Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 90 (2005) 503–511
Food Chemistry
www.elsevier.com/locate/foodchem

Anti-oxidant activity of isolates from acid hydrolysates of *Eucalyptus globulus* wood

J.M. Cruz, H. Domínguez *, J.C. Parajó

Departamento de Enxeñería Química, Facultade de Ciencias de Ourense, Universidade de Vigo (Campus Ourense), Edificio Politécnico, As Lagoas, Ourense 32004, Spain

Received 26 January 2004; received in revised form 6 May 2004; accepted 6 May 2004

Abstract

The ethyl acetate-soluble fraction of *Eucalyptus globulus* wood hydrolysates was fractionated on a Sephadex LH-20 column, using methanol as the mobile phase to give four fractions, and both the DPPH (α,α-diphenyl-β-picrylhydrazyl) radical-scavenging activity and composition of raw extracts and isolates were determined. One of the fractions isolated (denoted F4) showed a remarkable anti-oxidant activity (EC_{50} of 0.15 g/l, in comparison with 0.55 g/l for crude extracts), presenting a comparatively high phenolic content, with ellagic acid as the main component.

Keywords: *Eucalyptus globulus* wood; Acid hydrolysates; Ethyl acetate extract; Anti-oxidant activity

1. Introduction

During the production of hemicellulosic sugar solutions by mild acid hydrolysis of lignocellulosic materials, different inhibitors of microbial metabolism are also formed (Ando, Arai, Kiyoto, & Hanai, 1986; Boussaid et al., 2001; Martín, Galbe, Nilvebrant, & Jönsson, 2002). Extractives, aliphatic fatty acids, phenolic substances derived from lignin and sugar dehydration products were the major compounds identified in hydrolysates (Klinke, Schmidt, & Thomsen, 1998; Martín et al., 2002) and should ideally be removed in order to efficiently utilise sugars as carbon source for bioconversion processes (Clark & Mackie, 1984; Cruz, Domínguez, Domínguez, & Parajó, 1999; Parajo, Domínguez, & Domínquez, 1998). For this purpose, extraction with ethyl acetate (enabling the selective removal of phenolic compounds from sugar solutions) has been proposed (Clark & Mackie, 1984; Cruz et al., 1999; Martín et al., 2002). The search for practical applications of these residual fractions would benefit the integral use of the lignocellulosic materials.

Phenolic compounds in biomass hydrolysates come from the partial depolymerisation of lignin, a part of which is linked to hemicelluloses. The anti-oxidant activity of lignin-derived fractions and phenolic acids has been reported (Kasprzycka-Guttman & Odzeniak, 1994). Under acid hydrolysis conditions, compatible with the production of fermentable sugars, *Eucalyptus globulus* wood yields higher anti-oxidant and antimicrobial activities than other lignocellulosic materials (Cruz, Domínguez, Domínguez, & Parajó, 2001).

In a previous work (González, Cruz, Domínguez, & Parajó, 2004), the operational conditions leading to maximal phenolics recovery by hydrolysis-extraction of *Eucalyptus* wood were established. The phenolic content of crude extracts was in the range reported for extracts from hydrothermal treatments (Felizón, Fernández-Bolaños, Heredia, & Guillén, 2000) or from different plant materials proposed as "natural" anti-oxidants (Exarchou et al., 2002; Rodríguez de Sotillo, Hadley, & Holm, 1994). Benzoic and cinnamic acids and aldehydes were detected in liquors obtained in the mild-acidic processing of hardwoods (Garrote, Cruz, Moure, Domínguez, & Parajó, 2004), whereas tannins (proanthocyanidins and ellagitannins) (Cadahía, Conde, Fernández de Simón, & García-Vallejo, 1997), acids

* Corresponding author. Fax: +34-988-387001.
E-mail address: herminia@uvigo.es (H. Domínguez).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.05.018

(ferulic, ellagic, gallic, syringic and vanillic), aldehydes (syringaldehyde and sinapaldehyde) and flavonoids (naringenin and quercetin) have been detected in *Eucalyptus globulus* wood (Freire, Silvestre, & Nieto, 2002).

Ethyl acetate extracts of *Eucalyptus wood* acid hydrolysates are of dark colour and oily aspect, features that could hinder their direct application. A suitable purification process leading to the removal of fractions with limited anti-oxidant activity would overcome these inconveniences. In this context, it can be noted that phenolic fractions or purified extracts from plants can show higher anti-oxidant capacity than crude extracts (Watanabe, 1999; Watanabe, Ohshita, & Tsushida, 1997). Fractionation of plant extracts containing phenolic compounds has been successfully done with Sephadex LH-20 columns (Watanabe, 1999; Wettasinghe, Shahidi, & Amarowicz, 2002). The aim of the present work is to evaluate the phenolic content and the radical-scavenging capacity of fractions from the ethyl acetate-soluble compounds present in *Eucalyptus* wood hydrolysates.

2. Materials and methods

2.1. Manufacture of crude extracts

Milled *Eucalyptus globulus* wood samples were treated with 5% H_2SO_4 at 130 °C at a liquid:solid ratio (LSR) of 8:1 g/g for 60 min (González et al., 2004). The liquid phase was separated by filtration and neutralised with $CaCO_3$. The precipitate was removed by filtration before extraction with ethyl acetate at a hydrolysate:ethyl acetate volume ratio of 1:3 (v:v) in a single extraction stage (Cruz et al., 1999; Cruz et al., 2001). Ethyl acetate was removed by vacuum evaporation and reutilised, and the solid extract was freeze-dried and used for further characterisation. The average yield in ethyl acetate soluble compounds was 3.86 g/100 g dry wood.

2.2. Chromatographic fractionation

Sephadex LH-20 gel (Amersham Biosciences, Uppsala, Sweden) was used for fractionation by column chromatography. The ethyl acetate-soluble fraction of *Eucalyptus* wood hydrolysates (0.198 g) was redissolved in methanol (5 ml) and loaded on the column, which was eluted with methanol at a flow rate of 3 ml/min. Sampling was carried out using a Gilson FC 203B fraction collector with 3 ml test tubes. The samples were combined to obtain the fractions selected. Absorbance (280 nm) was measured on-line in a flow cell using an Agilent 8453 spectrophotometer.

2.3. Spectrophotometric determination of phenols

Total phenols were determined by two spectrophotometric methods (Folin–Denis and Folin–Ciocalteu). Absorbance readings were made at 745 nm in the case of the Folin–Denis method (AOAC, 1997) and 765 nm in the Folin–Ciocalteu assay (Singleton & Rossi, 1965). A standard curve made with gallic acid (Sigma Chem. Co.) was used for quantification in both cases.

2.4. HPLC determination of phenols

Ethyl acetate extracts (obtained from 25 ml hydrolysates) were dissolved in methanol (10 ml) and analysed by HPLC in a Hewlett–Packard 1050 instrument fitted with a 1050 DA detector using a Supelcosil LC-18 column (5 μm, 4.6 mm × 25 cm) operating at room temperature. Gradient elution was carried out at a flow rate of 1.0 ml/min using 0.01 M sodium citrate buffer (pH 5.4, adjusted with 50% acetic acid) (solution A) and methanol (solution B). The best separation was obtained using the following gradient: from 0 to 12 min, B increases from 2% to 4%; from 12 to 20 min, B increases from 4% to 13%; from 20 to 22 min, B is kept constant in 13% B; from 22 to 26 min, B decreases from 13% to 2%; from 26 to end, B is kept constant at 2%.

2.5. Qualitative GC–MS analysis

Samples were derivatised as reported by Quesada, Rubio, and Gomez (1997): around 50 mg of standard reagents or extracts were weighed into a 25 ml round bottom flask and trimethylsilylated by adding 200 μl of pyridine, 1 ml of BSTFA and 50 μl of TMCS. The round bottom flask was sealed, shaken vigorously, kept at 60 °C under stirring for 30 min in a water bath and cooled to room temperature before GC–MS analysis (injection volume, 1.5 μl). This procedure was successfully employed in the analysis of low molecular weight compounds (phenolics and carboxylic acids) from hydrolysates obtained by wet oxidation of wheat straw (Klinke, Ahring, Schmidt, & Thomsen, 2002), steam explosion and acid impregnation of sugarcane bagasse (Martín et al., 2002) and acidic processing of pinewood (Clark & Mackie, 1984). The identity of the compounds was confirmed by comparing both the retention time and the mass spectral data with those of pure compounds (similarity percentage higher than 85%).

2.6. Anti-oxidant activity (DPPH radical-scavenging activity)

Two ml of a 6×10^{-5} M methanolic solution of DPPH (α,α-diphenyl-β-picrylhydrazyl) were added to 50 μl of a methanolic solution of the anti-oxidant, and the decrease in absorbance at 515 nm was recorded in an