Antioxidant and Antimicrobial Effects of Extracts from Hydrolysates of Lignocellulosic Materials

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The antioxidant and antimicrobial activities of ethyl acetate extracts obtained from acid hydrolysates of several lignocellulosic materials (*Eucalyptus globulus* wood, barley bran, corn cobs, and corn leaves) were evaluated. The minimum inhibitory and bactericide concentrations (MIC and MBC, respectively) were determined against a selection of bacteria and yeasts. Extracts from *Eucalyptus* wood hydrolysates were the most active for inhibiting bacteria and yeast growth, with MIC in the range of $10^2-5 \times 10^3$ μg/mL and MBC in the range of 10^3-10^5 μg/mL. Bacteriogenic and bacteriostatic activities of extracts from *Eucalyptus* wood and barley bran acid hydrolysates were slightly higher than those of corn cobs and leaves. Both the radical scavenging capacity and the inhibition of the β-carotene bleaching caused by extracts were determined and compared with those of synthetic antioxidants. The antioxidant activity of extracts increased with their concentrations in the media, the stronger properties corresponding to those obtained from *Eucalyptus* wood hydrolysates.

Keywords: *Acid hydrolysates; antimicrobial activity; antioxidant activity; lignocellulosic materials*

INTRODUCTION

Mild acid hydrolysis (prehydrolysis) of lignocellulosic materials in the presence of a mineral acid (which acts as a catalyst) is a technology useful for obtaining sugar solutions derived from the hemicellulose fraction of the raw materials. In prehydrolysis steps, the hemicellulose polysaccharides (such xylan, mannan, and galactan) are converted into the correspondent monosaccharides and sugar degradation products, whereas a part of the phenolic fraction of the feedstocks (the acid-soluble lignin) is dissolved (*1*). The main components of the extracts derived from the lignin fraction are well-known (acids such as *p*-hydroxybenzoic acid and other hydroxyphenyl acids, ferulic acid, vanillic acid, syringic acid, and coumaric acid; aldehydes such as syringaldehyde, *p*-hydroxybenzaldehyde, and vanillin) (*2*, *3*).

The utilization of prehydrolysis liquors for making fermentation media is often hindered by the presence of antimicrobial components that act as fermentation inhibitors, including sugar degradation products or phenolics belonging to the soluble lignin fraction. To improve the fermentability of prehydrolysis-derived media, several methods have been proposed in the literature to remove lignin degradation products from hydrolysates. Among them, solvent extraction is especially interesting because of both the good susceptibility of detoxified hydrolysates to fermentation and the recovery of a phenolic fraction potentially valuable as a food additive owing to its antioxidant activity (*4*, *5*).

The antimicrobial activity of phenolics has been widely reported. Whereas such components are undesirable in fermentation media, they can be useful in the food industry to prevent rancidity and microbial spoilage. The substitution of synthetic food additives by "naturally derived" antimicrobial and/or antioxidant agents is desirable, although the safety of natural, alternative additives must be assessed before utilization (*6*).

The extracts evaluated here present the advantage of being generated from fractions of polymers naturally present in lignocellulosic materials simply by hydrolysis reactions catalyzed by hydronium ions. The released compounds also appear naturally in vegetable materials.

Some materials of residual nature have been proposed as cheap sources of antioxidants (*7*−*14*). Lignin dimers coming from the thermal degradation of lignin show antioxidant activity (*3*), and related studies dealing with the antioxidant activity of extracts bound to lignin and arabinoxylans have been reported (*15*, *16*). The antioxidant activity can be evaluated by chemical or biological tests either in vivo or in vitro (*17*, *18*).

Antimicrobial activity has been reported for phenolics from several sources, such as liquid smoke (*19*), potato peel (*20*), *Sempervivum* L. (*21*), or spices (*22*, *23*). The evaluation of antimicrobial activity has been carried out both in plates (*20*, *21*, *24*) and in food systems such as beef (*19*) or strawberry puree (*25*). Besides antioxidant and antimicrobial activities, other biological effects have been reported for phenolic compounds, including antiviral, antimutagenic, antiinflamatory, antiallergic, and anticarcinogenic activities and platelet inhibition as well as antiulcer and anticariogenic properties (*26*−*33*).

This study deals with the evaluation of both antimicrobial and antioxidant activities of ethyl acetate extracts from acid hydrolysates (EAH) of selected lignocellulosic materials, in order to assess their suitability as alternative food additivies. The lignocellulosic feedstocks for hydrolysis (*Eucalyptus globulus* wood, barley bran, corn cobs, and corn leaves) were selected on the basis of their high xylan contents, which upon hydrolysis lead to xylose-containing solutions useful as fermentation media after detoxification by solvent extraction.

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Table 1. Moisture Content (Percent Weight) and Proximal Composition (Percent Dry Weight) of the Lignocellulosic Materials and Hydrolysates Used in This Work

	Eucalyptus wood	barley bran	corn cobs	corn leaves
Lignocellulosic Raw Material				
moisture (%)	7.1	6.3	6.1	6.7
cellulose	46.3	23.0	31.7	37.6
hemicelluloses	17.1	32.7	34.7	34.5
lignin (acid soluble + acid insoluble)	22.9	21.4	20.3	12.6
acetyl groups	3.6	1.6	3.4	3.2
Hydrolysates				
xylose	17.1	35.6	35.3	22.5
glucose	1.7	5.9	3.2	2.0
arabinose	2.1	7.1	4.6	3.6
acetic acid	5.2	2.4	3.7	2.3
furfural	0.5	0.3	0.3	0.3
HMF	<0.1	<0.1	<0.1	<0.1
Extracts from Hemicellulose Hydrolysate				
ethyl acetate extraction yield (g of dry extract/g of raw material)	0.030	0.042	0.021	0.022

MATERIALS AND METHODS

Raw Material. *Eucalyptus globulus* wood chips were obtained from a local pulp mill, barley bran from malting was kindly provided by San Martín (Ourense, Spain), and corn cobs and leaves were collected from local farms. Their moisture contents and compositions (expressed as weight percents, oven-dry basis) are summarized in Table 1. These materials were stored in a dry and dark place at room temperature until utilization.

Analysis of the Raw Materials. Aliquots from the homogenized lots were submitted to moisture determination and to quantitative hydrolysis in two-stage, acid treatment (the first step with 72 wt % sulfuric acid at 30 °C during 1 h, and the second one after dilution of the media to 4 wt % sulfuric acid at 121 °C during 1 h) (*34*). The solid residue after hydrolysis was considered to be Klason lignin. Hydrolysates were assayed by high-performance liquid chromatography (HPLC) using an Interaction ION-300 column (mobile phase, 0.01 M H_2SO_4; flow rate, 0.4 mL/min; IR and UV detection). This method allowed the direct determination of glucose, xylose, arabinose, acetic acid, ethanol, xylitol, furfural, and hydroxymethylfurfural (HMF).

Prehydrolysis of the Lignocellulosic Materials and Solvent Extraction of Hydrolysates. Ground samples of *Eucalyptus* wood and barley bran were hydrolyzed with 3% H_2SO_4 during 15 min at 130 °C using a liquid/solid ratio (LSR) of 8:1 g/g. Milled corn cob samples were treated during 15 min with 2% H_2SO_4 at 130 °C using an LSR of 8:1 g/g, whereas corn leaves were treated with 3% H_2SO_4 during 15 min at 130 °C using an LSR of 16:1 g/g. Aliquots from the reaction media were analyzed by HPLC using the same procedure described above. The liquors obtained after hydrolysis were separated by filtration, and pH was adjusted to 3 with $CaCO_3$. The $CaSO_4$ precipitated was removed by filtration and centrifugation before extraction with ethyl acetate at a hydrolysate/ethyl acetate volume ratio of 1:3 (v/v) in a single extraction stage (*5*). Ethyl acetate was removed by vacuum evaporation, and the dry material (extracts from acid hydrolysates EAH) was used in experiments after yield calculation.

Microorganisms. The following microorganisms (kindly provided by the Spanish National Collection of Type Cultures, Valencia, Spain) were used to assess the antimicrobial activity of EAH: *Streptococcus bovis* CECT 213, *Escherichia coli* CECT 434, and *Staphylococcus aureus* CECT 59. Other microorganisms used for the same purpose were *Salmonella* sp. and *Enterococcus faecalis* (isolated from food and from a clinical uroculture, respectively) and the yeasts *Pichia stipitis* CBS 5773 and a *Saccharomyces cerevisiae* strain isolated from grape juice.

Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericide Concentration (MBC). The selected microorganisms were incubated for 24 h under favorable conditions for proliferation in media without added EAH. Concentrated EAH solutions were added to sterilized tubes containing Mueller Hinton medium (for the bacteria) or YPD (yeast extract, 15 g/L; peptone, 20 g/L; and glucose, 20 g/L) (for the yeasts) to reach final concentrations of 10^5, 5×10^4, 10^4, 5×10^3, 10^3, and 10^2 μg of EAH/mL of medium. Strain cultures in Mueller Hinton or YPD medium were diluted to give a final concentration in the test tube of $\sim 10^8$ colony-forming units (cfu)/mL. The tubes were incubated at 37 °C (for bacteria) or 25 °C (for yeasts) and checked after 24 h (4 days in *P. stipitis*) for the presence of turbidity. The MIC was determined as the lowest EAH concentration at which growth was prevented. Twenty microliters of media from the tubes in which no growth was observed was spotted onto Mueller Hinton or YPD agar plates, which were incubated at 37 °C (bacteria) or 25 °C (yeasts) and checked after 24 h (4 days in *P. stipitis*) for the presence of colonies. The MBC corresponded to the lowest EAH concentration at which fewer than five colonies were obtained.

Determination of the Antioxidant Activity. The β-carotene bleaching test was selected for antioxidant activity determination because it is carried out in an emulsion, a situation frequent in foods. On the other hand, it is generally agreed that the oxidation is initiated by free radical attack; therefore, assays to evaluate the radical scavenging activity are representative of the potential of a compound to retard oxidation. Among the radical scavenging assays, the one based on the utilization of DPPH was chosen due to its simplicity and worldwide acceptance for comparative purposes.

(a) β-Carotene Bleaching Method. The test was carried out following the spectrophotometric method of Miller (*35*), based on the ability of the different extracts to decrease the oxidative bleaching of β-carotene in a β-carotene/linoleic acid emulsion. A 2.0 mg sample of crystalline β-carotene was dissolved in 10 mL of chloroform, and 1 mL of this solution was pipetted into a round-bottom flask containing 20 mg of purified linoleic acid and 200 mg of Tween 40. After the removal of chloroform by evaporation, 50 mL of oxygenated, distilled water was added to the flask under vigorous stirring, and 5 mL aliquots of the aqueous emulsion formed were pipetted into test tubes containing 0.2 mL of ethanolic antioxidant solution. The absorbance readings at 470 nm against a control containing ethanol instead of EAH solution were recorded. The test and control tubes were stoppered and placed in a water bath at 50 °C, and subsequent readings were taken at regular intervals in a Beckman DU640 spectrophotometer until the carotene was decolored (~3 h). The antioxidant activity was measured by the antioxidant activity coefficient (AAC), which gives an estimate of the relative extent of oxidation in the presence of EAH with respect to the extent of the oxidation in their absence. The AAC was calculated as

$$\mathrm{AAC} = \frac{(A_{\mathrm{extract,120min}} - A_{\mathrm{control,120min}})}{(A_{\mathrm{control,0min}} - A_{\mathrm{control,120min}})} \times 1000$$

(b) α,α-Diphenyl-β-picrylhydrazyl (DPPH) Radical Scavenging Method. A minor modification of the method described by von Gadow et al. (*36*) was used. Two milliliters of a 6×10^{-5} M methanolic solution of DPPH was added to 50 μL of a methanolic solution of the antioxidant, and the decrease in absorbance (A) at 515 nm was recorded in a Beckman DU640 spectrophotometer for 16 min. The inhibition percentage (IP) of the DPPH radical was calculated as

$$\mathrm{IP} = \frac{(A_{t=0\mathrm{min}} - A_{t=16\mathrm{min}})}{A_{t=0\mathrm{min}}} \times 100$$

All tests and analyses were run in duplicate or in triplicate, and the average values are presented. Butylated hydroxyani-

Table 2. MIC[a] for Polyphenols Extracted from Hemicellulosic Hydrolysates against Bacteria and Yeasts

	extract concn (μg/mL)			
	Eucalyptus wood	barley bran	corn cobs	corn leaves
Salmonella sp. (from spoiled food)	5×10^3	5×10^3	10^4	10^4
Streptococcus bovis (CECT 213)	5×10^3	5×10^3	10^4	10^4
Enterococcus faecalis (from uroculture)	10^3	5×10^3	10^4	10^4
Escherichia coli (CECT 434)	5×10^3	10^4	10^4	10^4
Staphylococcus aureus (CECT 59)	10^2	5×10^3	5×10^3	10^3
Saccharomyces cerevisiae (from grape juice)	5×10^3	10^4	5×10^3	5×10^3
Pichia stipitis (CBS 5773)	5×10^{3b}	5×10^{3b}	5×10^{3b}	10^{3c}

[a] MIC: lowest concentration causing complete growth inhibition after 24 h of incubation (except for *P. stipitis*). [b] Incubation time = 4 days. [c] Incubation time = 2 days.

sole (BHA) and butylated hydorxytoluene (BHT) (Sigma Chemical Co., St. Louis, MO) were used as reference antioxidants.

RESULTS AND DISCUSSION

Extraction Yield. The lignocellulosic raw materials considered in this study were treated with sulfuric acid under the conditions cited in the previous section to obtain hydrolysates potentially useful as fermentation media (for example, for xylose bioconversion into ethanol or into xylitol). Table 1 lists data on the composition of both raw materials and hydrolysates. The extracts from acid hydrolysates EAH contain some compounds belonging to the extract fraction, which are solubilized in the prehydrolysis treatment, as well as aromatic compounds belonging to the acid soluble lignin fraction. The phenolic content of the hydrolysates was qualitatively measured with the analytical method proposed for soluble lignin (*37*) based on the determination of absorbances at 279 nm. In the solvent extraction of hydrolysates with ethyl acetate, the highest extraction yield (0.042 g of EAH/g of raw material) corresponded to barley bran, in comparison with 0.030 g of EAH/g of *Eucalyptus* wood, 0.021 g of EAH/g of corn cobs, and 0.022 g of EAH/g of corn leaves.

Antimicrobial Activity. All of the EAH tested in this work showed both bacteriostatic and batericide activities. Table 2 lists the MIC values against some bacteria and yeasts determined for EAH from *Eucalyptus* wood, barley bran, corn cobs, and corn leaves. The higher inhibitory action on microbial growth corresponded to EAH from *Eucalyptus* wood with MIC values ranging from 10^2 to 5×10^3 μg/mL, in comparison with 10^3-10^4 μg/mL for EAH from barley bran, corn cobs, and corn leaves. The maximum inhibitory activity was observed for *Eucalyptus* wood hydrolysates against *Staphylococcus aureus*, which was the most sensitive microorganism toward the assayed compounds. *St. aureus* was also the most sensitive against Egyptian propolis extracts (*38*), which showed MIC values in the range of $10^2-2.4 \times 10^4$ μg/mL. All of the EAH tested in this work caused similar growth inhibition on yeast. In comparative terms, a slightly higher inhibition of *P. stipitis* growth was determined for EAH from corn leaves, the lower effect corresponding to *Sac. cerevisiae* growth inhibition by EAH from barley bran hydrolysates.

Table 3. MBC after 1 or 4 Days[a] for Each of the Extracts from Hemicellulosic Hydrolysates

	extract concn (μg/mL)			
	Eucalyptus wood	barley bran	corn cobs	corn leaves
Salmonella sp. (from spoiled food)	10^4	10^4	5×10^4	$(0.5-1) \times 10^5$
Streptococcus bovis (CECT 213)	10^5	10^4	10^4	$(0.5-1) \times 10^5$
Enterococcus faecalis (from uroculture)	10^4	5×10^4	5×10^4	$(0.5-1) \times 10^5$
Escherichia coli (CECT 434)	5×10^{3b}	10^{4b}	5×10^4	10^{4b}
Staphylococcus aureus (CECT 59)	10^3	10^4	10^4	5×10^3
Saccharomyces cerevisiae (from grape juice)	5×10^4	10^{4b}	5×10^4	10^4
Pichia stipitis (CBS 5773)	5×10^3	5×10^3	10^4	10^4

[a] The data for *P. stipitis* were obtained after 4 days of incubation and for the rest of the microorganisms after 1 day. [b] MBC = MIC.

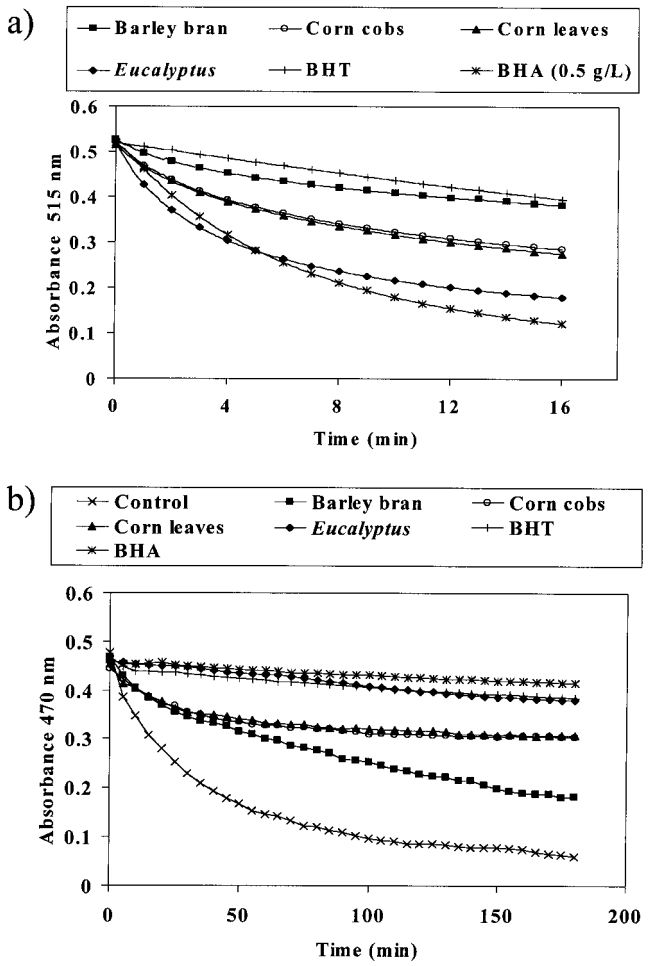

Figure 1. Course of antioxidant assays used in this work: (a) DPPH radical scavenging activity; (b) β-carotene bleaching inhibiting activity. Experiments were carried out with EAH or antioxidant concentration = 1 g/L of alcoholic solution (except in DPPH assay, in which the concentration of standard was 0.5 g of BHA/L).

In comparative terms, the MIC values determined for EAH were higher than those reported for nyasol isolated from the medicinal herb *Anemarrhena asphodeloides* (12.5−200 μg/mL) (*39*) or for the antibiotic substances produced by *Micromonospora coerulea* (which ranged from 0.3 μg/mL for *S. cerevisiae* to >50 μg/mL for *Cylindrocarpon destructans*, *Fusarium oxysporum*, *Candida albicans*, or *Bacillus subtilis*) (*40*). MIC values were

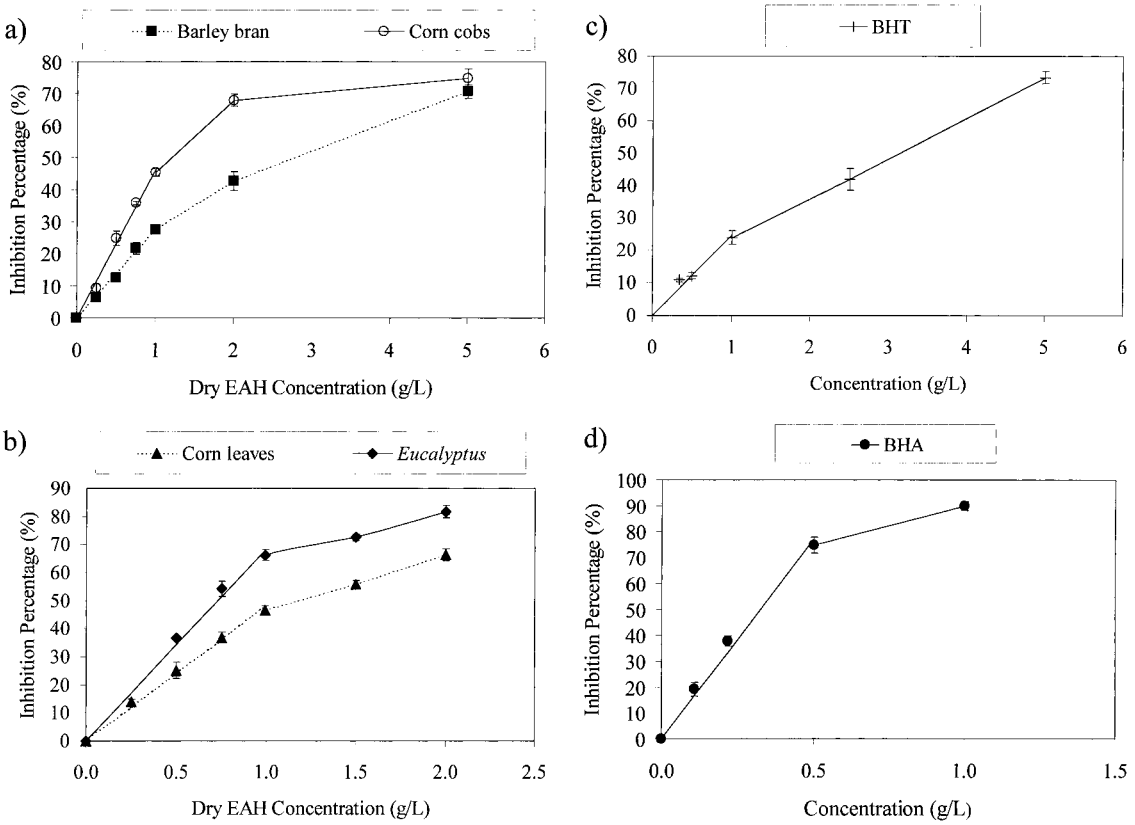

Figure 2. Effects of the concentrations of dry extracts from EAH and synthetic antioxidant on the DPPH radical scavenging activity. Concentration was measured as grams of dry EAH per liter of methanolic solution.

also higher than those of liquid smoke, which had values ranging from <2 mg/L for the Gram-negative bacteria *Vibrio vulnificus* to >80 mg/L for bacteria and yeasts (*24*). The phenolic compounds present in liquid smoke inhibited the growth of bacteria by prolonging the *lag* phase (*19*), the inhibition being related to the concentration of phenols (*24*).

On the other hand, the antimicrobial activity of the products tested was higher than the results reported for extracts from potato peel against *E. coli* and *Salmonella*, with MIC values of 10^5 μg/mL. These compounds did not show any effect on *St. aureus*, *Sac. cerevisiae*, or *Aspergillus niger* (*20*). The growth of bacteria and yeasts can be inhibited by 10 mg/mL of essential oils from mint and cinnamon. Oil from the latter feedstock was also inhibitory for most yeasts at a concentration of 1 mg/mL. Clove oil concentrations of 10 and 100 mg/mL were required for causing growth inhibition of yeasts and bacteria, respectively (*23*). In this study, the MBC values determined were 1−20 times higher than those required to inhibit growth (see Table 3).

Antioxidant Activity. Figure 1a shows the decrease in absorbance due to the scavenging of the DPPH radical by antioxidants, whereas Figure 1b shows the decrease in absorbance during the coupled oxidation of β-carotene and linoleic acid. The curves shown for the four types of EAH and BHT correspond to a concentration of 1 g/L, the concentrations of BHA being 0.5 g/L for data in Figure 1a and 1 g/L for EAH, BHT, and BHA in Figure 1b.

Figure 2 shows the DPPH radical scavenging activity of EAH from corn cobs and leaves, *Eucalyptus*, and barley bran as a function of the correspondent concentration in the methanolic solution. For comparative purposes, the same figure shows the effects of BHA and BHT. A linear effect of EAH concentration on the scavening activity was observed for the four substrates up to concentrations near 1 g/L. The inhibition percentage achieved in assays with methanolic solution containing 1 g of EAH/L ranged from 27.50 for barley bran (the weakest antioxidant) to 68.72 for *Eucalyptus* wood (the strongest one). The latter value corresponded to 44.5% of the BHA activity, whereas the four EAH showed higher DPPH radical scavenging activities than BHT (24.69). In related studies, Duh et al. (*41*) reported an almost linear dependence of the DPPH scavenging effect on the concentration of methanolic extracts from mung bean hulls in a given concentration range, whereas Yen and Wu (*42*) reported optimal DPPH radical scavenging activity (≈80% scavenging effect) of methanolic extracts of *Ganoderma* fruits at 500 ppm. The same optimal concentration was determined for chelating Fe^{2+} ions, but 10 times more substrate was needed for reaching maximum hydroxyl radical scavenging activity (in the range of 0.7−2.8 g/L, depending on the raw material considered). These values were remarkably lower than the optimal concentrations of pure phenolic compounds found in apple pomace (phloridzin and epicatechin dimer) reported by Lu and Foo (*43*). Ohta et al. (*15*) reported results on a related study dealing with the DPPH radical scavenging activity of fractions from acid-hydrolyzed corn bran isolated by chromatography, but the results are difficult to compare because literature assays were carried out at different reaction times. The DPPH scavenging activities found in this study for EAH from *Eucalyptus* wood are comparable to those reported for oolong and green teas using an extract to DPPH/mass ratio of 0.52 (*44*). Related results (*45*) have been obtained with methanolic extracts of peanut hull (89.3% activity at a concentration

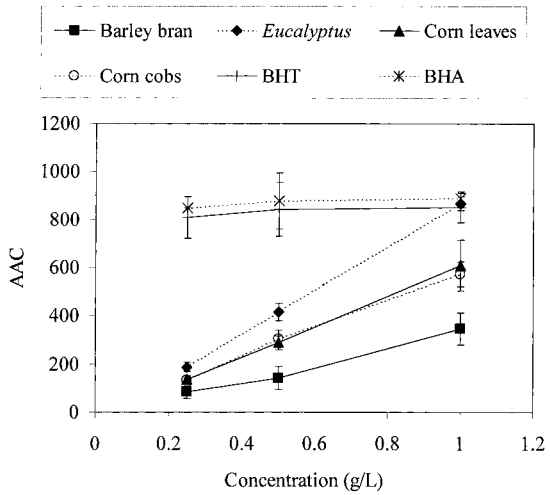

Figure 3. Effects of the concentrations of dry extracts from EAH and synthetic antioxidant concentration on the β-carotene bleaching inhibiting activity. Concentration was measured as grams of dry extract per liter of ethanolic solution.

of 1.5 mg/mL). Duh (*46*), dealing with water extracts of burdock roots, reported 40% DPPH scavenging effect at 5 g/L and 80% activity at 17.5 g/L in media containing 0.2 mM DPPH. Methanolic extracts of Yacon roots presented 93% DPPH scavenging activity using 2 mL of 50 g of extract/L and 2 mL of 2×10^{-4} mol of DPPH/L (*47*). DPPH scavenging activities >90% were reported for buckwheat groats, the optimal concentration ranging from 0.1 g/L for acetone and ethyl acetate extracts to 0.3 g/L for methanolic extracts (*48*). In the present study, a linear interrelationship between antioxidant activity and EAH concentration was observed at concentrations below these values.

The effect of antioxidant concentration on the AAC is shown in Figure 3. The values of the AAC determined in this work for EAH from barley bran at a concentration level of 1 g/L are similar to the results reported by von Gadow et al. (*49*) for the same concentration of tea phenols. According to experimental data, the same values of AAC can be obtained with 0.5 g/L of EAH from corn cob or leaves or with 0.4 g/L of EAH from *Eucalyptus* wood. It can be noted that the maximum values for BHA and BHT were reached at lower concentrations. A related situation has been reported for the effects of BHT on the oxidation of lipids in liposome membranes (*50*), for which the maximum inhibition corresponded to a concentration of 0.02 mM/L (5.6 mg/L).

The AAC values of EAH from *Eucalyptus* wood, corn cobs, and corn leaves determined in media containing 1 g/L were similar to the results reported by von Gadow et al. (*44*) for different teas at a concentration of 5 g/L, which varied in the range of 522−695. In a related study, AAC values in the range of 548−807 were obtained for 5 g of rooibos tea extracts/L depending on the extraction time (*49*). AAC values have also been determined for flavonoid glycosides; for example, in media containing 0.2 g/L of the considered compounds, the results for AAC were as follows: phloridzin, 283; quercetin, 717; and procyanidins (epicatechin monomers to tetramer procyanidins), 482 (*43*). Comparatively lower values of AAC (125−190) have been determined for the methanolic extracts of lupin seed flour extracts, depending on the extraction temperature (which also conditioned the extraction yield) (*51*).

In conclusion, the detoxification of hemicellulosic hydrolysates by solvent extraction leads to phenolic extracts with potential application as food additives. Even if further assessment of their acceptability and safety for food-related application is necessary, the results presented in this study confirm their potential as antimicrobial and/or antioxidant agents.

ABBREVIATIONS USED

EAH, extracts from acid hydrolysates; MIC, minimum inhibitory concentration; MBC, minimum bactericide concentration; LSR, liquid to solid ratio; DPPH, α,α-diphenyl-β-picrylhydrazyl; BHT, butylated hydroxytoluene; BHA, butylated hydroxyanisole; PG, propyl gallate; TBHQ, *tert*-butylhydroquinone; AAC, antioxidant activity coefficient.

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LITERATURE CITED

(1) Lora, J. H.; Wayman, M. Delignification of hardwoods by autohydrolysis and extraction. *TAPPI J*. **1978**, *61* (6), 47−50.
(2) Shahidi, F.; Naczk, N. In *Food Phenolics, Sources Chemistry Effects Applications*; Tecnomic Publishing: Lancaster, PA, 1995.
(3) Guillén, M. D.; Ibargoitia, M. L. New components with potential antioxidant and organoleptic properties, detected for the first time in liquid smoke flavoring preparations. *J. Agric. Food Chem*. **1998**, *46*, 1276−1285.
(4) Parajó, J. C.; Domínguez, H.; Domínguez, J. M. Xylitol production from *Eucalyptus* wood hydrolysates extracted with organic solvents. *Process Biochem*. **1997**, *32*, 599−604.
(5) Cruz, J. M.; Domínguez, J. M.; Domínguez, H.; Parajó, J. C. Solvent extraction of hemicellulosic wood hydrolysates: a procedure useful for obtaining both detoxified fermentation media and polyphenols with antioxidant activity. *Food Chem*. **1999**, *67*, 147−153.
(6) Hattori, M.; Yamaji-Tsukamoto, K.; Kimagai, H.; Feng, Y.; Takahashi, K. Antioxidative activity of soluble elastin peptides. *J. Agric. Food Chem*. **1998**, *46*, 2167−2170.
(7) Rodríguez de Sotillo, D.; Hadley, M.; Holm, E. T. Phenolics in aqueous potato peel extract: extraction, identification and degradation. *J. Food Sci*. **1994**, *59*, 649−651.
(8) Rodríguez de Sotillo, D.; Hadley, M.; Holm, E. T. Potato peel waste: stability and antioxidant activity of a freeze-dried extract. *J. Food Sci*. **1994**, *59*, 1031−1033.
(9) Visioli, F.; Romani, A.; Mulinacci, N.; Zarini, S.; Conte, D.; Vincieri, F. F.; Galli, C. Antioxidant and other biological activities of olive mill waste waters. *J. Agric. Food Chem*. **1999**, *47*, 3397−3401.
(10) Larrauri, J. A.; Rupérez, P.; Saura-Calixto, F. Effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape pomace peels. *J. Agric. Food Chem*. **1997**, *44*, 1390−1393.
(11) Larrauri, J. A.; Sánchez-Moreno, C.; Saura-Calixto, F. Effect of temperature on the free radical scavenging capacity of extracts from red and white grape pomace peels. *J. Agric. Food Chem*. **1998**, *46*, 2694−2697.
(12) Zandi, P.; Gordon, M. H. Antioxidant activity of extracts from old tea leaves. *Food Chem*. **1999**, *64*, 285−288.
(13) Azizah, A. H.; Nik Ruslawati, N. M.; Swee Tee, T. Extraction and characterization of antioxidant from cocoa by-products. *Food Chem*. **1999**, *64*, 199−202.
(14) Moure, A.; Cruz, J. M.; Franco, D.; Domínguez, J. M.; Sineiro, J.; Domínguez, H.; Núñez, M. J.; Parajó, J. C. Natural antioxidants from residual sources, a review. *Food Chem*. **2001**, *72*, 145−171.

(15) Ohta, T.; Yamasaki, S.; Egashira, Y.; Sanada, H. Antioxidative activity of corn bran hemicellulose fragments. *J. Agric. Food Chem*. **1994**, *42*, 653−656.
(16) Lehtinen, P.; Laakso, S. Effect of extraction conditions on the recovery and potency of antioxidants in oat fiber. *J. Agric. Food Chem*. **1998**, *46*, 4842−4845.
(17) Camire, M. E.; Dougherty, M. P. Added phenolic compounds enhance stability in extruded corn. *J. Food Sci*. **1998**, *63*, 516−518.
(18) Kanatt, S. R.; Paul, P.; D'Souza, S. F.; Thomas, P. Lipid peroxidation in chicken meat during chilled storage as affected by antioxidants combined with low-dose gamma irradiation. *J. Food Sci*. **1998**, *63*, 198−200.
(19) Estrada-Muñoz, R.; Boyle, E. A. E.; Marsden, J. L. Liquid smoke effects on *Escherichia coli* 0157:H7, and its antioxidant properties in beef products. *J. Food Sci*. **1998**, *63*, 150−153.
(20) Rodríguez de Sotillo, D.; Hadley, M.; Wolf-Hall, C. Potato peel extract a nonmutagenic antioxidant with potential antimicrobial activity. *J. Food Sci*. **1998**, *63*, 907−910.
(21) Abram, V.; Donko, M. Tentative identification of polyphenols in *Sempervivum tectorum* and assessment of the antimicrobial activity of *Sempervivum* L. *J. Agric. Food Chem*. **1999**, *47*, 485−489.
(22) Ejechi, B. O.; Souzey, J. A.; Akpomedaye, D. E. Microbial stability of mango (*Mangifera indica* L.) juice preserved by combined application of mild heat and extracts of two tropical spices. *J. Food Prot*. **1998**, *61*, 725−727.
(23) Leite, H. F.; Ferreira de S., S. J.; García-Cruz, C. H.; Vinturim, T. M.; Lima D. A. Determinaçao da actividade antimicrobiana "in vitro" de quarto oleos essenciais de condimentos e especiarias. *B. CEPPA, Curitiba* **1999**, *17*, 11−20.
(24) Suñen, E. Minimum inhibitory concentration of smoke wood extracts against spoilage and pathogenic microorganisms associated with foods. *Lett. Appl. Microbiol*. **1998**, *27*, 45−48.
(25) Cerruti, P.; Alzamora, S. M.; Vidales, S. L. Vanillin as an antimicrobial for producing shelf-stable strawberry puree. *J. Food Sci*. **1997**, *62*, 608−610.
(26) Ikken, Y.; Morales, P.; Martínez, A.; Marín, M. L.; Haza, A. I.; Cambero, M. I. Antimutagenic effect of fruit and vegetable ethanolic extracts against N-nitrosamines evaluated by the Ames test. *J. Agric. Food Chem*. **1999**, *47*, 3257−3264.
(27) Noguchi, Y.; Fukuda, K.; Matsushima, A.; Haishi, D.; Hiroto, M.; Kodera, Y.; Nishimura, H.; Inada, Y. Inhibition of Df-protease associated with allergic diseases by polyphenol. *J. Agric. Food Chem*. **1999**, *47*, 2969−2972.
(28) Carrol, K. K.; Kurowska, E. M.; Guthrie, N. Use of citrus limonoids and flavonoids as well as tocotrienols for the treatment of cancer. Int. Patent WO9915167, 1999.
(29) Kawaii, S.; Tomono, Y.; Katase, E.; Ogawa, K.; Yano, M. HL-60 differentiating activity and flavonoid content of the readily extractable fraction prepared from *Citrus* juices. *J. Agric. Food Chem*. **1999**, *47*, 128−135.
(30) Maeda-Yamamoto, M.; Kawahara, H.; Tahara, N.; Tsuji, K.; Hara, Y.; Isemura, M. Effects of tea polyphenols on the invasion and matrix metalloproteinases activities of human fibrosarcoma HT1080 cells. *J. Agric. Food Chem*. **1999**, *47*, 2350−2354.
(31) Miyazawa, M.; Okuno, Y.; Nakamura, S.; Kosaka, H. Antimutagenic activity of flavonoids from *Pogostemon cablin*. *J. Agric. Food Chem*. **2000**, *48*, 642−647.
(32) Vilegas, W.; Sanomimiya, M.; Rastrelli, L.; Pizza, C. Isolation and structure elucidation of two new flavonoid glycosides from the infusion of *Maytenus aquifolium* leaves. Evaluation of the antiulcer activity of the infusion. *J. Agric. Food Chem*. **1999**, *47*, 403−406.
(33) Tanabe, M.; Kanda, T.; Yanagida, A. Fruit polyphenol; process for production thereof; and antioxidant, hypotensive agent, antimutagenic agent, antiallergenic agent and anticariogenic agent, each comprising said polyphenol. Eur. Patent EP0657169, 1995.
(34) Vázquez, G.; Lage, M. A.; Parajó, J. C.; Vázque, G. Transformación de materiales lignocelulósicos: Composición, fraccionamiento y aprovechamiento. *Rev. Agroquim. Tecnol. Aliment*. **1991**, *31* (2), 143−164.
(35) Miller, H. E. A simplified method for the evaluation of antioxidants. *J. Am. Oil Chem. Soc*. **1971**, *45*, 91.
(36) von Gadow, A.; Joubert, E.; Hansmann, C. F. Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (*Aspalathus linearis*), α-tocopherol, BHT and BHA. *J. Agric. Food Chem*. **1997**, *45*, 632−638.
(37) Maekawa, E.; Ichizawa, T.; Koshijima, T. An evaluation of the acid-soluble lignin determination in analyses of lignin by the sulfuric acid method. *J. Wood Chem. Technol*. **1989**, *9*, 549−567.
(38) Ahmed, F. H.; Salem, H. M.; Abdel-Rahim, E. A.; Badawi, A. M. Antimicrobial activity of bee glue (Propolis) extracts. *Egypt. J. Microbiol*. **1997**, *31*, 423−435.
(39) Iida, Y.; Oh, K.-B.; Saito, M.; Matsuoka, H.; Kurata, H.; Natsume, M.; Abe, H. Detection of antifungal activity in *Anemarrhena asphodeloides* by sensitive BCT method and isolation of its active compound. *J. Agric. Food Chem*. **1999**, *47*, 584−587.
(40) Kim, B. S.; Moon, S. S.; Hwang, B. K. Isolation, antifungal activity, and structure elucidation of the glutarimide antibiotic, streptimidone, produced by *Micromonospora coerulea*. *J. Agric. Food Chem*. **1999**, *47*, 3372−3380.
(41) Duh, P.-D.; Yen, W. J.; Du, P.-C.; Yen, G.-C. Antioxidant activity of mung bean hulls. *J. Am. Oil Chem. Soc*. **1997**, *74*, 1059−1063.
(42) Yen, G.-C.; Wu, J.-Y. Antioxidant and radical scavenging properties of extracts from *Ganoderma tsugae*. *Food Chem*. **1999**, *65*, 375−379.
(43) Lu, Y.; Foo, L. Y. Antioxidant and radical scavenging activities of polyphenols from apple pomace. *Food Chem*. **2000**, *68*, 81−85.
(44) von Gadow, A.; Joubert, E.; Hansmann, C. F. Comparison of the antioxidant activity of rooibos tea (*Aspalathus linearis*) with green, oolong and black tea. *Food Chem*. **1997**, *60*, 73−77.
(45) Yen, G.-C.; Duh, P. D. Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species. *J. Agric. Food Chem*. **1994**, *42*, 629−632.
(46) Duh, P.-D. Antioxidant activity of Burdock (*Arctium lappa Linné*), its scavenging effect on free-radical and active oxygen. *J. Am. Oil Chem. Soc*. **1998**, *75*, 455−461.
(47) Yan, X.; Suzuki, M.; Ohnishi-Kameyama, M.; Sada, Y.; Nakanishi, T.; Nagata, T. Extraction and identification of antioxidants in the roots of yacon (*Smallanthus sonchifolius*). *J. Agric. Food Chem*. **1999**, *47*, 4711−4713.
(48) Pryzbylski, R.; Lee, Y. C.; Eskin, N. A. M. Antioxidant and radical-scavenging activities of buckwheat seed components. *J. Am. Oil Chem. Soc*. **1998**, *75*, 1595−1601.
(49) von Gadow, A.; Joubert, E.; Hansmann, C. F. Effect of extraction and additional heating on the antioxidant activity of rooibos tea (*Aspalathus linearis*) extracts. *J. Agric. Food Chem*. **1997**, *45*, 1370−1374.
(50) Gabrielska, J.; Oszmianski, J.; Lamer-Zarawska, E. Protective effect of plant flavonoids on the oxidation of lecithin liposomes. *Pharmazie* **1997**, *52*, 2−3.
(51) Tsaliki, E.; Lagouri, V.; Doxastakis, G. Evaluation of the antioxidant activity of lupin seed flour and derivatives (*Lupinus albus* ssp. *Graecus*). *Food Chem*. **1999**, *65*, 71−75.

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