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Influence of polysaccharides on wine protein aggregation

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ABSTRACT

Polysaccharides are the major high-molecular weight components of wines. In contrast, proteins occur only in small amounts in wine, but contribute to haze formation. The detailed mechanism of aggregation of these proteins, especially in combination with other wine components, remains unclear.

This study demonstrates the different aggregation behavior between a buffer and a model wine system by dynamic light scattering. Arabinogalactan-protein, for example, shows an increased aggregation in the model wine system, while in the buffer system a reducing effect is observed. Thus, we could show the importance to examine the behavior of wine additives under conditions close to reality, instead of simpler buffer systems. Additional experiments on melting points of wine proteins reveal that only some isoforms of thaumatin-like proteins and chitinases are involved in haze formation.

We can confirm interactions between polysaccharides and proteins, but none of these polysaccharides is able to prevent haze in wine.

1. Introduction

The aggregation of various wine components can cause haze and sediments in bottled wine. This represents a major optical defect especially in white and rosé wines leading to economic losses for a winery (Van Sluyter et al., 2015).

Protein haze can occur through the interaction and aggregation of proteins with metal ions like copper or phenolic components (Dietrich & Will, 1998; Fenchak, Kerr, & Corredig, 2002; Zhao, Diao, & Zong, 2013). Some grape pathogenesis-related proteins have been identified to be haze-related such as thaumatin-like proteins (TLP), chitinases and glucosidases, while the mechanism of aggregation and haze formation are not well understood yet (Esteruelas et al., 2009; Marangon, Van Sluyter et al., 2011; Pocock, Hayasaka, McCarthy, & Waters, 2000; Van Sluyter et al., 2015). Recently, Van Sluyter et al. (2015) reviewed the mechanism of protein haze formation and advances in prevention. They summarized the view that wine haze formation can be considered as a three-stage model including protein unfolding, protein self-aggregation and cross-linking of different aggregates.

In addition to proteins, other wine ingredient such as minerals, trace elements, phenols, polysaccharides and sulfites as well as different environmental conditions, namely pH, alcohol content, temperature, and ionic strength can affect haze formation (Batista, Monteiro, Loureiro, Teixeira, & Ferreira, 2009; Dufrechou, Vernhet, Roblin, Sauvage, & Poncet-Legrand, 2013; Dupin et al., 2000; Fenchak et al., 2002; Pocock, Alexander, Hayasaka, Jones, & Waters, 2007). However, high molecular weight polysaccharides like mannoproteins are thought to have protective influence on haze formation (Dupin et al., 2000; Van Sluyter et al., 2015; Waters, Pellerin, & Brillouet, 1994a). The identification of molecules involved in aggregation and haze formation is difficult due to the diversity and influence of environmental conditions on wine composition (Dufrechou, Poncet-Legrand, Sauvage, & Vernhet, 2012; Fenchak et al., 2002; Siebert, Carrasco, & Lynn, 1996).

To prevent haze formation proteins are often removed by the addition of bentonite, a cation exchanger which nonspecifically adsorbs positively charged molecules. Thus, not only the haze-related components like proteins are adsorbed and removed from wine, but also components beneficial for sensory properties like

Abbreviations: AGP, arabinogalactan-protein; CMC, carboxymethyl cellulose; DEAE, diethylethanolamine; DLS, dynamic light scattering; DSC, differential scanning calorimetry; FTIR, Fourier transform infrared spectroscopy; GA, gum arabic; HPLC, high-performance liquid chromatography; MA, mannan; NTU, nephelometric turbidity units; PDA, Photo Diode Array; RG, rhamnogalacturonan; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; TLP, thaumatin-like protein; T_m, melting temperature; TXRF, total reflection X-ray fluorescence.

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color and aroma leading to quality losses (Gonzalez-Neves, Favre, & Gil, 2014; Jaeckels et al., 2015; Lambri, Dordoni, Silva, & De Faveri, 2010; Segad, Jonsson, Akesson, & Cabane, 2010). Therefore, alternative and more efficient fining strategies need to be developed. The understanding of the interaction of wine components and the mechanisms involved in haze formation represent the basis. In recent years, some stabilization strategies have been tested such as protein degradation by proteases or the usage of novel fining agents with partly promising results but the application of bentonite is still necessary (Van Sluyter et al., 2015).

Different macromolecules are present in wine which could influence aggregation behavior. White wine contains high molecular weight polysaccharides (50–560 kDa) in concentrations of 400 to –600 mg/l (Moreno-Arribas, Pueyo, Nieto, Martin-Alvarez, & Polo, 2000; Resende, Catarina, Geraldes, & de Pinho, 2013; Vidal, Williams, Doco, Moutounet, & Pellerin, 2003) while proteins are only present in concentrations between 20 to and 300 mg/l in red wine (Dambrouck et al., 2003; Ferreira, Picarra-Pereira, Monteiro, Loureiro, & Teixeira, 2002). Due to the excess of polysaccharides in wine it can be assumed that these may interact with proteins and influence aggregation behavior as consequence.

For this reason and because polysaccharides are discussed to reduce haze formation in wine (Schmidt et al., 2009; Waters, Pellerin, & Brillouet, 1994a) our study focuses on the interaction of polysaccharides and wine proteins. Therefore, we tested the haze influencing effect of five different polysaccharides (arabinogalactan-protein, rhamnogalacturonan, gum arabic, carboxymethyl cellulose, yeast mannan) by investigating the temperature dependent aggregation with wine proteins comparing a buffer system with a model wine system which is close to reality.

2. Materials and methods

2.1. Materials

All chemicals were of high purity and purchased from commercial companies. Acrylamide, ammonium persulfate, SDS, tris(hydroxymethyl)aminomethane, glycine, di-sodium-hydrogenphosphate, Coomassie brilliant blue G-250, sodium chloride, methanol, citric acid and sodium citrate were from Carl Roth (Karlsruhe, Germany), protein molecular weight standard from Bio-Rad (Munich, Germany), bromophenol blue from Serva (Heidelberg, Germany).

2.2. Wine proteins and polysaccharides

For our research lyophilised Auxerrois wine colloids were used. Colloids, which include wine components larger than 10 kDa like polysaccharides and proteins, were isolated from 150 l of Auxerrois wine (highly unstable wine, bentonite demand 1000 g/hl). The wine was concentrated in a Sartocon beta ultrafiltration system (Sartorius, Göttingen, Germany) using a molecular cutoff of 10 kDa at a feed pressure of 2.5 bar and a back pressure of 1.0 bar. The effective membrane area was 1.2 m^2 (Sartocon ~~Slice~~ beta Hydrosart, Sartorius, Göttingen, Germany) and the retentate temperature was kept below 40 °C throughout the whole process. The resulting wine retentate was diafiltered with citrate buffer (5 g/l, pH 4, 40 l) and 50 l of distilled water. After diafiltration, the retentate was quantitatively removed from the system and spin freezed, vacuum dried and finally weighed giving the gravimetric colloid content (mg/l). The freeze-dried sample was stored dry at 20 °C. These wine colloids are large molecular weight substances, which contains polysaccharides and proteins. The total wine colloid content was 720 mg/l wine which consisted of 86.6 mol% and 13.4 mol% protein. For the experiments a stock solution containing 17 mg/ml colloid was applied. The protein concentration in the stock solution was 1 mg/ml. The protein concentration was calculated in the water solved sample as described before (Jaeckels et al., 2015).

Arabinogalactan-protein (AGP) was isolated from a cider apple juice by ion exchange chromatography on DEAE-Sepharose CL-6B (GE Healthcare, Freiburg, Germany). Mainly, AGP is composed of arabinose and galactose and contained 1.7% protein. Rhamnogalacturonan was also isolated from apple juice using a similar method (DEAE-Sepharose Fast Flow, GE Healthcare, Freiburg, Germany) (Will, Mischler, & Dorreich, 1994). Mannan was isolated from baker yeast using ethanol precipitation (Peat, Edwards, & Whelan, 1961). Gum arabic (SIHA Gummi Arabicum Granulat, Lot. No. 854671) was kindly provided by Eaton Technologies GmbH (Langenlonsheim, Germany) and carboxymethyl cellulose (Cellulose Gum Charge 6 K 531) was obtained by Dow Wolff Cellulosics (Bomlitz, Germany).

The experiments were performed either in 0.1 M citrate buffer pH 3.5 or in a Riesling permeate (MWCO < 10 kDa, 12.5% vol. alcohol, vintage 2013, Geisenheim, Germany) pH 3.5 which should give a more realistic approach to the behavior of proteins in wine. No changes in pH value could be detected when heating the solutions.

2.3. Temperature dependent aggregation performed with dynamic light scattering (DLS)

Temperature depended aggregation of proteins was monitored as the function of temperature and particle size (mean hydrodynamic radius, R_S) using different polysaccharides in different colloid-polysaccharides-ratios (1:1; 1:0.75; 1:0.5; 1:0.25). A ratio of 1:1 indicates a colloid concentration of 17 mg/ml, which contains 1 mg/ml total protein, and a polysaccharide concentration of 17 mg/ml. The experiments ($n = 3$) were performed with the Zetasizer Nano S Size (Malvern Instruments Ltd., Worcestershire, United Kingdome). Therefore, we need a high protein concentration to get stable signals at the beginning of the measurements. Samples were heated from 26 °C to 82 °C in 2 °C steps. A deviation of ±0.1 °C was calculated. At each step the 100 µl samples were equilibrated before measurement for 3 min. Additionally, in the model wine system using Riesling permeate instead of citrate buffer the samples had to be overlaid with paraffin to prevent strong evaporation.

2.4. Temperature dependent denaturation of wine proteins

Differential Scanning Calorimetry (DSC) was used to determine the denaturation temperatures of wine proteins (VP-DSC Microcalorimeter, MicroCal, Northampton, USA). Samples were heated from 20 °C to 120 °C with a scan rate of 60 °C/h. For each measurement ($n = 2$) a sample volume of 500 µl was applied and changes in heat capacity C_P between reference and sample were recorded. For these experiments the stock solution was diluted to a total protein concentration of 0.13 mg/ml. Accordingly polysaccharide concentrations were reduced to obtain the ratios 1:1; 1:0.75.

2.5. SDS–PAGE

SDS–PAGE was performed using the method by Laemmli (1970). We used homemade 12.5% polyacrylamide gels pH 8.8 with 3% stacking gels pH 6.8. Before SDS–PAGE, 30 µl sample were mixed with 10 µl SDS sample buffer (25% (v/v) 0.5 M Tris (pH 6.8)/ 20% (v/v) glycerin/ 4% (w/v) SDS, with a spade point of bromophenol blue) and the gel was loaded with 30 µl of each mixture. As protein standard the Precision Plus Protein™ Standard from