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Centrifugal partition chromatography applied to the isolation of oak wood aroma precursors

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ABSTRACT

Flavours extracted from oak wood during barrel ageing contribute to the organoleptic character of wines and spirits. The aim of this work was to identify the glycosidic precursors of the key volatile compounds responsible for oak wood aroma. Oak extract is a very complex matrix and, furthermore, precursors are present in very small quantities. Preparative centrifugal partition chromatography (CPC) is a promising solution for purifying the oak extract. The solvent system was selected on the basis of the partition coefficient of glycosidase enzyme activity (K_{ca}). Thanks to the efficacy of CPC separation, three glucoside gallates were subsequently isolated by HPLC chromatography. Vanillin-(6′-*O*-galloyl)-*O*-β-D-glucopyranoside, 3,4,5-trimethoxyphenyl-(6′-*O*-galloyl)-*O*-β-D-glucopyranoside, and (6R,9R)-3-oxo-α-ionol-9-*O*-(6′-*O*-galloyl)-β-glucopyranoside (macarangioside E) were isolated and identified. This was the first time that vanillin-(6′-*O*-galloyl)-*O*-β-D-glucopyranoside was identified and the first time that macarangioside E was isolated from oak wood. Heating macarangioside E resulted in the formation of megastigmatrienone, which has an aroma reminiscent of tobacco.

1. Introduction

Wines and alcohol beverages are traditionally aged in wooden barrels, most commonly made of oak. During this phase, volatile oak compounds are extracted and add woody notes to the product. The free volatile compounds in oak responsible for these woody aromas have been extensively studied. Vanillin, oak lactone, and volatile phenols play a prominent role in the aromas of wood-aged wines (Boidron, Chatonnet, & Pons, 1988; Chatonnet, Boidron, & Pons, 1990; Chatonnet, Dubourdieu, & Boidron, 1992; Jarauta, Cacho, & Ferreira, 2005; Pocock, Sefton, & Williams, 1994; Spillman, Pollnitz, Liacopoulos, Skouroumounis, & Sefton, 1997). However, there has been little research into bound volatile compounds in oak wood. It is well known that during wood seasoning and cooperage processes (Chatonnet, 1995; Jarauta, Cacho, & Ferreira, 2005; Maga, 1985; Nishimura, Onishi, Masuda, Koga, & Matsuyama, 1983; Petruzzi et al., 2010; Roulland, Snakkers, & Cantagrel, 1999; Sarni, Moutounet, Puech, & Rabier, 1990; Sefton, Francis, Pocock, & Williams, 1993), the concentrations of several flavours are increased and others are produced by heat treatment. Lignin depolymerisation is considered to cause an increase in phenolic aldehydes during toasting. However, it has been demonstrated that vanillin and other phenolic aldehydes also increase during malolactic fermentation in barrel (de Revel, Bloem, Augustin, Lonvaud-Funel, & Bertrand, 2005). Bacteria glycosidase activity was proved to be involved in this phenomenon, suggesting the existence of glycosidic flavour precursors in oak wood (Bloem, Lonvaud-Funel, & de Revel, 2008). In fact, two glycosidic oak-lactone precursors had previously been isolated from oak wood: (3S,4S)-3-methyl-4-*O*-(6'-*O*-galloyl)-β-D-glucopyranosyloctanoic acid (galloylglucoside) (Masson, Baumes, La Guernevé, & Puech, 2000) and (3S,4R)-3-methyl-4-*O*-β-D-glucopyranosyloctanoic acid (Hayasaka, Wilkinson, Elsey, Raunkjær, & Sefton, 2007).

Other oak aroma precursors were investigated by GC–MS analysis of trifluoroacetylated oak extract (Nonier, Vivas de Gaulejac, Vivas, & Vitry, 2005). The authors postulated that vanillin, syringaldehyde, and megastigmatrienone were directly linked to sugar, but identification was not confirmed.

The aim of this work was to isolate and identify in oak wood the glycosidic precursors of a powerful aroma like vanillin. We also looked for the precursors of 3-oxo-α-ionol and megastigmatrienon. These molecules are involved in the tobacco aroma of oak wood.

In a preliminary step, oak extract was purified by centrifugal partition chromatography (CPC) and semipreparative HPLC,

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resulting in three purified molecules, identified by ESI-MS and NMR spectroscopy as vanillin-(6′-*O*-galloyl)-*O*-β-glucopyranoside; 3,4,5-trimethoxyphenyl-(6′-*O*-galloyl)-*O*-β-glucopyranoside, and (6R,9R)-3-oxo-α-ionol-9-*O*-(6′-*O*-galloyl)-β-glucopyranoside (macarangioside E). CPC is widely used to isolate natural substances but has rarely been used in wine and grape research. Some recent papers report interesting applications of this chromatography technique in enology research (Amira-Guebailia et al., 2009; Bisson et al., 2011; Delaunay, Castagnino, Chèze, & Vercauteren, 2002; Marchal, Waffo-Téguo, Génin, Mérillon, & Dubourdieu, 2011).

2. Materials and method

2.1. Chemicals and reagents

Pure water for HPLC analysis was obtained using an Elga (Elga Process Water Ltd., Marlow, UK) water purification system with a resistivity of at least 18 MΩ cm^{-1}. Formic acid, vanillin, 3,4,5-trimethoxyphenol, eugenol, and 2-dodecanol were purchased from Sigma–Aldrich (St. Louis, MO) at the highest purity available. Megastigmatrienone was kindly provided by Symrise AG (Holzminden, Germany). Ethyl acetate (EtOAc) and dichloromethane (CH_2Cl_2) were purchased from Fisher Scientific (Loughborough, UK). Acetonitrile (ACN), acetone and cyclohexane were purchased from VWR (Fontenay-sous Bois, France). Methanol-d_4 and acetone-d_6 for NMR analysis were purchased from Euriso-top (Gif-sur-Yvette, France).

2.2. Extraction of oak aroma precursors from wood

Dried French oak wood (1.5 kg) was cut into small pieces (maximum length <2 cm) and macerated three times under agitation in 2 L aqueous acetone (1:1) solution for 24 h. Extracts were pooled and evaporated under reduced pressure (T <30 °C). The remaining extract was separated by liquid/liquid extraction in 3 stages, using cyclohexane (3 × 300 mL), CH_2Cl_2 (5 × 300 mL), and EtOAc (5 × 300 mL). The combined extract from each phase was freeze-dried.

2.3. Enzymatic hydrolysis of oak wood aroma precursors

From each CPC and semiprep-HPLC fraction an aliquot of 500 μL was taken and added with 0.8 mL of sodium acetate buffer (pH 5.0, 10 mM), and 200 μL of a glycosidase rich enzyme preparation (AR2000, Delft, Netherlands; 70 mg/mL in sodium acetate buffer); the mixture was incubated at 27 °C for 24 h. 2-Dodecanol (10 μL at 410 mg/L) was added to the mixture as an internal standard. The released aglycones were extracted once with 2 mL CH_2Cl_2 (Silva, Gunata, Lepoutre, & Odoux, 2011).

2.4. CPC purification of wood extract

2.4.1. CPC apparatus

Centrifugal partition chromatography (CPC) was performed at room temperature on an FCPC 1000 apparatus from Kromaton® Technologies (Angers, France). The rotor consisted of 45 stainless steel disks containing 1440 cells, with a total rotor volume of 940 mL. A Gilson 321-H1 2-way binary high-pressure gradient pump was used. Injection was via a Rheodyne® injection valve with a 50 mL sample loop. Effluent was monitored using a Kromaton UV–vis detector (Angers, France) and fractions were collected with an Advantec CHF 122SC fraction collector (Advantec MFS Inc., Dublin, CA).

2.4.2. CPC solvent system selection

A 50-mg sample of EtOAc oak extract was dissolved in the biphasic system (2 mL from the upper layer and 2 mL from the lower layer) and shaken vigorously. An aliquot (0.9 mL) of each phase was evaporated and reconstituted with 0.2 mL water/MeOH (9:1) mixture and subjected to enzymatic hydrolysis, as described at Section 2.3, followed by GC–MS analysis. The activity partition coefficient (K_{ca}) was calculated as the ratio of vanillin, oak lactone, and 3-oxo-α-ionol released in each phase.

$$K_{ca}^{X} = \frac{A_{star}^{X}}{A_{mob}^{X}} \quad (1)$$

K_{ca}^{X}: Activity partition coefficient of X (aglycone) between the two CPC phases

A_{phase}^{X}: Quantity (Arbitrary unit) aglycone (X) realeased in each CPC phase

Ternary system EtOAc/propan-2-ol/H_2O (Köhler, Wray, & Winterhalter, 2008) in several proportions and Arizona systems B, F, K (Foucault, 1994; Foucault & Chevolot, 1998) were tested. The water/propan-2-ol/ethyl acetate (40/1/40) system (Köhler, Wray, & Winterhalter, 2008) was selected on the basis of equal distribution of released volatile compounds between the upper and lower layers, corresponding to a K_{ca} close to 1.

2.4.3. CPC conditions and procedures

CPC conditions were optimised by the authors. A mixture of EtOAc/propan-2-ol/H_2O (40:1:40, v/v/v) was shaken vigorously in a separating funnel and left to stand at room temperature for about 1 h. The lower phase was used as the stationary phase and the upper as the mobile phase at a flow rate of 20 mL/min. Rotation speed was set to 1000 rpm. Eight grams EtOAc oak extract were dissolved in 40 mL of stationary phase, filtered and injected. Detection was carried out at 254, 280 and 313 nm. Fractions were collected at 1-min intervals.

2.5. HPLC fractionation of CPC primary fraction

The HPLC purification was developed by the authors. HPLC fractionation was performed using a Waters 600 system (St-Quentin Yvelines, France) equipped with a Waters 600 controller, a Rheodyne® injection valve with a 600-μL sample loop, and a Waters 2487 UV detector at 280 and 313 nm. A Nucleodur C18ec 250 × 10 mm, 5 μm column was used Macherey–Nagel GmbH & Co. KG, Düren, Germany. Chromatographic conditions: flow rate 2 mL/min; solvent A: H_2O and 0.5% HCOOH; solvent B: ACN. Eluent was collected using a Foxy Jr. collector from Teledyne Isco (Lincoln, NE).

2.6. HPLC–ESI-MS apparatus

HPLC–ESI-MS method was optimised by the authors. The Agilent 1200 chromatography system from Agilent Technologies (Santa Clara, CA) consisted of an autosampler module, a degasser, a binary pump, a column heater/selector, and a diode array detector. The column was a Nucleodur C18 ec 125 mm × 4.6 mm, 5 μm. Fractions and library compounds were eluted at 0.8 mL/min with a gradient of water-0.1% formic acid (solvent A) and acetonitrile-0.1% formic acid (solvent B), according to the following gradient program (v/v): 0 min 17% B linear, 5 min 17% B, 25 min 30% B, 35 min 38% B, 45 min 100% B linear for 10 min, followed by 10 min for rebalancing. This HPLC was coupled to an Esquire 3000 + ion trap mass spectrometer using an ESI source from Bruker Daltonics (Billerica, MA). The HPLC output flow of 0.8 mL/min was split using a passive splitter with an average 1:100 ratio, depending on the flow solvent viscosity and rate. Drying gas (N_2) was