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Short communication

Sorption of 4-ethylphenol and 4-ethylguaiacol by suberin from cork

Joan-Josep Gallardo-Chacón [a], Thomas Karbowiak [b,*]

[a] CARINSA GROUP & Centre Especial de Recerca, Planta Tecnologia dels Aliments (CERPTA), UAB Veterinary Building, Campus UAB, 08193 Cerdanyola del Vallès, Catalonia, Spain
[b] UMR PAM, Équipe PAPC, Agrosup Dijon/Université de Bourgogne, 1 esplanade Erasme, F-21078 Dijon, France

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ABSTRACT

Cork shows an active role in the sorption of volatile phenols from wine. The sorption properties of 4-ethylphenol and 4-ethylguaiacol phenols in hydro-alcoholic medium placed in contact with suberin extracted from cork were especially investigated. To that purpose, suberin was immersed in model wine solutions containing several concentrations of each phenol and the amount of the compound remaining in the liquid phase was determined by SPME–GC–MS. Sorption isotherms of 4-ethylguaiacol and 4-ethylphenol by suberin followed the Henry's model. The solid/liquid partition coefficients (K_{SL}) between the suberin and the model wine were also determined for several other volatile phenols. Suberin displayed rather high sorption capacity, which was positively correlated to the hydrophobicity of the volatile. Finally, the capacity of suberin to decrease the concentration of 4-ethylphenol and 4-ethylguaiacol was also tested in real wines affected by a *Brettanomyces* character. It also lead to a significant reduction of their concentration in wine.

1. Introduction

Cork is the natural product obtained from the bark of *Quercus suber* L. (oak tree). Its alveolar structure and its specific chemical composition confers to this material a strong capacity for insulation from heat, sound and polar liquids. These properties lead to a wide range of uses for cork. Among them, the most important is the manufacture of stoppers for oenology (Pereira, 2007). Cork is mainly composed of suberin (~50 wt%), followed by lignin (15–29 wt%), polysaccharides (6–25 wt%) and other extractive substances (Silva et al., 2005). Different studies have been performed to clarify the role of traditional cork closures in wine evolution, considering especially oxygen ingress (Karbowiak, Gougeon, et al., 2010). Some interactions have also been reported between cork and some molecules that could be found in the headspace of a wine bottle. Thus, the propensity of cork to sorb water (Lequin et al., 2010), ethanol (Lequin, Chassagne, Karbowiak, & Bellat, 2013) or sulphur dioxide (Lequin, Karbowiak, Brachais, Chassagne, & Bellat, 2009) has been evaluated, showing physical and/or chemical interactions with these substances.

Furthermore, volatile phenols have a very important role in the wine aroma formation. Among them, 4-ethylphenol (EP) and 4-ethylguaiacol (EG) have a special interest for producers because at low concentration, they both increase wine complexity. However, they develop animal related flavours at concentrations higher than approximately 0.4 ppm (Chatonnet, Dubourdieu, Boidron, & Pons, 1992). This fact is closely related to the effect of *Brettanomyces* yeast metabolism. The importance of these compounds in terms of sensory impact resulted in different studies in order to decrease their concentration in wine. Thus, reverse osmosis was assayed, displaying a significant lowering in the levels of both volatile compounds. However, it is not a very selective process and there is also a concomitant depletion for all of the other of the aroma compounds (Ugarte, Agosin, Bordeu, & Villalobos, 2005). Testing was also recently conducted using sorption on esterified cellulosic polymers (Larcher, Puecher, Rohregger, Malacarne, & Nicolini, 2012). Other works focused on the sorptive capacity of materials related to wine production, such as oak wood (Barrera-García, Gougeon, Voilley, & Chassagne, 2006) and cork (Karbowiak, Mansfield, Barrera-García, & Chassagne, 2010), showing in both cases a significant amount sorbed. Although cork has a heterogeneous chemical composition, its major fraction (suberin) could play an important role in the sorption of such compounds due to its hydrophobic character.

Suberin is a lipophilic biopolymer (insoluble in water) that protects plants against environmental damage. Its structure, despite being studied in different works (Cordeiro, Belgacem, Silvestre, Pascoal Neto, & Gandini, 1998; Graça & Santos, 2007; Lopes, Gil, Silvestre, & Neto, 2000; Santos Bento et al., 2001), is still not yet fully clarified. It appears to be composed of an aliphatic polyester domain covalently bound to a phenolic matrix. The aliphatic

* Corresponding author. Tel.: +33 3 80 77 23 88; fax: +33 3 80 77 40 47.
E-mail address: thomas.karbowiak@u-bourgogne.fr (T. Karbowiak).

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fraction contains in decreasing order of importance ω-hydroxyacids, α,ω-diacids, 9,10,18-trihydroxyoctadecanoic acid, 9,10-epoxy-18-hydroxyoctadecanoic acid, dihydroxyoctadecanedioic, alkanoic acids, 9,10-epoxyoctadecanodioic acid and alkanols (Santos Bento et al., 2001). Finally, this cork moiety has also been explored for its physico-chemical properties and to some extent for technological applications such as an additive to offset printing inks (Gandini, Pascoal Neto, & Silvestre, 2006).

The objective of this study was to evaluate the suberin capacity for sorption of EP and EG, along with some other compounds from the homologous guaiacol series, in a model wine system and also under oenological conditions. To that end, suberin was extracted after cork methanolysis. This product was then used to perform sorption isotherms of EP and EG in model wine, those two aroma compounds being of interest in oenology as off-flavours related molecules. Finally, two contaminated wines were analysed to determine the real sorbing effect of this material.

2. Materials and methods

2.1. Materials

Guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-propylguaiacol, eugenol, 3,4-dimethylphenol and 4-ethylphenol, as well as other classical chemical compounds and solvents, were purchased from Sigma–Aldrich (St. Louis, MO, USA). The purity of those standards was minimum 98%.

The model wine used for sorption measurements was composed of 12.5% V/V ethanol, malic acid (3 g/L), acetic acid (0.1 g/L), potassium sulphate (0.1 g/L), and magnesium sulphate (0.025 g/L). The pH of the model wine was adjusted to 3.5.

Raw cork stoppers, from *Quercus suber L.* oak trees in the Mora (Portugal) production area, were supplied by Bouchons Trescases S.A. (Boulou, France). Cork was neither washed nor surface treated (with paraffin or silicone) prior to use.

2.2. Suberin extraction

Suberin from raw cork stoppers was extracted after methanolysis following the method described by Pereira (1988) and modified as follows: four stoppers were grated and sieved through a 500 μm pore size sieve in order to eliminate big particles. To obtain the Free Extractive Cork (FEC), approximately 3 g of this cork powder were extracted in a Soxhlet device with dichloromethane (6 h), ethanol (6 h) and water (8 h), successively, and then dried at 105 °C to constant weight. The FEC represents 84.2% w/w (±1.0) of the cork sample, the rest being eliminated in the previously cited solvents.

1.5 g of FEC were then boiled under reflux heating with 250 mL of 3% sodium methoxide ($NaOCH_3$) in methanol during 3 h. $NaOCH_3$ has been used as strong nucleophile for transesterification with methanol to give fatty acid methyl esters, which is referred to as suberin extract. The suspension was filtered and the residue washed 15 min with methanol under reflux heating. This residue corresponds to the lignocellulosic fraction and was dried and weighed. The amount of suberin was determined by difference: it represents 37.0% w/w (±2.7) of the FEC sample. The methanolic solution containing the extracted suberin fraction was acidified to pH 6 with H_2SO_4 and dried in rotating evaporator. The residue was re-suspended with 25 mL of Milli-Q water and extracted 3 times with 25 mL of chloroform. The solution was filtered through sodium sulphate and evaporated under vacuum. Finally, the rest of the solvent was removed under nitrogen flux and this extracted suberin fraction was stored in a dry atmosphere (under P_2O_5) at room temperature.

2.3. Sorption isotherms of volatiles on suberin

To perform the sorption isotherms of 4-ethylguaiacol and 4-ethylphenol by suberin, 2 mL of model wine with concentrations of volatile phenols between 1 and 20 ppm (w/w) were added to suberin. The suspensions were placed at 25 °C under stirring with a magnetic bar. No significant differences were observed for the sorption of 4-ethylphenol and 4-ethylguaiacol between samples over 2, 17 and 24 h. This means that the time to reach the thermodynamic equilibrium is rather short. In the further experiments, samples were therefore left overnight (12 h) to be sure equilibrium was attained. After that time, the suberin was removed by centrifugation at 14,000 rpm during 10 min. The same concentration of each aroma compound in model wine without suberin was used as a blank. The sorption capacity of suberin is determined by concentration difference with the blank. The solid/liquid partition coefficient (K_{SL}) between the solid phase (Suberin) and the liquid phase (Model Wine) was calculated as follows:

$$K_{SL} = \frac{C_S}{C_{MW}}$$

where C_S is the quantity of volatile phenol sorbed by suberin divided by the initial weight of suberin (mg/kg) and C_{MW} is the mass fraction of volatile phenol in model wine after sorption by suberin (mg/kg).

Moreover, in order to avoid any suberin aggregation during the sorption experiment, the following procedure was used: approximately 20 mg of the suberin material was placed in a 4 mL vial and dissolved in 1 mL ethanol. Then, 200 mg of glass beads were added and they were vortex mixed. Finally, the ethanol was evaporated under nitrogen flux. These glass beads coated with suberin were used for all sorption experiments, as well in model wine as in wine contaminated with *Brettanomyces.*

2.4. Solid phase microextraction (SPME) and chromatographic (GC–MS) analysis

The SPME analyses were performed following the optimised conditions for temperature and time of extraction as described by Vichi, Romero, Tous, Tamames, and Buxaderas (2008). Briefly, 1 mL of the liquid phase sample was analysed in a 4 mL vial closed with a silicone septum. 10 μL of 3,4-dimethylphenol (from a 1000 ppm solution) was used as internal standard. The sample was placed in an oil bath at 60 °C under magnetic stirring (700 rpm). The sample was conditioned for 10 min and afterwards, the fibre (PDMS/Carboxen 75 μm) was exposed for 30 min in gas phase. Finally, the fibre was put in the chromatograph injector at 220 °C for 15 min, afterwards the split valve was opened and then the fibre was kept in the injection port for minimum 15 min more for a cleaning step under septum purge.

The identification and quantification of volatile phenols in wine and model solution was performed using a gas chromatography device (GC Trace Ultra, Thermo Electron Corporation, San José CA, USA) with a quadrupole mass selective spectrometry detector. Separation of analytes was performed in a CP-WAX 57CB column (Varian, Palo Alto, CA; 25 m 0.25 mm i.d.; 0.2 μm bonded phase) with splitless injection at 250 °C. Oven temperature was held at 50 °C for 5 min, then increased to 220 °C at 10 °C/min and held 2 min at 220 °C. Helium was used as carrier gas, at a constant flow of 1.5 mL/min. The temperature was 230 °C for ion source and 230 °C for the transferline. Positive electron ionisation mass spectra were recorded at 70 eV ionisation energy, at 0.8170 scan/s. The range of m/z analysed was 50–650. Base peak ions were used for quantification of compounds, using response factor.