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Production of antioxidants from *Eucalyptus globulus* wood by solvent extraction of hemicellulose hydrolysates

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Abstract

Eucalyptus globulus wood samples were subjected to acid prehydrolysis, and the ethyl acetate-soluble fraction of hydrolysis liquors was assayed for antioxidant activity. The total yield was measured gravimetrically, the yield in phenolics was determined in terms of gallic acid equivalents, and the antioxidant activity was measured, using both the DPPH test for radical scavenging capacity and the inhibition of β-carotene bleaching assay. Up to 0.43 g of gallic acid equivalents/100 g dry wood were recovered by ethyl acetate extraction. The antioxidant activity of this fraction ($EC_{50} = 0.39$ g/l) corresponded to 61% of that presented by BHA, and was 12 times higher than that of BHT. When the acid hydrolysis was carried out under conditions leading to extracts with maximal antioxidant specific activity, the EC_{50} (0.23 g/l) was comparable to that of BHA. The freeze-dried extracts were stable for two months at 4 °C in the darkness, whereas the antioxidant capacity was reduced by 30 and 48% after 2 months storage at 40 and 50 °C, respectively. The aqueous phase, after ethyl acetate extraction of hydrolysates, was suitable for use as a xylose-based fermentation medium.

Keywords: *Eucalyptus globulus* wood; Acid hydrolysates; Ethyl acetate extraction; Antioxidant activity; Xylose

1. Introduction

The utilization of natural antioxidants as substitutes for those from chemical synthesis is being encouraged by factors such as contradictory data on the safety of synthetic compounds, growing demand and increased consumer preferences for natural food additives. However, the origin does not guarantee absence of toxicological or allergenic effects of natural antioxidants, and their experimental evaluation is needed.

During the past few years, a number of vegetal materials have been considered as sources of antioxidants. The search for cheap and widespread feedstocks for this purpose led to the evaluation of residual materials (Moure et al., 2001), including potato peel waste (Rodríguez de Sotillo, Hadley, & Holm, 1994), olive rape (Sheabar & Neeman, 1988), grape pomace and peels (Bonilla, Mayen, Merida, & Medina, 1999; Lu and Foo, 1999), citrus seeds and peels (Manthey & Grohmann, 2001), carrot pulp waste (Chen & Tang, 1998), old tea leaves (Zandi & Gordon, 1999) and cocoa by-products (Azizah, Nik Ruslawati, & Swee Tee, 1999).

The fibrous part of vegetal biomass can yield antioxidants after hydrolytic processing (Cruz, Domínguez, Domínguez, & Parajó, 2001; Ohta, Yamasaki, Egashira, & Sanada, 1994). Mild acid hydrolysis is currently used for breaking the hemicellulose fraction of lignocellulose into monosaccharides (which can be used as carbon sources in the formulation of fermentation media), leaving cellulose and lignin in the solid phase. The acid hydrolysates contain sugars, sugar-dehydration products (furfural or hydroxymethylfurfural), acetic acid (generated from acetyl groups) and compounds derived from the soluble lignin fraction. Purification of sugar solutions by solvent extraction yields a phenolic-rich extract and improves their fermentability by yeasts (Cruz, Domínguez, Domínguez, & Parajó, 1999). A variety of lignocellulosic materials, suitable for the formulation of xylose-based fermentation media, have been explored as possible antioxidant sources after acid prehydrolysis. In this context, fractions obtained from

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Eucalyptus globulus wood showed high antimicrobial and antioxidant ability (Cruz et al., 2001).

Partial depolymerization of lignin and lignin-hemicellulose linkages occurs during acid hydrolysis. Lignin is covalently linked to polysaccharides via sugar residues of phenolic acids esterified to polysaccharides. Even if most of the lignin is acid-insoluble (Klason lignin), a part of it can be solubilized in acidic media (acid-soluble lignin). Hot water can extract the free phenolic acids and acid hydrolysis can release simple esterified phenolic acids. The whole acid-soluble lignin fraction is assumed to be of a phenolic (or polyphenolic) nature. Lignin monomers and dimers were observed to be active antioxidants (Barclay, Xi, & Norris, 1997). The hydrolytic degradation of hemicelluloses in media, cataysed by acids or enzymes (Ohta et al., 1994; Weinberg, Akiri, Potoyevski, & Kanner, 1999), increases the recovery of phenols, and the combination of both was proposed for analytical purposes (Yu, Vasanthan, & Temelli, 2001).

Association of hydrolysable tannins and lignin has been studied in hardwoods (Helm, Ranatunga, & Chandra, 1998). Both proanthocyanidins and ellagitannins are the tannin groups present in *Eucalyptus globulus* wood (Cadahía, Conde, Fernández de Simón, & García-Vallejo, 1997). Conde, Cadahía, Díez-Barra, and García-Vallejo (1996) reported the presence of acids (gallic, vanillic and ellagic), aldehydes (syringaldehyde and sinapaldehyde) and naringenin and quercetin, in relative amounts dependent on the tree species and provenace. The presence of ferulic acid, homovanillyl alcohol and syringic acid was recently detected in the *Eucalyptus globulus* lipophilic fraction (Freire, Silvestre, & Nieto, 2002). Ellagitannins can be hydrolysed to yield ellagic acid (Viriot, Scalbert, Lapierre, & Moutounet, 1993), which is made up of gallic acid. Both antioxidant and biological properties have been reported for gallic acid and ellagic acid (Aruoma, Murcia, Butler, & Halliwell, 1993; Priyadarsini, Khopde, Kumar, & Mohan, 2002).

The utilisation of crude extracts as antioxidants, instead of pure compounds or purified fractions, is a frequent approach (Xing & White, 1997; Zandi & Gordon, 1999). This alternative is more favourable from an economic point of view, and in some cases crude extracts have proved to be superior to synthetic mixtures of the main components (Onyeneho & Hettiarachchy, 1992; Rodríguez de Sotillo et al., 1994), even though the opposite behaviour has also been reported (Watanabe, Ohshita, & Tsushida, 1997).

Data on the mild acid hydrolysis of *Eucalyptus globulus* wood, dealing with the production of xylose solutions, was previously published (Parajó, Vázquez, Alonso, Santos, & Domínguez, 1993, 1994). In this work, prehydrolysis was explored in a wider temperature range in order to assess the total yield of acetate-soluble compounds, the yield in phenolics and the antioxidant activity of these fractions. The antioxidant activity was measured by both the DPPH radical scavenging capacity and the β-carotene bleaching inhibition tests. The compatibility of the operational conditions employed in the acid hydrolysis step for producing both xylose and antioxidants is also considered.

2. Materials and methods

2.1. Raw material

Eucalyptus globulus wood chips were obtained from a local pulp mill and stored in a dry and dark place at room temperature until used. The composition (measured by standard methods and expressed as weight percent, oven-dry basis) was: cellulose 46.3, hemicelluloses 17.1, Klason lignin 22.9, acid soluble lignin 4.1, ash 0.2, extractives 2.7 and others (by difference) 6.7.

2.2. Prehydrolysis and solvent extraction of hydrolysates

Ground samples of *Eucalyptus globulus* wood were hydrolysed with 2.5–5% H_2SO_4 at 100–130° C using a liquid:solid ratio (LSR) of 8:1 g/g. For comparative purposes, a control experiment, without externally added acid (autohydrolysis reaction), was also performed. Hydrolysates were separated from the solid phase (made up of cellulose and lignin) by vacuum filtration. Xylose in liquors was determined by HPLC analysis of hydrolysates using an Interaction ION-300 column (mobile phase, H_2SO_4 0.01 M; flow rate, 0.4 ml/min; IR and UV detection).

The ethyl acetate extraction was carried out using a hydrolysate:ethyl acetate volume ratio of 1:3 (v/v) in a single extraction stage (Cruz et al., 1999). Ethyl acetate was removed by vacuum evaporation and reutilised, and the solid extract was used in experiments after yield calculation. Fig. 1 summarises the processing of samples.

Total phenols in the extracts obtained after ethyl acetate evaporation were determined by absorbance readings at 745 nm of the complex formed with the Folin–Denis reagent and the extracts. A standard curve with gallic acid (Sigma Chem. Co.) was used to express the concentrations of phenolics as gallic acid equivalents.

2.3. Determination of the antioxidant activity

2.3.1. DPPH radical scavenging activity test

The ability of extracts to scavenge the α,α-diphenyl-β-picrylhydrazyl (DPPH) radical was measured as reported by von Gadow, Joubert, and Hansmann (1997). Two millilitres of a 6×10^{-5} M methanolic solution of DPPH were added to 50 μl of a methanolic solution of extracts, and the decrease in absorbance at 515 nm was

recorded for 16 min in an Agilent 8453 spectrophotometer. The inhibition percentage (IP) of the DPPH radical was calculated as:

$$\mathrm{IP} = \frac{(\mathrm{Absorbance}_{t=0\,\mathrm{min}} - \mathrm{Absorbance}_{t=16\,\mathrm{min}})}{\mathrm{Absorbance}_{t=0\,\mathrm{min}}} \cdot 100$$

The parameter EC_{50} was calculated from the IP data as the amount of ethyl acetate soluble extracts (redissolved in methanol) causing a 50% inhibition of the DPPH radical.

2.3.2. β-Carotene bleaching test

The ability of the extracts to protect against the oxidation of β-carotene in a β-carotene/linoleic acid emulsion test was carried out as reported by Miller (1971). Two milligrammes of β-carotene (Sigma, Chemical Co.) were dissolved in 10 ml of chloroform, and 1 ml of this solution was pipetted into a round-bottomed flask containing 20 mg of purified linoleic acid (Fluka) and 200 mg of Tween 40 (Fluka). After removing chloroform by evaporation, 50 ml of oxygenated, distilled water were added to the flask under vigorous stirring, and 5 ml aliquots of the emulsion were transferred into test tubes containing 0.2 ml of ethanolic antioxidant solution. The absorbance readings at 470 nm against a control containing ethanol instead of antioxidant were recorded.

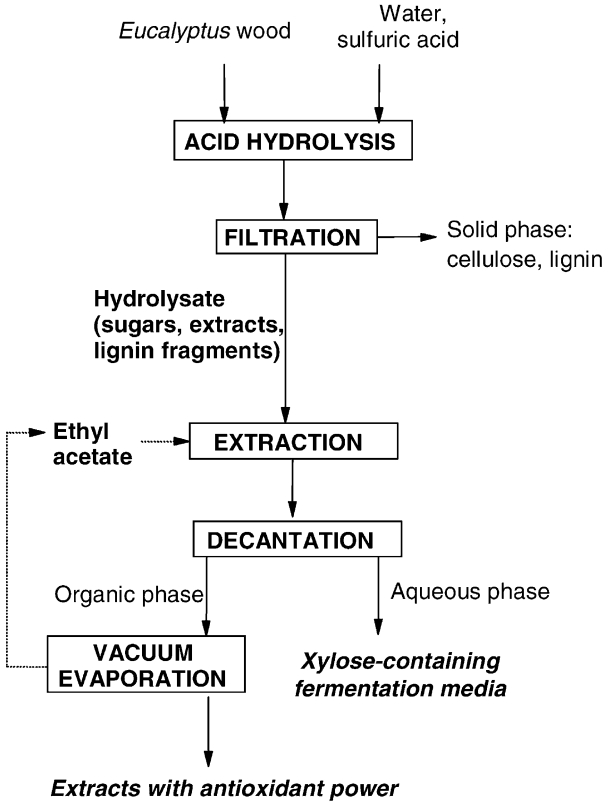

Fig. 1. Scheme of the process used to extract and recover compounds with antioxidant activity from *Eucalyptus globulus* wood.

The test and control tubes were stoppered and placed in a water bath at 50 °C, and absorbance readings were taken at regular intervals until complete decoloration. The antioxidant activity was measured by the antioxidant activity coefficient, AAC, which gives an estimate of the relative extent of oxidation in the presence and in the absence of extracts. The AAC was calculated as:

$$\mathrm{AAC} = \frac{(\mathrm{Absorbance\ of\ extract}_{120\ \mathrm{min}} - \mathrm{Absorbance\ of\ control}_{120\ \mathrm{min}})}{(\mathrm{Absorbance\ of\ control}_{0\ \mathrm{min}} - \mathrm{Absorbance\ of\ control}_{120\ \mathrm{min}})} \cdot 1000$$

All tests and analyses were run in duplicate or in triplicate and the average values are presented. For comparative purposes, additional assays were carried out with the reference antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Sigma Chemical Co.).

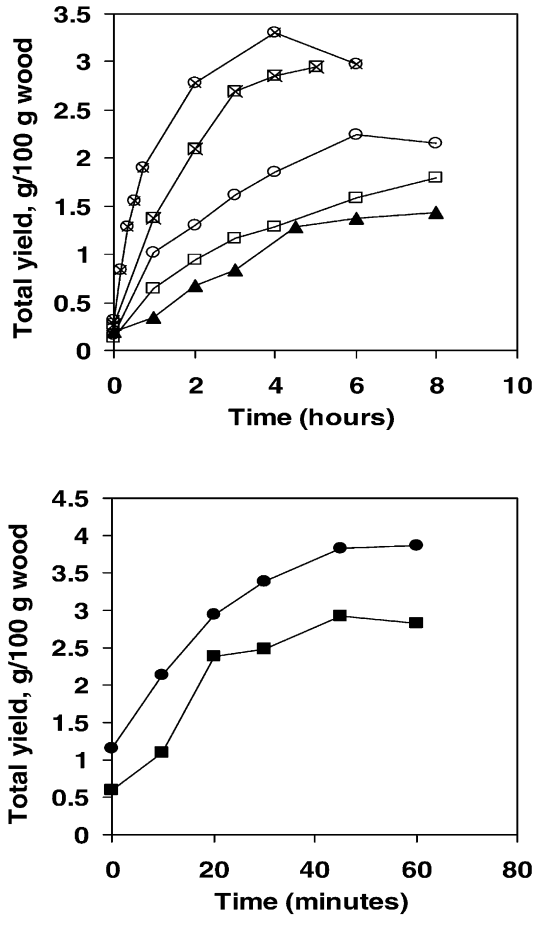

Fig. 2. Time course of the total yield of ethyl acetate-soluble compounds for experiments carried out at 100 °C—5% sulfuric acid (○), 100 °C—2.5% sulfuric acid (□), 115 °C—5% sulfuric acid (⊗), 115 °C—2.5% sulfuric acid (⊠), 130 °C—5% sulfuric acid (●), 130 °C—2.5% sulfuric acid (■) and 130 °C without externally added acid (▲).

3. Results and discussion

3.1. Extraction yield

Fig. 2 shows the dependence of the yield of ethyl acetate-soluble compounds (total yield, expressed as percentage of dry weight of the initial sample) on the reaction time for experiments carried out at 100, 115 or 130 °C in media containing 0–5% sulfuric acid. The data concerning the beginning of the isothermal reaction stage (at which the reaction time was set to zero) showed that detectable amounts of extractables were released during the heating period. The extraction yield was influenced by the severity of the acid hydrolysis: in the studied range, the higher the temperature and/or acid concentration employed, the higher was the yield. As a general trend, the yield increased with the reaction time at the beginning of the isothermal reaction stage to reach a maximal value (which depended on temperature and, in a minor degree, on the acid concentration). The reaction time needed to achieve maximal yields was significantly shorter in experiments carried out at 130 °C. The highest yield in ethyl-acetate extracts (3.9 g/100 g dry wood) was obtained under the severest conditions assayed. Results in this range (yields of 3.0, 2.1, 2.2 and 4.2 g/100 g raw material) have been reported for the acid hydrolysis of *Eucalyptus* wood (3% H_2SO_4, 15 min, 130 °C), corn cobs (2% H_2SO_4, 15 min, 130 °C), corn leaves (3% H_2SO_4, 15 min, 130 °C) and barley hulls (3% H_2SO_4, 15 min, 130 °C), respectively (Cruz et al., 2001).

Fig. 3 shows the time course of the yield in Folin-Denis phenols (expressed as g of gallic acid equivalents/100 g raw material) for the same experiments. Gallic acid was selected as standard in order to provide comparative data with the literature, although the response of gallic is different from those of other phenolic compounds such as ellagic acid (Julkunen-Tiito, 1985). The yield of Folin-Denis phenols increased during the initial reaction stages to reach a stable final value closely dependent on the severity of treatments. The yield in Folin–Denis phenols accounted for 10–20% of the total yield, depending on the hydrolysis conditions. In the experiment with no acid added at 130 °C and in assays at 100 °C, short reaction periods resulted in improved selectivity of phenolics extraction. However, the low total yields achieved under these conditions make these conditions unfavourable for the purposes of this work.

The origin of the phenolic compounds obtained in the prehydrolysis-extraction process seems to be different depending on the severity conditions, in the present work varying from autohydrolysis to acid hydrolysis. Under autohydrolysis, hemicelluloses become solubilized as a result of an autocatalysed process where the active species are the hydronium ions coming from acetic acid. The fact that high yields are obtained at short times suggests that the ethyl acetate-soluble fraction is obtained by solubilisation of the corresponding compounds. This behaviour is consistent with that expected for free phenolic acids, most of which can be extracted with hot water. Under acidic conditions, degradation of tannins occurs (Andlauer, Stumpf, & Fürst, 2000) although this fact may not be reflected in a higher content of Folin–Ciocalteau phenols (Martin, Galbe, Nilvebrant, & Jönsson, 2002).

The maximum yield of Folin-Denis phenols (0.43 g of gallic acid equivalents/100 g of dry wood) was achieved after 4 h at 115 °C or after 45 min at 130 °C. Comparatively, up to 0.76 g of gallic acid equivalents/100 g of dry wood were obtained by autohydrolysis of the same feedstock at temperatures over 260 °C (Garrote, Cruz, Domínguez, & Parajó, 2003). In related literature studies, Folin–Denis phenols obtained by steam explosion of olive cake and further ethyl acetate extraction led to up to 1.5 g of caffeic acid equivalents/100 g (Felizón, Fernández-Bolaños, Heredia, & Guillén, 2000). The same treatment yielded up to 3.2 g/100 g from whole olive stones and 4.21 g/100 g and 4.12 g/100 g

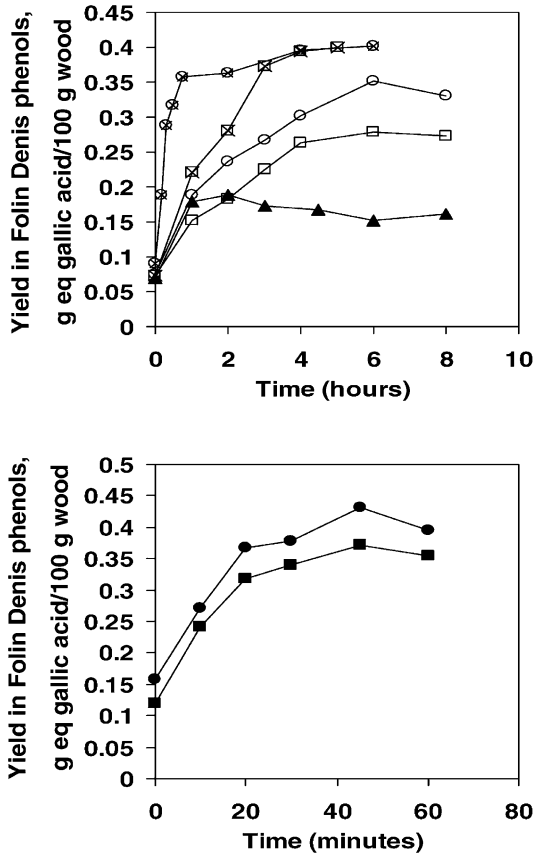

Fig. 3. Time course of the yield of Folin–Denis phenols (expressed as gallic acid equivalent) for acid hydrolysis of *Eucalyptus globulus* wood at 100 °C—5% sulfuric acid (○), 100 °C—2.5% sulfuric acid (□), 115 °C—5% sulfuric acid (⊗), 115 °C—2.5% sulfuric acid (⊠), 130 °C—5% sulfuric acid (●), 130 °C—2.5% sulfuric acid (■) and 130 °C without externally added acid (▲).

in steam-explosion (carried out in the absence or presence of externally added acid) of olive seed husks, expressed as vanillic acid, vanillin, syringic acid and syringaldehyde (Fernández-Bolaños Felizón, Brenes, Guillén, & Heredia, 1998). The concentration of total phenolics was not affected by sulphuric acid impregnation during steam explosion of sugarcane bagasse, but the product distribution was different (Martín et al., 2002).

Eucalyptus globulus wood from the same provenance as that used in the present work contains 3.86% methanol-extractable compounds (including 0.7–1.6% phenols, expressed as quercetin equivalent) (Conde et al., 1996). The phenolic fraction, accounting for 1.04 g gallic acid equivalents/100 g wood, is characterized by the presence of proanthocyanidins and ellagitannins with a wide diversity and abundance of molecular structures (Cadahía, Muñoz, Fernández de Simón & García-Vallejo, 2001).

During ageing of spirits in hardwood barrels, the contents of both ellagitannins and lignin are higher than those of simple phenols (Viriot et al., 1993; Cahadía et al. 2001). The three monomers making part of lignin are *p*-coumaryl, coniferyl and sinapyl alcohol. Hardwood lignin, with sinapyl alcohol as a monomer, is easier to break down than other lignin components, since there is no site on the aryl ring where free radical coupling can occur due to the presence of two methoxyl groups. Methoxyl is the major functional group of these lignins, followed by benzyl alcohol groups (which account for a third of the methoxyl groups) and the phenolic hydroxyl groups (which account for one tenth). *E. globulus* lignin is of the type S:G with up to 86% syringyl units (Evtuguin et al., 2001).

Ethyl acetate extraction allows the removal of a variety of potentially valuable compounds, coming from extractives and acid-soluble lignin. In literature studies (Fernández-Bolaños et al., 1998; Guillén & Ibargoitia, 1998; Shahidi & Naczk, 1995), lignin-derived products (such as *p*-hydroxybenzoic acid and other hydroxyphenyl acids, including ferulic, vanillic, syringic and coumaric acids as well as aldehydes such as syringaldehyde, *p*-hydroxybenzaldehyde and vanillin) have been identified. Preliminary GC–MS analysis (data not shown) proved that gallic acid, vanillic acid, syringic acid, syringaldehyde and protocatechuic acid were the predominant phenols in extracts obtained from prehydrolysis liquors of *Eucalyptus globulus* wood under conditions leading to maximal yield of Folin–Denis phenols (130 °C, 5% sulfuric acid, 45 min).

Unlike *Eucalyptus* wood, other woods present a significant fraction of extractables that could be selectively removed by solvent extraction. A wide range of phenolic yields, resulting from the direct solvent extraction of woody materials, has been reported. *Acacia confusa* heartwood contains 53 mg gallic acid equivalents/100 g (Chang et al., 2001). Using gallic acid as a standard and the Folin–Ciocalteau method for phenols, Scalbert, Monties, and Janin (1989) measured up to 6.3 g total phenols/100 g in the aqueous extracts from *Quercus* species, 5.3 g/100 g *Castanea sativa* wood and 2.4 g/100 g *Eucalyptus globulus* Labill wood.

3.2. Antioxidant activity

Fig. 4 presents data on the the antioxidant activity of extracts obtained by prehydrolysis-extraction of *Eucalyptus* wood (measured by the Inhibition Percentage, IP, determined from the DPPH radical scavenging assays). The antioxidant activity of hydrolysates increased with the concentration of phenolic compounds, leading to increased IP for extracts obtained at higher severities. In order to compare the antioxidant activity of the extracts on a weight basis (specific activity), Fig. 5 shows the IP values determined in assays carried out with a fixed extract concentration (0.25 g extract/l). The specific

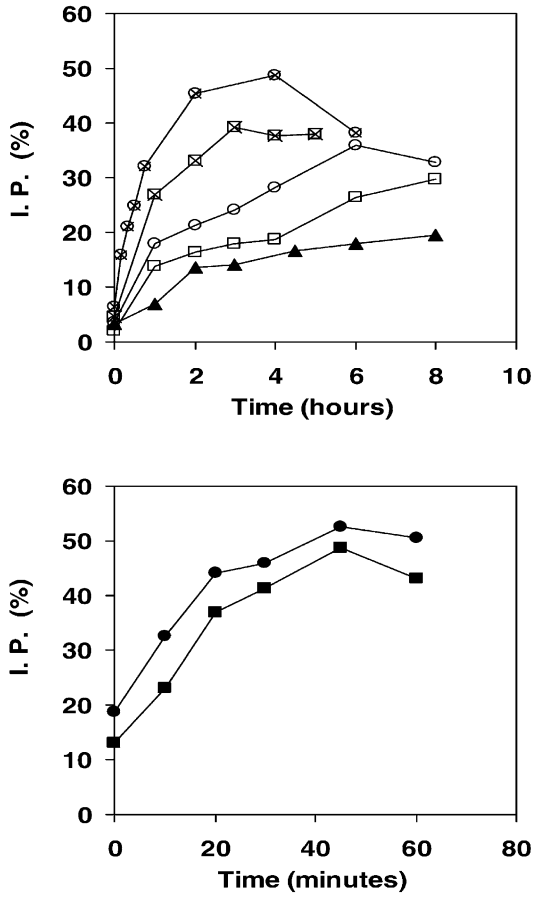

Fig. 4. Inhibition percentage of the DPPH radical by the extracts (present in 8.33 ml of hydrolysates and redissolved in 100 ml of ethanol) obtained in treatments at 100 °C—5% sulfuric acid (○), 100 °C—2.5% sulfuric acid (□), 115 °C—5% sulfuric acid (⊗), 115 °C—2.5% sulfuric acid (⊠), 130 °C—5% sulfuric acid (●), 130 °C—2.5% sulfuric acid (■) and 130 °C without externally added acid (▲).

activity was maximal for extracts obtained at short reaction times in prehydrolysis experiments performed at the highest temperature considered (130 °C). For autohydrolysis at 130 °C and acid hydrolysis with 2.5% sulfuric acid at 100 and 115 °C, maximal specific activities were achieved after 1–2 h. Longer reaction times in the prehydrolysis step led to extracts with decreased specific antioxidant activity, suggesting a different nature and/or structure of the active compounds. A similar behaviour has been reported for extracts coming from autohydrolysis of *Eucalyptus* wood carried out at 180–260 °C (Garrote et al., 2003).

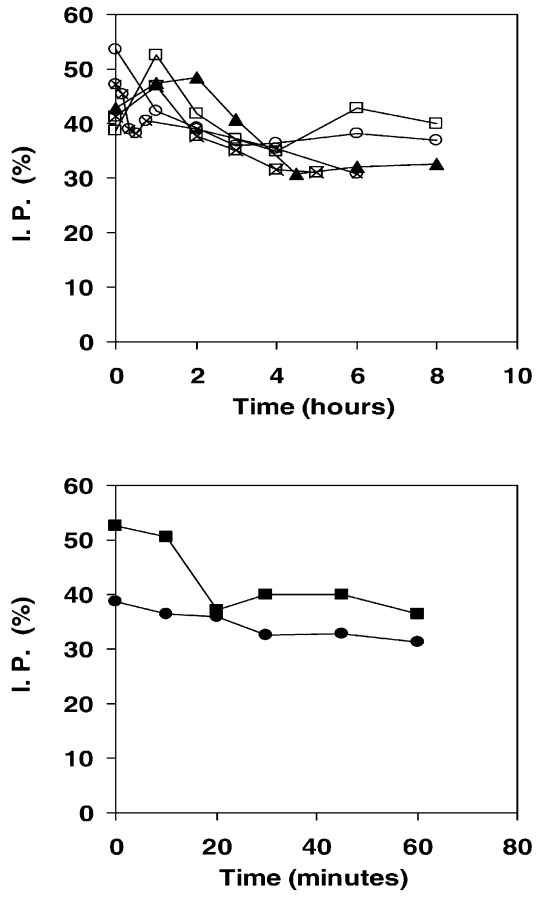

Fig. 5. Inhibition percentage of the DPPH radical achieved by a solution containing 0.25 g extracts/L obtained in treatments at 100 °C—5% sulfuric acid (○), 100 °C—2.5% sulfuric acid (□), 115 °C—5% sulfuric acid (⊗), 115 °C—2.5% sulfuric acid (⊠), 130 °C—5% sulfuric acid (●), 130 °C—2.5% sulfuric acid (■) and 130 °C without externally added acid (▲).

Ethyl acetate extracts contain monomeric and oligomeric compounds from lignin, extractives and products derived from hemicelluloses. The severity of the process can be directly related to changes in the major phenolics present in the crude extracts. Increased severities in the steam-explosion of olive cake resulted in increased yields of low molecular weight phenols (Felizón et al., 2000). On this basis, the higher antioxidant activity of the extracts produced after a short prehydrolysis time could be ascribed to the presence of oligomeric fractions. According to data reported for model compounds, lignin tetramers, trimers and dimers are more potent antioxidants than monomers (Barclay et al., 1997). Similar findings have been reported for compounds present in liquid smoke (Guillén & Ibargoitia, 1998).

Table 1 summarises the maximal EC_{50} determined for the extracts produced from *Eucalyptus globulus* wood. As additional information, the same table includes the total yields and the AAC of the corresponding extracts (measuring their antioxidant ability according to the β-carotene bleaching method). For comparative purposes, the EC_{50} and AAC of two synthetic antioxidants (BHA and BHT) are also included in Table 1. The DPPH radical scavenging capacity of extracts obtained under the operational conditions leading to the highest specific activity was similar to that of BHA and 12 times higher than that of BHT. The activity of extracts obtained under the conditions leading to maximal extraction yield (i.e. corresponding to a maximal volumetric activity of hydrolysates), was 61% of that presented by BHA and 7 times higher than the activity of BHT. When the extracts produced at 130 °C during 45 min with 5% sulphuric acid were used at a concentration of 0.5 g/l, the corresponding AAC values were 57 and 63.4% of those determined for BHT and BHA, respectively. The extracts produced at 100 °C showed higher AAC, but the yield was low. The different antioxidant capacities

Table 1
Results obtained for the parameters EC_{50} and AAC in assays with extracts produced by hydrolysis-extraction of *Eucalyptus globulus* wood and in assays with the reference antioxidants BHA and BHT. The data concerning the β-carotene bleaching assay were obtained with solutions containing 0.5 g of extracts or synthetic antioxidant/l of methanolic solution

Extract or antioxidant	EC_{50}	AAC	g EASC[a]/100 g raw material
Eucalyptus wood (100 °C, 0 h, 5% H_2SO_4)	0.23	737	0.17
Eucalyptus wood (130 °C, 45 min, 5% H_2SO_4)	0.39	542	3.83
Eucalyptus wood (130 °C, 60 min, 5% H_2SO_4)	0.39	504	3.87
BHT	2.78	854	–
BHA	0.24	876	–

[a] EASC: Ethyl acetate soluble compounds.

observed with the different methods are due to the close dependence of the antioxidant activity on the polarity of the testing system (Brand-Williams, Cuvelier, & Berset, 1995; von Gadow et al., 1997).

The extracts produced under conditions leading to maximal antioxidant activity in volumetric terms (130 °C, 5% sulfuric acid, 45 min) were freeze-dried and stored at 30 and 4 °C in the darkness. The DPPH radical scavenging capacity of a solution containing 0.5 g extract/l was 52.7% ± 1.4 and remained constant for 70 days at 4 °C in the dark, both in the presence and absence of air. The solution was also stable for at least 14 days at 30 °C in the presence of light. The antioxidant activity of freeze-dried extracts was not altered after 60 days storage at 30 °C. Upon storage at 40 and 50 °C for 60 days, the antioxidant activity was reduced by 24.2 and 36.8%, respectively.

The detoxification of hemicellulosic hydrolysates by solvent extraction leads to phenolic extracts with potential application as food additives. This extract also offers the possibility of commercial development in cosmetic fields. The toxicological safety of synthetic antioxidants has been questioned and the search for cheap, renewable sources of natural ones is gaining interest. So, even if further assessment of the acceptability and safety of theses natural extracts for food-related application is necessary, the results presented in this study confirm their potential as antioxidant agents.

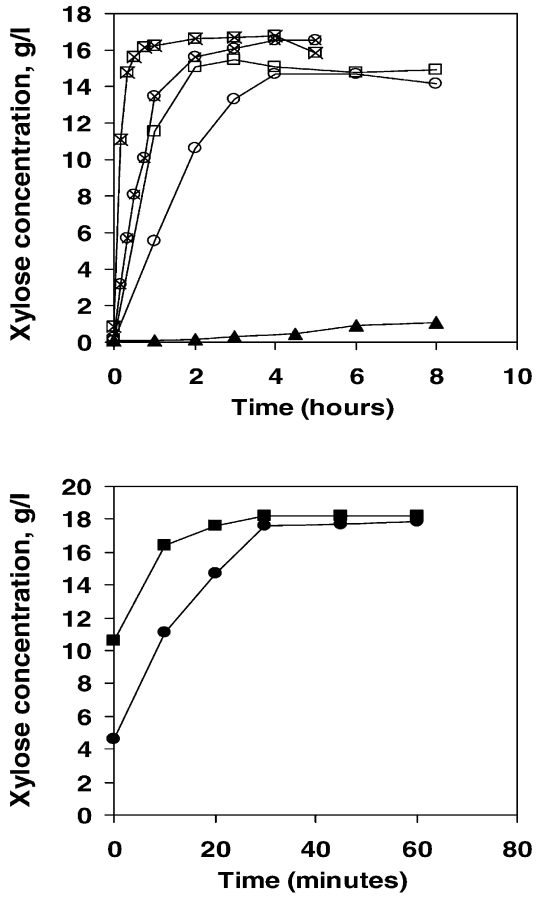

Fig. 6. Time course of xylose concentration in treatments at 100 °C—5% sulfuric acid (○), 100 °C—2.5% sulfuric acid (□), 115 °C—5% sulfuric acid (⊗), 115 °C—2.5% sulfuric acid (⊠), 130 °C—5% sulfuric acid (●), 130 °C—2.5% sulfuric acid (■) and 130 °C without externally added acid (▲).

3.3. Xylan solubilization

An exhaustive analysis of the acid prehydrolysis of *Eucalyptus globulus* wood for producing xylose solutions has already been published (Parajó et al., 1994). Liquors obtained under the conditions leading to maximal xylose concentrations were neutralised, supplemented with nitrogen sources, sterilized and used for the fermentative xylitol production (Parajó, Domínguez, & Domínguez, 1997a). The direct fermentation of these solutions was hindered by reaction byproducts contained in the reaction medium (which include microbial inhibitors such as furfural, hydroxymethylfurfural, acetic acid and compounds derived from the acid-soluble lignin fraction) (Ando, Arai, Kiyoto, & Hanai, 1986; Boussaid, Robinson, Cai, Gregg, & Saddler, 1999; Taherzadeh, Millati, & Niklasson, 2001). The solvent extraction process used in the present work to separate antioxidants is an efficient detoxification method allowing the production of fermentable xylose solutions (Parajó, Domínguez, & Domínguez, 1997b; Cruz *et al.*, 1999). The xylose concentration profiles, corresponding to hydrolysates produced under the conditions assayed in this work are presented in Fig. 6. The xylose concentrations in liquors (in the range 15–18 g/l) make possible the formulation of fermentation media with or without previous vacuum concentration. The concentrations of microbial inhibitors in ethyl acetate-extracted prehydrolsis liquors (less than 5 g acetic acid/l, 0.5 g furfural/l and 0.1 g HMF/l) are below the threshold causing microbial inhibition.

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References

Andlauer, W., Stumpf, C., & Fürst, P. (2000). Influence of the acetification process on phenolic compounds. *Journal of Agricultural and Food Chemistry*, *48*, 3533–3536.

Ando, S., Arai, I., Kiyoto, K., & Hanai, S. (1986). Identification of aromatic monomers in steam-exploded poplar and their influences

on ethanol fermentation by *Saccharomyces cerevisiae*. *Journal of Fermentation Technology*, *64*, 567–570.

Aruoma, O. I., Murcia, A., Butler, J., & Halliwell, B. (1993). Evaluation of the antioxidant and prooxidant actions of gallic acid and its derivatives. *Journal of Agricultural and Food Chemistry*, *41*, 1880–1885.

Azizah, A. H., Nik Ruslawati, N. M., & Swee Tee, T. (1999). Extraction and characterization of antioxidant from cocoa by-products. *Food Chemistry*, *64*, 199–202.

Barclay, L. R. C., Xi, F., & Norris, J. Q. (1997). Antioxidant properties of phenolic lignin model compounds. *Journal of Wood Chemistry and Technology*, *17*(1 & 2), 73–90.

Bonilla, F., Mayen, M., Merida, J., & Medina, M. (1999). Extraction of phenolic compounds from red grape marc for use as food lipid antioxidants. *Food Chemistry*, *66*, 209–215.

Boussaid, A., Robinson, J., Cai, Y., Gregg, D. J., & Saddler, J. N. (1999). Fermentability of the hemicellulose-derived sugars from steam-exploded softwood (Doglar Fir). *Biotechnology and Bioengineering*, *64*, 284–289.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaft und Technologie*, *28*, 25–30.

Cadahía, E., Conde, E., Fernández de Simón, B., & García-Vallejo, M. C. (1997). Tannin composition of *Eucalyptus camaldulensis, E. globulus* and *E. rudis*. Part I. *Holzforschung*, *51*, 119–124.

Cadahía, E., Muñoz, L., Fernández de Simón, B., & García-Vallejo, M. C. (2001). Changes in low molecular weight phenolic compounds in spanish, French and American oak woods during natural seasoning and toasting. *Journal of Agricultural and Food Chemistry*, *49*, 1790–1798.

Chang, S. T., Wu, J. H., Wang, S. Y., Kang, P. L., Yang, N. S., & Shyur, L. F. (2001). Antioxidant activity of extracts from *Acacia confusa* bark and heartwood. *Journal of Agricultural and Food Chemistry*, *49*, 3420–3424.

Chen, B. H., & Tang, Y. C. (1998). Processing and stability of carotenoid powder from carrot pulp waste. *Journal of Agricultural and Food Chemistry*, *46*, 2312–2318.

Conde, E., Cadahía, E., Díez-Barra, R., & García-Vallejo, M. C. (1996). Polyphenolic composition of bark extracts from *Eucalyptus camaldulensis, E. globulus*, and *E. rudis*. *Holz als Roh- und Werkstoff*, *54*, 175–181.

Cruz, J. M., Domínguez, J. M., Domínguez, H., & Parajó, J. C. (1999). Solvent extraction of hemicellulosic wood hydrolysates: a procedure useful for obtaining both detoxified fermentation media and polyphenols with antioxidant activity. *Food Chemistry*, *67*, 147–153.

Cruz, J. M., Domínguez, J. M., Domínguez, H., & Parajó, J. C. (2001). Antioxidant and antimicrobial effects of extracts from hydrolyzates of lignocellulosic materials. *Journal of Agricultural and Food Chemistry*, *49*, 2459–2464.

Evtuguin, D. V., Neto, C. P., Silva, A. M. S., Dominguez, P. M., Amado, F. M. L., Robert, D., & Faix, O. (2001). Comprehensive study on the chemical structure of dioxan lignin from plantation *Eucalyptus globulus* wood. *Journal of Agricultural and Food Chemistry*, *49*, 4952–4961.

Felizón, B., Fernández-Bolaños, J., Heredia, A., & Guillén, R. (2000). Steam-explosion pre-treatment of olive cake. *Journal of the American Oil Chemists' Society*, *77*, 15–22.

Fernández-Bolaños, J., Felizón, B., Brenes, M., Guillén, R., & Heredia, A. (1998). Hydroxytyrosol and tyrosol as the main compounds in the phenolic fraction of steam-exploded olive stones. *Journal of the American Oil Chemists Society*, *75*, 1643–1649.

Freire, C. S. R., Silvestre, A. J. D., & Nieto, C. P. (2002). Identification of new hydroxy fatty acids and ferulic acid esters in the wood of *Eucalyptus globulus*. *Holzforschung*, *56*, 143–149.

Garrote, G., Cruz, J. M., Domínguez, H., & Parajó, J. C. (2003). Valorisation of waste fractions from autohydrolysis of selected lignocellulosic materials. *Journal of Chemistry Technology and Biotechnology*, *78*, 392–398.

Guillén, M. D., & Ibargoitia, M. L. (1998). New components with potential antioxidant and organoleptic properties, detected for the first time in liquid smoke flavouring preparations. *Journal of Agricultural and Food Chemistry*, *46*, 1276–1285.

Helm, R. F., Ranatunga, T. D., & Chandra, M. (1998). Lignin-hydrolyzable tannin interaction in wood. *Journal of Agricultural and Food Chemistry*, *45*, 3100–3106.

Julkunen-Tiito, R. (1985). Phenolic constituents in the leaves of northern willows, methods for the analysis of certain phenolics. *Journal of Agricultural and Food Chemistry*, *33*, 213–217.

Lu, Y., & Foo, L. Y. (1999). The polyphenol constituents of grape pomace. *Food Chemistry*, *65*, 1–8.

Manthey, J. A., & Grohmann, K. (2001). Phenols in citrus peel byproducts. Concentrations of hydroxycinnamates and polymethoxylated flavones in citrus peel molasses. *Journal of Agricultural and Food Chemistry*, *49*, 3268–3273.

Martín, C., Galbe, M., Nilvebrant, N. O., & Jönsson, L. F. (2002). Comparison of the fermentability of enzymatic hydrolyzates of sugarcane bagasse pretreated by steam explosion using different impregnating agents. *Applied Biochemistry and Biotechnology*, *98–100*, 699–716.

Miller, H. E. A. (1971). Simplified method for the evaluation of antioxidants. *Journal of the American Oil Chemists' Society*, *45*, 91.

Moure, A., Cruz, J. M., Franco, D., Domínguez, J. M., Sineiro, J., Domínguez, H., Núñez, M. J., & Parajó, J. C. (2001). Natural antioxidants from residual sources, a review. *Food Chemistry*, *72*, 145–171.

Ohta, T., Yamasaki, S., Egashira, Y., & Sanada, H. (1994). Antioxidative activity of corn bran hemicellulose fragments. *Journal of Agricultural and Food Chemistry*, *42*, 653–656.

Onyeneho, S. N., & Hettiarachchy, N. S. (1992). Antioxidant activity of durum wheat bran. *Journal of Agricultural and Food Chemistry*, *40*, 1496–1500.

Parajó, J. C., Vázquez, D., Alonso, J. L., Santos, V., & Domínguez, H. (1993). Prehydrolysis of *Eucalyptus* wood with dilute sulphuric acid: operation at atmospheric pressure. *Holz als Roh und Werkstoff*, *51*, 357–363.

Parajó, J. C., Vázquez, D., Alonso, J. L., Santos, V., & Domínguez, H. (1994). Prehydrolysis of *Eucalyptus* wood with dilute sulphuric acid: operation in autoclave. *Holz als Roh und Werkstoff*, *52*, 102–108.

Parajó, J. C., Domínguez, H., & Domínguez, J. M. (1997a). Improved xylitol production with *Debaryomyces hansenii* Y-7426 from raw or detoxified wood hydrolysates. *Enzyme Microbiology Technology*, *21*, 18–24.

Parajó, J. C., Domínguez, H., & Domínguez, J. M. (1997b). Xylitol production from Eucalyptus hydrolysates extracted with organic solvents. *Procces Biochemistry*, *32*, 599–604.

Priyadarsini, K. I., Khopde, S. M., Kumar, S. S., & Mohan, H. (2002). Free radical studies of ellagic acid, a natural phenolic antioxidant. *Journal of Agricultural and Food Chemistry*, *50*, 2200–2206.

Rodríguez de Sotillo, D., Hadley, M., & Holm, E. T. (1994). Phenolics in aqueous potato peel extract: extraction, identification and degradation. *Journal of Food Science*, *59*, 649–651.

Scalbert, A., Monties, B., & Janin, G. (1989). Tannins in wood: comparison of different estimation methods. *Journal of Agricultural and Food Chemistry*, *37*, 1324–1329.

Shahidi, F., & Naczk, N. (1995). *Food phenolics, sources chemistry effects applications*. Lancaster, PA: Technomic Publishing Co Inc.

Sheabar, F. Z., & Neeman, I. (1988). Separation and concentration of natural antioxidants from the rape of olives. *Journal of the American Oil Chemists' Society*, *65*, 990–993.

Taherzadeh, M. J., Millati, R., & Niklasson, C. (2001). Continuous cultivation of dilute-acid hydrolysates to ethanol by immobilized *Saccharomyces cerevisiae*. *Applied Biochemistry and Biotechnology*, *95*, 45–57.

Viriot, C., Scalbert, A., Lapierre, C., & Moutounet, M. F. (1993). Ellagitannins and lignins in aging of spirits in oak barrels. *Journal of Agricultural and Food Chemistry*, *41*, 1872–1879.

von Gadow, A., Joubert, E., & Hansmann, C. F. (1997). Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (*Aspalathus linearis*), α-tocopherol, BHT and BHA. *Journal of Agricultural and Food Chemistry*, *45*, 632–638.

Watanabe, M., Ohshita, Y., & Tsushida, T. (1997). Antioxidant compounds from buckwheat (*Fagopyrum esculentum* Möench) hulls. *Journal of Agricultural and Food Chemistry*, *45*, 1039–1044.

Weinberg, Z. G., Akiri, B., Potoyevski, E., & Kanner, J. (1999). Enhancement of polyphenol recovery from rosemary (*Rosmarinus officinalis*) and Sage (*Salvia officinalis*) by enzyme-assisted ensiling (ENLAC). *Journal of Agricultural and Food Chemistry*, *47*, 2959–2962.

Xing, Y., & White, P. (1997). Identification and function of antioxidants from oat groats and hulls. *Journal of the American Oil Chemists' Society*, *74*, 303–307.

Yu, J., Vasanthan, T., & Temelli, F. (2001). Analysis of phenolic acids in barley by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry*, *49*, 4352–4358.

Zandi, P., & Gordon, M. H. (1999). Antioxidant activity of extracts from old tea leaves. *Food Chemistry*, *64*, 285–288.