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Reducing the negative sensory impact of volatile phenols in red wine with different chitosans: Effect of structure on efficiency

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ABSTRACT

"Brett character" is a negative sensory attribute acquired by red wines when contaminating *Dekkera/Brettanomyces* yeasts produce 4-ethylphenol and 4-ethylguaiacol, known as volatile phenols (VPs), from cinnamic acid precursors. In this study, chitins and chitosans with different structural features, namely deacetylation degree (5–91%) and molecular weight (24–466 kDa) were used for the reduction of this sensory defect. Chitins and chitosans decreased 7–26% of the headspace abundance of VPs without changing their amounts in wines. The efficiency of reduction increased with the deacetylation degree and applied dose. Reduction of headspace abundance of VPs by chitosans enabled significant decreases in the negative phenolic and bitterness attributes and increased positive fruity and floral attributes. Results show that chitosan with high deacetylation degrees, including fungal chitosan, which is already approved for use in wines, is an efficient approach for reducing the negative sensory impact of VPs in red wines.

1. Introduction

"Brett character" is a negative sensory attribute that wines acquire when 4-ethylphenol (4-EP) and 4-ethylguaiacol (4-EG) are formed above their sensory threshold, primarily through the action of contaminating *Dekkera/Brettanomyces* yeast from phenolic precursors present in wine, namely, *p*-coumaric and ferulic acids (Chatonnet, Dubourdieu, Boidron, & Poins, 1992; Chatonnet, Viala, & Dubourdieu, 1997) and their ethyl esters when *Dekkera/Brettanomyces* present ethyl esterase activity (Hixson et al., 2012). This sensory defect has been reported in several wine styles around the world, especially, premium wines (Campolongo, Siegumfeldt, Aabo, Cocolin, & Arneborg, 2014), and thus volatile phenols (VPs) are a global problem in winemaking. Red wines are more susceptible to *Dekkera/Brettanomyces bruxellensis* contamination and proliferation due to their lower acidity and frequent ageing in wood barrels (Campolongo et al., 2014). These VPs create an unpleasant, strong "phenolic", "animal", "horsey" or "stable" aromas and a decrease or elimination of fruity and varietal aromas in wine (Petrozziello et al., 2014). Even subliminal concentrations of these VPs have been shown to affect the fruity notes (Tempère et al., 2016). This sensory defect is considered negative by both professionals and consumers (Lattey, Bramley, & Francis, 2010). A series of preventive measures to avoid *Dekkera/Brettanomyces* wine contamination, proliferation and VPs formation have been developed, but they do not appear to have a widespread use, adherence and/or efficiency, as there are globally wines marketed with high concentrations of VPs (Pollnitz, Pardon, & Sefton, 2000). Therefore, a series of remediation treatments have been developed to remove already-formed VPs from wines and decrease or eliminate their negative sensory impact. These treatments can be divided into two main groups: those intended to decrease the headspace contents by decreasing their partition coefficients to the gas phase without changing the total VPs contents of wines (non-extractive techniques; Milheiro, Filipe-Ribeiro, Cosme, & Nunes, 2017; Petrozziello et al., 2014), and those intended to remove the VPs from wines, decreasing their content and their headspace concentration (extractive techniques). The oenological products already authorized by the OIV and tested in red wines include activated carbons (Lisanti, Gambuti, Genovese, Piombino, & Moio, 2017; Milheiro et al., 2017) yeast cell walls (Chassagne, Guilloux-Benatier, Alexandre, & Voilley, 2005; Nieto-Rojo, Ancín-Azpilicueta, & Garrido, 2014), potassium caseinate and egg albumin (Milheiro et al., 2017). Of these, those that appear to be efficient within the legal limits are activated carbons, egg albumin and potassium caseinate. The impact of these techniques on wine quality has been only studied in a few reports, but these studies

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will ultimately dictate the suitability and the choice by winemakers. The non-extractive remediation approach to reduce the sensory impact of VPs in wines has been much less studied (Milheiro et al., 2017; Petrozziello et al., 2014), although it could present some advantages, such as lower or no impact on global wine composition, as observed for many extractive methods already tested (Chassagne et al., 2005; Filipe-Ribeiro, Milheiro, Matos, Cosme, & Nunes, 2017a; Lisanti et al., 2017; Milheiro et al., 2017; Nieto-Rojo et al., 2014). The reasoning of this approach is that by modifying the partition coefficients of the VPs for the gas phase in equilibrium with wine by adding substances that can bind these VPs (Milheiro et al., 2017; Petrozziello et al., 2014), their sensory impact will be lowered or even eliminated. It has been shown that chitosan can decrease the headspace abundance of VPs, but no sensory analyses of the wines were performed to access the impact of this treatment on wine sensory profiles (Milheiro et al., 2017). The use of chitosan in winemaking has been authorized by the EU for heavy metals and contaminant removal, prevention of cloudiness, and reduction of undesirable *Brettanomyces* spp. populations (EC Regulation 53/2011). Only chitosan from fungal origin was authorized, but fungal chitin has unique features with respect to chemical structure and biosynthesis (Bowman & Free, 2006) compared to crustacean chitins. However, a major difference results from the fact that fungal chitin is associated with other polysaccharides that do not occur in the exoskeleton of arthropods (Bowman & Free, 2006; Hameda, Özogul, & Regenstein, 2016). Furthermore, chitins and chitosans present a diversity of structural features, such as deacetylation degree (DD) and molecular weight (MW), that can affect properties such as charge density and solubility (Bowman & Free, 2006; Hameda et al., 2016). Thus, the aim of this work was to study the efficiency of chitins and chitosans with different structural features, namely, DD and MW and different origins (crustacean or fungi), in the reduction of the wine headspace abundance of VPs. The impact of the different chitins and chitosans and different application doses on the phenolic and headspace aroma composition of wines was addressed. The overall impact of chitosan treatment on the sensory profile of VPs-contaminated wines was studied to determine the suitability of chitosans for reduction or elimination of the negative “Brett character” sensory profile of red wines.

2. Materials and methods

2.1. Chitin and chitosan samples and production

Commercial crustacean chitin (CHTN, chitin from shrimp shells, Sigma C9213), two commercial crustacean chitosans (CHTB, chitosan with high MW, Sigma 419419 and CHTD, chitosan with 100000–300000 Da MW, Acros 34905500) and one fungal chitosan (No Brett Inside, Lallemand) were used. One additional chitin (CHTNA) and one additional chitosan (CHTC) were produced by alkaline deacetylation of CHTN and CHTB, respectively. For deacetylation of chitin and chitosan, 15 g of the initial material was dispersed in 150 mL NaOH solution (50% w/v) with $NaBH_4$ (10 g/L) and heated for 12 h under reflux with stirring at 130-150 °C under nitrogen (Liang, Chang, Tsai, Lee, & Fu, 1997). For chitin deacetylation, commercial chitin was previously ground to a particle size less than 0.15 mm (obtained by sieving). After cooling to room temperature, the solution was neutralized to pH 6–8 with HCl 12 M, and ethanol was added until 75% (v/v) for chitosan precipitation. The precipitate was washed thoroughly with ethanol at 75% (v/v). The material was dried at 50 °C in a forced air oven for 24 h.

2.2. Chitin and chitosan chemical characterization

2.2.1. Chitin and chitosan deacetylation degree and viscosity-average MW

Chitin and chitosan DD were determined by potentiometric titration (Jiang, Chen, & Zhong, 2003). The MW of chitosan was determined using an Ubbelohde capillary viscometer (N° 0B, ASTM-D2515) at 25 °C with a flow time for the solvent of 195 s (t_0). Chitosan solutions of different concentrations (0.1 to 1 g/L or 0.4 g/L to 4.0 g/L) in a 2% acetic acid, 0.2 mol/L sodium acetate (pH 4.5) solution were prepared (Kasaai, Arul, & Charlet, 2000). The MW of chitosan was obtained according to the Mark-Houwink equation (Kasaai et al., 2000), using the K and a characteristic constants of the polymer-solvent system of 1.38×10^{-5} and 0.85, respectively (Gamzazade et al., 1985). Analyses were performed in triplicate.

2.2.2. Sugar composition and content analysis

Neutral sugars were determined by anion-exchange chromatography after acid hydrolysis of chitin and chitosans using the method described by Ribeiro, Fernandes, Nunes, Filipe-Ribeiro, and Cosme (2014). Analyses were performed in triplicate.

2.2.3. Mineral composition and water content of chitin and chitosan

Potassium and sodium were determined by atomic emission flame spectrophotometry, and calcium, magnesium and iron were measured by atomic absorption flame spectrophotometry after dissolution of chitin and chitosans in a 2% acetic acid solution. Chloride content was determined by the colourimetric method (Iwasaki, Utsumi, Hagino, & Ozawa, 1956). The water content of chitin and chitosan was determined by freeze-drying chitin and chitosan until constant weight. Analyses were performed in triplicate.

2.2.4. FTIR analysis of chitins and chitosans

Chitin and chitosan FTIR spectra were recorded in the range of wavenumbers 4000–450 cm^{-1}, and 128 scans were taken at 2 cm^{-1} resolution using a Unicam Research Series FTIR spectrometer. KBr pellets were used. Analyses were performed in duplicate.

2.2.5. X-ray diffraction analysis of chitins and chitosans

Powder X-ray diffraction (XRD) data were recorded on solid samples (chitins and chitosans) using a PANalytical X’Pert Pro X-ray diffractometer equipped with an X’Celerator detector and secondary monochromator. The measurements were performed using Cu Kα radiation (40kV; 30 mA) in Bragg-Bentano geometry at a 7–60° 2θ angular range. Analyses were performed in duplicate.

2.3. Experimental design

To study the effect of DD on chitin and chitosan headspace VPs reduction performance, two chitins and four chitosans were used at 10 g/hL (CHTN10, CHTNA10, CHTB10, CHTC10, CHTD10 and CHTF10). The wine was previously contaminated at two levels of 4-EP (750 and 1500 μg/L) and 4-EG (150 and 300 μg/L) according to the ranges usually found in the literature (Chatonnet et al., 1992; Pollnitz et al., 2000). Chitins and chitosans were added at 10 g/hL to 250 mL of wine in graduated cylinders. All chitins and chitosans were prepared according to the recommendation of the commercial fungal chitosan, 1 part of chitosan to 10 parts of wine, thoroughly mixed followed by addition to the wine. The wine was allowed to remain in contact with chitins and chitosans for 6 days at 20 °C without shacking. To study the effect of chitosan application dose, the chitosans CHTD and CHTF were also tested in a second trial at 10, 100 and 500 g/hL (CHTD10, CHTD100, CHTD500, CHTF10, CHTF100 and CHTF500). After 6 days, the wine was centrifuged at 10,956*g*, 10 min and 20°C for analysis. Experiments were performed in duplicate.

2.4. Wine samples

Two blend red wines from Douro Valley (vintage 2015) were used in this work. The main characteristics of the wine used in the first assay (CHTN10, CHTNA10, CHTB10, CTHC10, CHTD10, CHTF10), were as follows: alcohol content (% v/v) 13.3, specific gravity (20 °C) (g/mL)