkowski, Roi, &

Abbreviations: DLS, dynamic light scattering; GST, grape skin tannins; WP, wine pool; WT, wine tannins; YPE, yeast protein extract; D_H, average hydrodynamic diameter; I_S, intensity scattered.

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Vernhet, 2015). Interactions between polyphenols and cell walls were observed, but purified cell walls resulted in about 10-fold lower polyphenol retention than that obtained with whole yeast. We demonstrated later by means of confocal fluorescence and transmission electron microscopy that polyphenols cross the cell envelope barrier (cell wall and plasma membrane) of dead yeast and interact with cytoplasm constituents (Mekoue Nguela, Vernhet, Sieczkowski, & Brillouet, 2015). This explained the quantitative differences observed between whole inactivated cells and cell walls. As proteins in yeast represent 40–50% of the cell dry weight (Wiederhold, Veenhoff, Poolman, & Slotboom, 2010), it was supposed that the cytoplasmic constituents involved were mainly proteins. These microscopic observations clearly showed that mannoproteins interact with tannins, but nothing could be concluded concerning β-glucans.

The present study has aimed to further explore interaction mechanisms between polyphenols and yeast components by: (i) studying interactions in solution between polyphenols and a yeast protein extract and (ii) comparing YPE/polyphenol interactions with mannoprotein/polyphenol and β-glucan/polyphenol interactions in solution. Interactions were studied using three different polyphenol fractions: grape skin tannins, a total polyphenol extract purified from a red wine and tannins purified from this extract.

2. Materials and methods

2.1. Yeast protein extract

The YPE was provided by Lallemand (Montreal, Canada). It was obtained from a biomass of a commercial *Saccharomyces cerevisiae* strain used in enology. The yeast biomass was produced according to the industrial process, in aerobic conditions. Intracellular proteins were extracted using a treatment likely to avoid their hydrolysis and the extract was spray-dried. Residual water accounted for about 6% (w/w) of the powder, which was stored at 4 °C before use. The YPE powder was first rehydrated in water under stirring for 15 min at a concentration of 100 g L^{-1}. It contained an insoluble fraction, which was removed by centrifugation (15,000*g*, 15 min, 15 °C) and represented 13% w/w (weighed after lyophilization) of the initial YPE powder. The soluble fraction (87% w/w) was further characterized and used for interaction studies.

Soluble polymers were separated from oligomers by ultrafiltration. To this end, 10 mL of YPE (concentration 87 g L^{-1}) were introduced in a 50 mL centrifugal filter unit equipped with a 10 kDa membrane (Amicon, Millipore) and centrifuged at 6000*g* during 10 min, at a temperature of 15 °C. The permeate was recovered and the retentate diafiltrated several times to remove the residual small solutes. Samples were freeze-dried to determine the proportion of oligomers (molecular weight <10 kDa) and polymers (molecular weight >10 kDa) and used for further analyses: total nitrogen, proteins and carbohydrates). Total nitrogen was determined by the Kjeldahl method using a Büchi digestion unit K-435 system and a Büchi distillation unit K-314 system. 30 mg of samples were used for this analysis. Proteins in the YPE were analyzed by 1D SDS-PAGE electrophoresis, performed on a 14% acrylamide resolving gel (gel length, 60 mm). The YPE soluble (10 g L^{-1}) was dissolved twice in a Laemmli 2× buffer and 30 µL were loaded on the gel. A low molecular weight calibration kit (14.4–97 kDa, Pharmacia, Biotech) was included in the electrophoretic run. Gels were stained with 0.1% Coomassie Brilliant Blue R-250 (Biorad) in 40% of ethanol, 10% acetic acid and destained overnight in 10% acetic acid. Gels were then scanned at 300 dpi with an image scanner (GE Biosciences). Neutral glycosyl-residues were quantified in the YPE fractions after hydrolysis by converting the monosaccharides into their alditol acetate derivatives (Albersheim, Nevins, English, & Karr, 1967). Analyses were performed on 2 mg of samples. Inositol and allose (100 µL of a 1 mg mL^{-1} solution) were added as internal standards before reduction and acetylation. The quantification was performed by GC analysis using a fused silica DB-225 (temperature programming: 170 °C for 15 min, 170–210 °C at 1 °C min^{-1}) capillary column (30 m × 0.25 µm × 0.25 mm ID) with H_2 as the carrier gas and a SHIMADZU GC-2010-Plus gas chromatograph. Alditol acetate derivatives were identified on the basis of their retention time by reference to standard monosaccharides. Neutral sugar amount was calculated relative to the allose (Albersheim et al., 1967).

2.2. Mannoproteins

A commercial solution of *Saccharomyces cerevisiae* mannoproteins, commonly used in enology, was fractionated by ultrafiltration on a 10 kDa membrane (GE-Healthcare, Hollow fiber cartridge filter, VFP-10-C-4M) to recover the high molecular weight fraction (>10 kDa). Residual small solutes and oligosaccharides in the retentate obtained by UF were removed by diafiltration with water (up to a final dilution factor of 1000). The high molecular weight fraction was conserved at 4 °C with sodium azide (0.02% w/v) until use. The dry weight per milliliter of solution, 66 mg mL^{-1}, was determined by freeze-drying of aliquots. Neutral sugars (Dallies, François, & Paquet, 1998) and Kjeldhal analyses indicated a mannose content of 55% (w/w) and a total nitrogen content of 1.6% (w/w), indicating an average protein moiety in the mannoproteins of 10%. The molecular weight distribution of the mannoproteins, between 14 and 500 kDa, was determined by high-resolution size exclusion chromatography (Ducasse et al., 2010).

2.3. Laminarin

Laminarin from *Laminaria digitata* (brown seaweed) was purchased from Sigma-Aldrich. Laminarin is a low molecular weight (6 kDa) and water-soluble β-glucan, composed of a linear β-(1,3)-glucan chain with β-(1,6)-linkages. It was used in this study to evaluate interactions between tannins and β-glucans.

2.4. Polyphenol isolation and analysis

A wine polyphenol extract **WP** was purified from a Merlot wine elaborated in 2012 at the INRA Experimental Unit of Pech Rouge (Gruissan, France), as previously described (Mekoue Nguela, Sieczkowski, et al., 2015). The **WP** was stored at −80 °C under argon atmosphere before further use. Spectrophotometric analyses were made with a SAFAS UV-MC^2 spectrophotometer (Monaco) on the powder dissolved in a model wine at a concentration of 5 g L^{-1}. This provided an absorbance at 280 nm of 55.0 (Total Polyphenol Index, TPI), corresponding to that of the initial wine. The color intensity ($A_{420nm} + A_{520nm} + A_{620nm}$, optical path 1 cm) of the reconstituted wine was 16.1 ± 0.5 and its tint (A_{420nm}/A_{520nm}, optical path 1 cm) was 0.55 ± 0.12. Accounting for the presence of residual sugars and nitrogenous compounds in the fraction (15%), a TPI of 55.0 corresponded to a whole polyphenol concentration of 4.3 g L^{-1}. This value was used to convert TPI into polyphenol amount. Polyphenol monomers were analyzed by HPLC-DAD according to the procedure described in Ducasse et al. (2010). Tannins were analyzed by HPLC after acid-catalyzed depolymerization reaction in the presence of a nucleophilic agent. Considering the low yield obtained with usual phloroglucinolysis (Mekoue Nguela, Sieczkowski, et al., 2015), the acid-catalyzed cleavage was carried out in the presence of excess 2-mercaptoethanol, according to the protocol developed by Roumeas, Aouf, Dubreucq, and Fulcrand (2013). Mercaptolysis was carried out

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