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Purified Phenolics from Hydrothermal Treatments of Biomass: Ability To Protect Sunflower Bulk Oil and Model Food Emulsions from Oxidation

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ABSTRACT: The phenolic fractions released during hydrothermal treatment of selected feedstocks (corn cobs, eucalypt wood chips, almond shells, chestnut burs, and white grape pomace) were selectively recovered by extraction with ethyl acetate and washed with ethanol/water solutions. The crude extracts were purified by a relatively simple adsorption technique using a commercial polymeric, nonionic resin. Utilization of 96% ethanol as eluting agent resulted in 47.0−72.6% phenolic desorption, yielding refined products containing 49−60% w/w phenolics (corresponding to 30−58% enrichment with respect to the crude extracts). The refined extracts produced from grape pomace and from chestnut burs were suitable for protecting bulk oil and oil-in-water and water-in-oil emulsions. A synergistic action with bovine serum albumin in the emulsions was observed.

KEYWORDS: phenolic compounds, polymeric resins, oxidative stability, emulsions

INTRODUCTION

Autohydrolysis processing, based on the utilization of hot, compressed water, has been studied as an extraction method for selected feedstocks (including wood and byproducts or wastes from agriculture or industry). Autohydrolysis liquors obtained under suitable operational conditions contain xylooligomers with prebiotic activity, monosaccharides, sugar dehydration compounds, and nonsaccharide products (mainly derived from extractives and acid-soluble lignin). Ethyl acetate extraction of autohydrolysis liquors led to a crude product containing 30% w/w phenolics (mainly benzoic and cinnamic acids), which showed a radical scavenging capacity comparable to that of synthetic antioxidants.[1,2] Crude extracts can be refined to obtain concentrates with enhanced purity and antioxidant activity, suitable for specific food applications.

Adsorption is a scalable separation technology, enabling the recovery of adsorbed target products upon regeneration. In the past decade, resins have been widely used for the separation and enrichment of a variety of products from a number of raw materials.[3−5] In a previous paper, we reported on the adsorption and desorption of phenolics from barley husk hydrolysates.[1]

The protective action of antioxidants has been frequently studied in oils, model foods, foods, and cosmetic emulsions. The influence of proteins on antioxidant capacity has been scarcely studied, despite their potential to affect the activity of antioxidants in food or cosmetic applications. Bovine serum albumin (BSA) enhanced the antioxidant effect of virgin olive oil phenolics in oil-in-water emulsions[6] and the ability of chlorogenic acid to inhibit oxidation of human low-density lipoproteins (LDL).[7]

This paper deals with the following topics: (i) assessment of the adsorption and desorption of phenolic compounds from hydrothermal liquors onto a selected commercial polymeric, nonionic resin, with the aim of developing an efficient method for obtaining purified extracts; (ii) evaluation of the antioxidant activity and phenolic profile of these extracts; and (iii) influence of BSA on the effectiveness of selected products to protect both sunflower bulk oil and emulsified lipid substrates from oxidation.

MATERIALS AND METHODS

Feedstocks. Corn (*Zea mays*) cobs were collected from a local farm, eucalypt (*Eucalyptus globulus*) wood chips were obtained from a local pulp mill (ENCE, Pontevedra), almond (*Prunus amygdalus*) shells were kindly provided by Borges S.A. (Tárrega, Lleida, Spain), chestnut (*Castanea sativa*) burs were collected in Ribeira Sacra (Ourense, Spain), and white grape (*Vitis vinifera*) pomace (the waste solid residue after spirit distillation stage in wineries) was kindly supplied by Cooperativa Vitivinícola do Ribeiro (Ourense, Spain).

Adsorbent. Sepabeads SP700, a nonionic PS-DVB copolymer, supplied by Resindion SRL (Mitsubishi Chemical Corp.), has been selected due to its adsorption capacity under different conditions.[1] The data provided by the manufacturer include pore volume (2.2 mL/g), specific surface area (1200 m^2/g), mean particle size (450 μm), average pore radius (85 Å), and moisture content (65% w/w). The resin was mixed with 2 bed volumes (BV) of methanol, shaken for 1 min, and stirred (175 rpm) at 25 °C for 15 min. The resin was rinsed with 5 BV of deionized water before use. To check the moisture content of the rinsed resins, a portion of resins was used to determine the moisture content by oven-drying at 100 °C until constant weight.

Preparation of Crude Extracts. Extracts of corn cobs (CCE), eucalypt wood (EWE), almond shells (ASE), chestnut burs (CBE), and grape pomace (GPE) were obtained by nonisothermal autohydrolysis,

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extraction with ethyl acetate, and washing with ethanol/water as described previously.[2] Extracts were freeze-dried and resuspended in water at a concentration of 15 g gallic acid equivalents (GAE)/L in all solutions (see below for details on the Analytical Methods).

Static Adsorption Test. Batch adsorption tests of phenolics were performed by contacting 20 g of wet resin with aqueous solutions of extract (relation moist solid/liquid, 3 g/5 mL) with shaking in Erlenmeyer flasks (175 rpm) at 25 °C for 20 min.

Dynamic Adsorption and Desorption Tests. Dynamic adsorption and desorption were carried out on a glass column (300 mm × 11 mm), wet-packed with 20 g of wet SP700 resin (BV, 44 mL) coupled to a BioLogic DuoFlow system (BIO-RAD). Sample solutions flowed through the glass column at a flow rate of 1 mL/min, and the concentration of phenolic compounds in the effluents collected at 3 mL interval was analyzed. Unadsorbed compounds were removed by washing with 140 mL of deionized water (flow rate = 1 mL/min). Desorption was performed by elution with 100 mL of 96% ethanol (pH 6) at 35 °C, at a reverse flow rate of 1 mL/min. Eluent absorbance was recorded online using a QuadTec UV−vis detector, and fractions were collected at fixed time intervals using a BioFrac fraction collector. The desorbed extracts were freeze-dried, resuspended in water, and analyzed by high-performance liquid chromatography (HPLC) on an Agilent 1100 series HPLC. Resin regeneration was carried out by contacting the resin overnight with 1 M NaOH, followed by further washing with deionized water.

Calculation of Adsorption Capacity and Adsorption and Desorption Ratios. The concentration of total solids and total phenolics in the liquid phases before and after adsorption and desorption was analyzed gravimetrically and by the Folin−Ciocalteu method, respectively. The capacity of adsorption and the adsorption and desorption ratios were calculated as follows.

adsorption capacity at equilibrium (mg/g resin), q_e

$$q_e = \frac{(C_0 - C_e) \times V_i}{W}$$

adsorption ratio (percentage of total solute being adsorbed at adsorption equilibrium), E

$$E = \frac{(C_0 - C_e)}{C_0} \times 100\%$$

where C_0 and C_e are the initial and equilibrium concentrations of solutes in the liquid phase (mg/mL), V_i is the volume of the feed solution (mL), and W is the dry weight of resin (g).

desorption ratio (percentage of total desorbed substrate with respect to the mass present in the loaded resin), D

$$D = \frac{C_d \times V_d}{(C_0 - C_e) \times V_i} \times 100\%$$

where C_d is the concentration of the solutes of the desorption effluent (mg/mL), V_d is the volume of the desorption solution (mL), and C_0, C_e, and V_i are as above.

Analytical Methods. *Spectrophotometric Determination of Phenols.* The total phenolic content was determined by using the Folin−Ciocalteu colorimetric method,[8] and expressed as GAE.

Antioxidant Activity. (a) α,α-Diphenyl-β-picrylhydrazyl (DPPH) Radical Scavenging. The DPPH assay was carried out according to the method of von Gadow et al.[9] 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 at 515 nm after 16 min was recorded in an Agilent 8453E UV−visible spectrophotometer. Equivalent concentration (EC_{50}) was calculated as the extract concentration required to quench 50% of the initial DPPH radical after 16 min. Assays were performed in triplicate, and data were statistically analyzed.

(b) Trolox Equivalent Antioxidant Capacity (TEAC). This assay, proposed by Re et al.,[10] is based on the scavenging of ABTS radical 2, 2′-azinobis(3-ethylbenzothiazoline-6-sulfonate). ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate (final concentration). The mixture was allowed to stand in the dark at room temperature for 12−16 h before use and then diluted with phosphate buffer saline (PBS) (pH 7.4) to an absorbance of 0.70 at 734 nm and equilibrated at 30 °C. After the addition of 1.0 mL of diluted $ABTS^{\bullet+}$ solution to 10 μL of antioxidant compounds or Trolox standards in ethanol or PBS, the absorbance readings were recorded for 6 min. Appropriate solvent blanks were run in each assay, and the percentage of absorbance inhibition at 734 nm was calculated as a function of the concentration of extracts and Trolox. The extract concentration leading to a 50% drop of the initial ABTS concentration was defined as EC_{50}. Assays were performed in triplicate, and data were statistically analyzed.

(c) Ferric Reducing Antioxidant Power (FRAP). The method was originally developed by Benzie and Strain[11] to measure the ferric reducing ability of plasma. The method is based on the ability of a compound to reduce Fe(III), which is relevant due to the role of this cation as an initiator of oxidation processes in organisms. The reagent was made by mixing 25 mL of 300 mmol/L acetate buffer (pH 3.6) and 2.5 mL of a 10 mmol/L 2,4,6-tripyridyl-*s*-triazine (TPTZ) solution in 40 mmol/L HCl and 20 mmol/L $FeCl_3 \cdot 6H_2O$ in distilled water. The reagent was always freshly prepared and used as a blank. Aqueous solutions of ascorbic acid and $FeSO_4 \cdot 7H_2O$ were used for calibration. Samples (100 μL) were mixed with 3 mL of the reagent, and the absorbance was monitored at 593 nm. Assays were performed in triplicate, and data were statistically analyzed.

(d) Reducing Power. The reducing power of samples was determined according to the method proposed by Oyaizu,[12] based on the chemical reduction of Fe(III) into Fe(II). One milliliter of extract (dissolved in methanol) was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1.0% w/v potassium ferricyanide, and the mixture was incubated at 50 °C for 30 min. After the addition of 2.5 mL of 10% w/v trichloroacetic acid, the mixture was centrifuged, and the supernatant (2.5 mL) was mixed with water (2.5 mL) and 0.5 mL of 0.1% w/v ferric chloride before the absorbance was determined at 700 nm. Results are expressed as ascorbic acid equivalents. Assays were performed in triplicate, and data were statistically analyzed.

(e) β-Carotene Bleaching Method. The spectrophotometric method of Miller,[13] based on the ability of the different extracts to decrease oxidative losses of β-carotene in a β-carotene/linoleic acid emulsion, was used. The oxidative bleaching of β-carotene in a β-carotene/linoleic acid emulsion was measured using the following procedure: 1 mL of a solution prepared with a 2.0 mg sample of crystalline β-carotene in 10 mL of chloroform was pipetted into a flask containing 20 mg of purified linoleic acid and 200 mg of Tween 40. After removal of chloroform by evaporation, 50 mL of oxygenated, distilled water was added to the flask with vigorous stirring, and 5 mL aliquots of the emulsion formed were pipetted into test tubes containing 0.2 mL of ethanolic antioxidant solution. The test and control (ethanol) tubes were stoppered and placed in a water bath at 50 °C. Absorbance readings at 470 nm were taken at regular intervals until the β-carotene was decolorized. Assays were performed in triplicate, and data were statistically analyzed. The antioxidant activity was quantified by the antioxidant activity coefficient (AAC), calculated as

$$\mathrm{AAC} = \frac{(\text{abs of extract}_{120\text{min}} - \text{abs of control}_{120\text{min}})}{(\text{abs of control}_{0\text{min}} - \text{abs of control}_{120\text{min}})} \times 1000$$

(f) Oxygen Radical Absorbance Capacity (ORAC-Fluorescein) Assay. The method proposed by Dávalos et al.[14] was used as follows: The reaction was carried out in 75 mM phosphate buffer (pH 7.4), and

Table 1. Adsorption Yield and Adsorption Capacities onto SP700 Resin

material	adsorbed mass (%)	phenolic adsorption (%)	adsorption capacity, q (mg GAE/g dry resin)
CCE	74.3	97.3	69.7
EWE	84.0	97.8	70.2
ASE	75.9	99.0	71.3
CBE	73.3	90.8	64.8
GPE	72.9	93.7	67.1

the final reaction mixture was 200 μL. The mixture of antioxidant (20 μL) and fluorescein (120 μL; 70 nM, final concentration) was preincubated for 15 min at 37 °C. 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (AAPH) solution (60 μL; 12 mM final concentration) was added rapidly. Fluorescence was recorded every minute for 120 min (excitation wavelength, 485 nm; emission wavelength, 520 nm) in an F-2500 fluorescence spectrophotometer. Results were calculated on the basis of the differences in areas under the fluorescein decay curve between the blank and the sample and are reported as Trolox equivalents using a standard curve (2−40 μM Trolox). Assays were performed in triplicate, and data were statistically analyzed.

Antioxidant Activity in Food Emulsions. *(a) Removal of Tocopherols from Sunflower Oil.* Tocopherols were removed from sunflower oil by column chromatography using activated alumina, as described by Yoshida.[15]

(b) Emulsion Samples. Oil-in-water emulsions (OW, 30% w/w oil) were prepared by dissolving Tween-20 (1% w/w) in acetate buffer (0.1 M, pH 5.4), either with or without BSA (0.2% w/w) and chestnut bur or grape pomace purified extracts (denoted CBPE and GPPE, respectively) (60 mg GAE/kg emulsion). Water-in-oil emulsions (WO, 20% w/w water) were prepared by dissolving Dimodan HR distilled monoglyceride (1% w/w) in acetate buffer (0.1 M, pH 5.4), either with or without BSA (0.2% w/w) and CBPE or GPPE extracts (containing 60 mg GAE/kg emulsion). Emulsions were prepared by dropwise addition of oil to the water phase, with sonication by a LABSONIC P ultrasonic (Sartorius Stedim Biotech) during cooling in an ice bath for 10 min.

(c) Bulk Oil Samples. Selected extracts (CBPE and GPPE) were resuspended in ethanol and added to bulk oil at a ratio of 500 mg GAE/kg oil. Ethanol was evaporated under vacuum.

(d) Oxidation Experiments. All emulsions were stored in triplicate in 25 mL glass bottles in the dark at 50 °C. Bulk sunflower oil samples were oxidized at 120 °C to simulate fat frying, which is one of the most commonly used procedures for the manufacture and preparation of foods in the world. Aliquots of each sample were removed periodically and assayed for peroxide value and *p*-anisidine value.

(e) Spectrophotometric Determination of Peroxide Value (PV). Emulsion (0.3 mL) was added to isooctane/2-propanol (3:2 v/v, 1.5 mL), and the mixture was treated in a vortex mixer (three cycles, 10 s/cycle). After centrifugation (2 min, 1000*g*), the clear upper layer was collected, and peroxides were quantified as per Díaz et al.[16] Lipid peroxide concentration was determined using cumene hydroperoxide as a standard for quantification. Assays were performed in triplicate, and data were statistically analyzed.

(f) Spectrophotometric Determination of p-Anisidine Value (PA). The PA value was determined according to AOCS Official Method cd 18-90.[17]

(g) Total Oxidative Value (TOTOX). The TOTOX value, employed to assess the oxidative deterioration of lipids, was defined as

$$\text{TOTOX} = 2\text{PV} + \text{PA}$$

where PV and PA are the peroxide and *p*-anisidine values defined above.

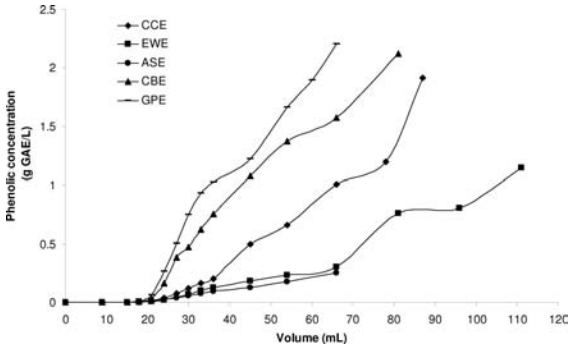

Figure 1. Dynamic leakage curves of phenolic compounds from the extracts of lignocellulosic materials subjected to autohydrolysis onto a column packed with SP700 resin.

(h) Synergy. Synergy was evaluated on the basis of the time (induction period, IP) needed to achieve a given level of deterioration in control samples (c) and in samples containing antioxidant (a), protein (p) and antioxidant + protein ($a + p$), according to the following equation:

$$\%\ \text{synergism} = 100\frac{[\text{IP}(a+p) - \text{IP}(c)] - [(\text{IP}a - \text{IP}c) + (\text{IP}p - \text{IP}c)]}{[(\text{IP}a - \text{IP}c) + (\text{IP}p - \text{IP}c)]}$$

HPLC Identification and Quantification. Purified extracts were analyzed using an Agilent HPLC 1100 instrument equipped with a Waters Spherisorb ODS-2 column (5 μm, 250 mm × 4.6 mm) and diode array detector (DAD), operating at 30 °C at a flow rate of 1 mL/min. Gradient elution was generated using solvent A (acetonitrile/water/formic acid, 10:85:5) and solvent B (acetonitrile/water/formic acid, 90:5:5) in the following proportions: 0 min, 100% A, 0% B; 40 min, 85% A, 15% B; 45 min, 0% A, 100% B; 60 min, 100% A, 0% B. Extracts were analyzed in triplicate. The triplicate determinations were used to calculate the mean and standard deviation. Phenolic compounds were identified by comparison of the retention times and spectral features with those of authentic compounds. Quantification was performed from calibration curves obtained with standard compounds diluted in methanol. Solvents and samples were filtered through 0.45 μm membranes before HPLC analysis.[2]

RESULTS AND DISCUSSION

Static Adsorption. The adsorption capacity of the adsorbent and the adsorption ratio for the extracts from the five lignocellulosic materials studied fell in the ranges of 64.8−71.3 mg/g and 90.8−99.0%, respectively (Table 1).

Dynamic Leakage Curve. Adsorption on macroporous resins depends on a number of factors, including surface interactions, sieve classification, surface electrical properties, and hydrogen bonding. When adsorption reaches the break point, the adsorption affinity decreases and even disappears, resulting in leaking of solutes. Breakthrough curves enable the calculation of the amount of resin needed for processing a given volume of feed solution with defined solute concentration. Dynamic leakage curves on SP700 resin were obtained for phenolic compounds based on the volume of effluent liquid and the concentration of solute herein (Figure 1). In general, adsorption presumably reached saturation when the concentration in effluent was 5% of the original concentration. The breakthrough volumes for GPE, CBE, CCE, EWE, and ASE solutions containing 0.75 g GAE/L were 31, 37, 57, 87, and >66 mL. In general, adsorption reached saturation when volumes >33.3 mL of extract solutions, which is the fixed volume

Table 2. Concentrations of Phenolic Compounds Removed from the Sorbent by Washing with Water and Desorption with 96% Ethanol

	washing		desorption (96% ethanol)	
extract	solids (%)	phenolics (%)	solids (%)	phenolics (%)
CCPE	22.8	13.5	55.7	66.3
EWPE	20.8	9.7	57.3	68.1
ASPE	15.3	5.4	37.9	47.0
CBPE	32.5	13.5	59.0	55.2
GPPE	24.8	19.1	48.0	72.6

used in static adsorption studies, were flowed through the glass column.

Dynamic Desorption Curve. The operational costs of adsorption are strongly dependent on the adsorbent reutilization, making the regeneration step highly influential on the overall economic balance. A number of regeneration techniques have been reported in the literature, including thermal procedures and treatments involving the utilization of acids, alkalis, or solvents. The best regeneration method depends on the physical and chemical characteristics of both adsorbate and sorbent. Solvent regeneration is particularly effective for polymeric sorbents when adsorbates are soluble in the regeneration solvent and when the solvating forces are higher than the physical interaction between adsorbent and adsorbate. In this study, 96% ethanol was used for desorbing the target compounds.[1]

The dynamic desorption curves were obtained by considering the volume of the desorption solution and the total concentration of phenolic compounds. As a preliminary step (see Table 2), washing with deionized water (about 140 mL or 3 BV) enabled the removal of unadsorbed solutes. Further elution with 96% ethanol (about 100 mL or 2 BV) resulted in complete desorption of phenolic compounds from the SP700 resin, yielding purified extracts (denoted CBPE, EWPE, ASPE, CCPE, and GPPE) (Figure 2).

Phenolic Content and Antioxidant Activity of Purified Extracts. Fractions (20−50 mL) collected from the desorption effluents from the processing of the various raw materials were dried under vacuum and freeze-dried, yielding stocks of purified extracts. Samples of these stocks were resuspended in water and assayed for phenolic content and antioxidant activity. The purified fractions showed higher phenolic content and antioxidant activity than the corresponding crude extracts (see Table 3). Purified samples contained 49−60% (w/w) phenolics, in comparison to a range of 32−43% (w/w) for the crude extracts.[2] The increases in phenolic concentrations were 30.2% for CBPE, 39.5% for EWPE, 52.8% for ASPE, 53.1% for CCPE, and 57.9% for GPPE.

The experimental results confirmed the suitability of macroporous resin adsorption for the efficient separation, recovery, and concentration of phenolic components from ethyl acetate extracts of autohydrolysis liquors. In related work, extracts of pigeon pea roots processed with ADS-5 macroporous resins increased the content of genistein and apigenin in the refined product by factors of 9.36−11.09, with recovery yields of 89.8−93.4%, respectively.[18] Effective recovery of antioxidant spinach flavonoids from an aqueous crude extract was achieved using polystyrenic and acrylic resins (XAD 16 HP and XAD 7 HP).[19] Crude extracts of *Inga edulis* leaves were purified by adsorption on macroporous resins,[20] whereas the recovery of narirutin from water extracts of *Citrus unshiu* peels was easily and effectively achieved by adsorption−desorption cycles performed with Amberlite XAD-7, resulting in a recovery yield of about 84%.[21]

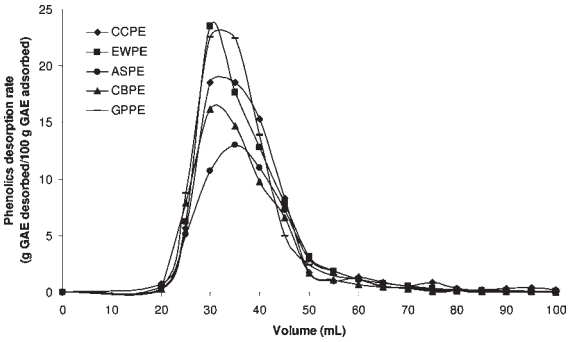

Figure 2. Dynamic desorption curves of phenolic compounds from the extracts of lignocellulosic materials subjected to autohydrolysis.

Identification and Quantification of Phenolic Compounds. Table 4 summarizes the results obtained in the HPLC-DAD analysis of purified extracts considered in this work. The compounds have been classified in four groups: phenolic acids, aldehydes, flavonoids (all of them of phenolic nature), and sugar-derived compounds of nonphenolic nature.

As a general trend, adsorption−desorption resulted in increased concentrations of phenolics from depolymerization of the acid-soluble lignin fraction. As this latter kind of compound, together with sugar dehydration products (for example, furfural and hydroxymethylfurfural), can be detrimental for the further bioconversion of biomass-derived hydrolysates, their selective removal by ion exchange and/or adsorption on polymeric resins has been proposed.[22,23]

HPLC analysis revealed different affinities for individual compounds, enabling a selective enrichment of concentrates in components with preferred properties. The concentration of phenolic acids (*p*-coumaric and ferulic acids) was significantly higher in the CCPE extract, whereas the rest of the purified extracts showed similar or even lower concentrations of phenolic acids than the respective crude extracts. Decreased proportions of 4-hydroxybenzoic acid, chlorogenic acid, and vanillic acid were observed in all of the refined extracts. An opposite behavior was observed for aldehydes, which showed higher concentrations in purified extracts than in crude solutions.[2] The contents of 3,4-dihydroxybenzaldehyde and vanillin reached in the GPPE extract were 3.96- and 5.36-fold higher than those in the initial extract, respectively, whereas the contents of syringaldehyde in the EWPE and ASPE purified extracts were 5.03- and 5.64-fold higher than the ones in the respective crude extracts. Adsorption−desorption also resulted in the removal of undesired impurities, revealed by the increased proportions of phenolic compounds in the concentrates. Sugar degradation in hydrothermal processing resulted in the formation of limited amounts of furfural and HMF (from dehydration of pentoses and hexoses, respectively). Both components were removed by 40.2−86.8% upon adsorption−desorption. The contents of sugar dehydration compounds varied in the following order: GPPE < CBPE < ASPE < CCPE < EWPE.

Antioxidant Activity in Model Food Emulsions and in Bulk Oil. To accelerate the oxidative damage, emulsions and bulk oil samples were stored at 50 and 120 °C, respectively. Oxidative stability was assessed by periodic analysis of primary and secondary oxidation products (measured by the peroxide and anisidine

Table 3. Phenolic Concentrations and Antioxidant Capacity of Purified Extracts

extract	PC[a] (g GAE/g extract)	DPPH, EC_{50} (mg extract/L)	TEAC EC_{50} (mg extract/L)	TEAC (g Trolox/g extract)	ORAC (μM Trolox)	FRAP (μM $FeSO_4 \cdot 7H_2O$)	FRAP (mM ascorbic acid equiv)	reducing power (mM ascorbic acid equiv)	β-carotene (CAA)
CCPE	0.49 (53.13)[b]	415.35	384.64	0.90	39.93[d]	500.03[e]	0.24[e]	0.17[e]	470.79[f]
EWPE	0.53 (39.47)[b]	355.01	286.27	1.21	29.96[d]	693.40[e]	0.34[e]	0.25[e]	730.92[f]
ASPE	0.55 (52.78)[b]	351.89	282.33	1.26	20.90[c]	758.13[e]	0.37[e]	0.24[e]	539.53[f]
CBPE	0.56 (30.23)[b]	296.83	314.49	1.13	22.54[c]	574.59[e]	0.28[e]	0.24[e]	769.04[f]
GPPE	0.60 (57.89)[b]	170.35	189.44	1.46	24.33[c]	786.75[e]	0.38[e]	0.32[e]	692.01[f]

[a] Phenolic concentration in the test solution. [b] Enrichment percentage with respect to the crude extracts. [c] 2.5 ppm extract. [d] 5 ppm extract. [e] 100 ppm extract. [f] 1000 ppm extract.

Table 4. Results Obtained in the HPLC-DAD Analysis of Purified Extracts

		mg compound/g extract				
t_R (min)	compound	CCPE	EWPE	ASPE	CBPE	GPPE
phenolic acids		83.42	20.02	4.54	19.22	21.94
4.28	gallic acid	ND[a]	0.68 ± 0.02	ND	10.86 ± 0.14	20.14 ± 0.14
8.74	4-hydroxybenzoic acid	ND	ND	ND	1.18 ± 0.06	ND
9.78	chlorogenic acid	ND	ND	1.24 ± 0.02	ND	1.49 ± 0.01
11.05	vanillic acid	0.56 ± 0.02	0.57 ± 0.04	0.79 ± 0.06	1.11 ± 0.02	0.25 ± 0.03
13.02	syringic acid	ND	8.47 ± 0.25	2.51 ± 0.39	2.01 ± 0.00	ND
17.45	*p*-coumaric acid	66.06 ± 2.42	ND	ND	3.47 ± 0.06	ND
21.80	ferulic acid	16.80 ± 0.07	ND	ND	0.59 ± 0.12	ND
28.97	ellagic acid	ND	10.30 ± 0.06	ND	ND	0.06 ± 0.05
aldehydes		46.94	20.44	60.27	ND	25.58
7.86	3,4-dihydroxybenzaldehyde	21.04 ± 0.03	6.34 ± 0.03	17.59 ± 0.03	ND	23.33 ± 0.44
11.73	4-hydroxybenzaldehyde	6.80 ± 0.00	ND	1.73 ± 0.01	ND	ND
15.68	vanillin	19.10 ± 0.09	3.58 ± 0.00	18.21 ± 0.08	ND	2.25 ± 0.00
19.02	syringaldehyde	ND	10.52 ± 0.38	22.74 ± 0.13	ND	ND
flavonoids		10.60	7.87	11.80	14.70	11.99
11.74	(−)-epicatechin	ND	ND	ND	ND	10.41 ± 6.01
28.70	rutin	ND	ND	ND	4.34 ± 0.04	ND
45.96	quercetin	10.60 ± 0.17	7.87 ± 0.07	11.80 ± 0.91	9.66 ± 0.09	2.37 ± 0.13
46.93	apigenin	ND	ND	ND	0.70 ± 0.02	ND
sugar-derived compounds		23.55	78.75	20.89	10.86	0.68
5.87	hydroxymethylfurfural	23.00 ± 0.31	78.75 ± 0.49	19.11 ± 0.15	10.02 ± 0.24	ND
8.46	2-furfuraldehyde	0.55 ± 0.02	ND	1.78 ± 0.08	0.84 ± 0.02	0.68 ± 0.01

[a] ND, not detected.

values, respectively). Hydroperoxides are formed when molecular oxygen and unsaturated fatty acids are combined in the presence of heat, light, or catalysts such as iron, copper, or enzymes. On the other hand, peroxides are reactive and are consumed by reactions with fats, leading to the formation of new oxidizing products. Secondary oxidation products (alkanes, alkenes, aldehydes, and ketones) are formed mainly in the final reaction stages. The anisidine value is a measure of the production of aldehydes during oxidation of fats or oils and can be used as an indicator of the "oxidative history" of the product.

The PV increased during the early stages of oxidation, when the rate of hydroperoxide formation exceeds the decomposition rate (see Figure 3). As shown in Table 5, the PA values for the OW emulsions were lower than those of the analogous WO emulsions. The TOTOX value (see results in Figure 4) measured the overall oxidative stability. After 35 days of storage, purified extracts inhibited lipid oxidation more effectively in water-in-oil emulsions (62.8%) and in bulk oil (84.2%) than in oil-in-water emulsions (30.4%). CBPE extract was more effective than GPPE extract in inhibiting lipid oxidation in oil-in-water emulsions (35.4%), but showed limited ability to protect water-in-oil emulsions (43.3%) or bulk oil from oxidation (40.4%). This finding confirmed the results obtained previously in the assay of oxidative bleaching of β-carotene in emulsion.

The antioxidant capacity of natural extracts in food emulsions has been ascribed to a number of influential factors, including the different polarities and antiradical activities of mixed phenolics. The presence of water in an emulsion results in the partition of antioxidants between polar and apolar phases, a fact influencing the antioxidant activity. According to the "polar paradox", hydrophilic

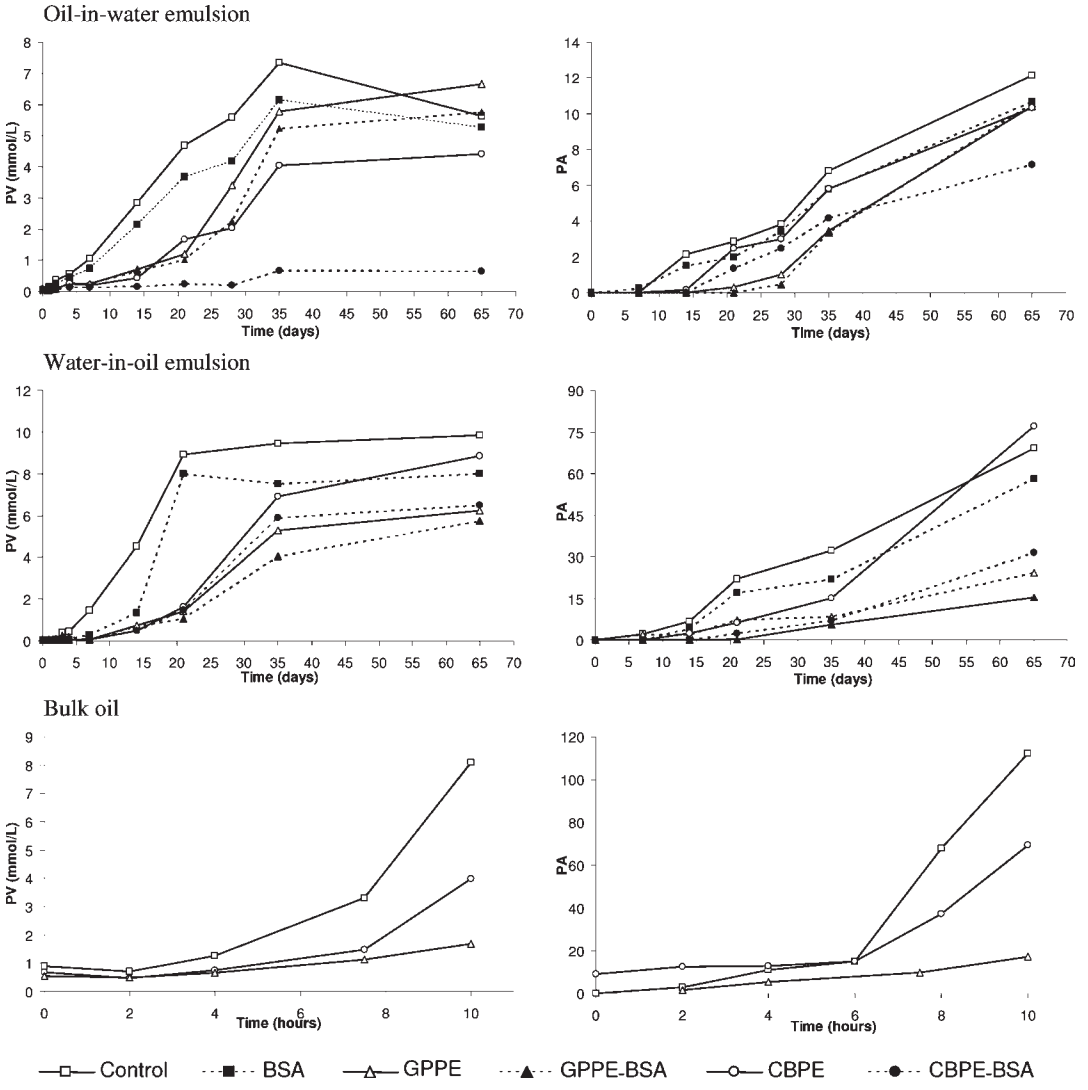

Figure 3. Changes in peroxide value (PV) and *p*-anisidine value (PA) of oil-in-water emulsions and water-in-oil emulsions during storage at 50 °C and in bulk oil during storage at 120 °C.

antioxidants are more effective in nonpolar media, whereas lipophilic compounds are better antioxidants in polar media.[24] However, some authors have reported that some compounds do not comply with the polar paradox and interpreted the behavior of phenolic compounds in emulsified systems according to a different approach known as the "cutoff theory".[25] Rice Evans et al.[26] provided molar TEAC values for pure flavonoids and phenolic acids and provided evidence that the values in mixtures are additive. Gallic acid, epicatechin, quercetin, and rutin are the components in the extracts with reported antioxidant activity. Using the TEAC values reported by Rice Evans[26] and the concentrations in Table 4, it can be calculated that the contribution of these components to the molar TEAC value for GPPE is 0.48 mM Trolox, whereas for CBPE it is 0.36 mM Trolox. This is consistent with the higher experimental TEAC value for the GPPE compared to CBPE. Gallic acid contributes 73.8% to the molar TEAC value for GPPE but only 53.5% to the molar TEAC value for CBPE. Due to its polarity and water solubility, the contribution of gallic acid to the antioxidant capacity in an OW emulsion will be small according to the polar paradox, and this was observed experimentally by Schwarz et al.[27] The contribution of gallic acid to the antioxidant capacity of GPPE in a homogeneous solution is higher due to its higher concentration, so the inversion in the order of activity of the GPPE and CBPE on changing from a homogeneous solution to an emulsion can be attributed, at least in part, to the reduced contribution of gallic acid in the emulsion. The higher concentration of quercetin and rutin in CBPE compared to GPPE contributes to its higher antioxidant capacity in the emulsion. Enrichment with polyphenols increases the shelf life of processed foods by preventing lipid oxidation and protecting samples from oxidative damage, resulting in benefits for human health. Phenolic extracts from fruit, leaves, and pomace of olive plant showed remarkable antioxidant activity for retarding the oxidative rancidity of sunflower oil,[28] whereas extracts of grape waste increased the induction time of sunflower oil from 7.45 to 15.3 h, an effect similar to that of common antioxidants used as food additives including butylated hydroxytoluene (BHT), ascorbyl palmitate, and vitamin E.[29]

The antioxidant potential of natural extracts may be due to the presence of different active phenolic compounds, accompanying components, and synergism among them. A strong synergistic inhibition of lipid oxidation in oil-in water emulsions was observed between CBPE extract and BSA. In the presence of

Table 5. Time (in Days) Needed To Reach the Indicated Values of PV, PA, and TOTOX at 50 °C (or 120 °C for Bulk Oil)

oil-in-water emulsion	PV (0.63 mM hydroperoxide)	PA (7.16)	TOTOX (8.42)
OW	4.44	36.88	14.92
OW−BSA	5.94	43.49	19.21
OW−GPPE	13.00	51.06	28.57
OW−GPPE−BSA	13.78	50.89	30.76
% synergy	–	–	–
OW−CBPE	15.10	43.99	29.39
OW−CBPE−BSA	65.0	65.0	65.0
% synergy	397.79	105.07	166.86
water-in-oil emulsion	PV (5.73 mM hydroperoxide)	PA (15.43)	TOTOX (26.89)
WO	15.94	17.97	17.23
WO−BSA	18.62	21.10	19.35
WO−GPPE	49.30	48.24	48.36
WO−GPPE−BSA	65	65	65
% synergy	36.12	45.15	43.65
WO−CBPE	31.93	35.11	33.50
WO−CBPE−BSA	34.49	45.43	44.58
% synergy		42.49	48.64

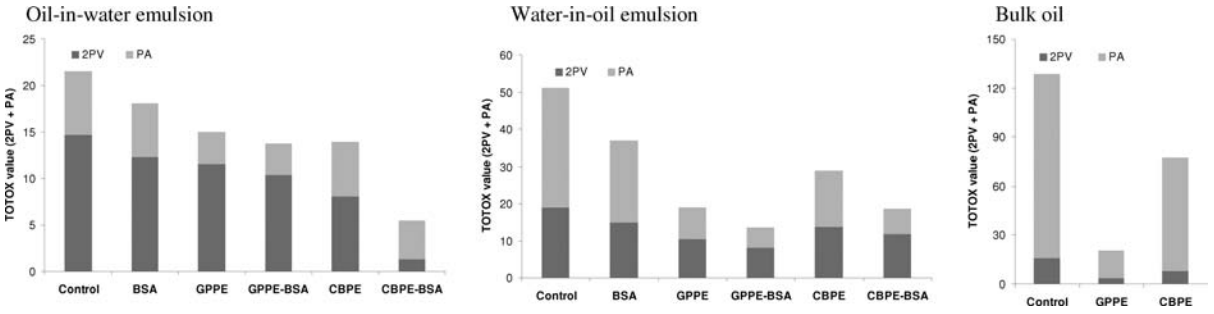

Figure 4. TOTOX value of oil-in-water emulsions and water-in-oil emulsions containing antioxidants during storage (50 °C) at 35 days and of bulk oil during storage (120 °C) at 10 h .

BSA, the stability of the OW emulsions containing CBPE extract increased by 167% (based on the TOTOX value = 8.42), but BSA did not result in significant increases in stability of WO emulsions containing CBPE extract (based on TOTOX results). Model oil-in-water emulsions containing green tea extract, epicatechin (EC), and epigallocatechin gallate (EGCG) showed a synergistic stability increase in emulsions containing added albumin. EGCG showed 35% higher synergy than EC with ovalbumin.[30] Stability during storage of emulsions containing tea extracts was greatly increased by the presence of BSA. The combination of BSA with phenolics from virgin olive oil showed 58−127% synergy, depending on the analytical method employed.[6] Contrarily, the phenols present in rooibos infusion did not increase stability in the presence of BSA, indicating that no synergistic effects occurred.[31]

In conclusion, phenolic fractions obtained from selected biomass sources by autohydrolysis−-extraction were purified by adsorption−desorption using the nonionic polymeric resin Sepabeads SP700. The experimental results confirmed that the approach considered in this work was successful for obtaining concentrates with increased phenolic content. Operating under selected conditions, the refined products contained 30−58% higher phenolic content than the respective crude extracts. HPLC analysis revealed a selective enrichment of concentrates in components with preferred properties. Among the samples studied, the refined products derived from the processing of grape pomace (denoted GPPE) and chestnut burs (denoted CBPE) showed remarkable antioxidant activity and might be useful for lipid stabilization in processed foods.

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■ ABBREVIATIONS USED

AAPH, 2,2′-azobis(2-methylpropionamidine) dihydrochloride; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate); ASE, almond shells; ASPE, almond shell purified extract; BHT, butylated hydroxytoluene; BSA, bovine serum albumin; BV, bed volume; CBE, chestnut burs; CBPE, chestnut bur purified extract; CCE, extracts of corn cobs; CCPE, corn cob purified extract peroxide value; DAD, diode array detector; DPPH, α, α-diphenyl-β-picrylhydrazyl; EC, epicatechin; EC_{50}, equivalent concentration; ECGC, epigallocatechin gallate; EWE, eucalypt wood; EWPE, eucalypt wood purified extract; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalents; GPE, grape pomace; GPPE, grape pomace purified extract; HMF, 5-(hydroxymethyl)furfural; HPLC, high-performance liquid chromatography; IP, induction period; LDL, human low-density lipoproteins; ORAC, oxygen radical absorbance capacity; OW, oil-in-water; PA, *p*-anisidine value; PBS, phosphate buffer saline; PS-DVB, polystyrene−divinylbenzene; SP700, Sepabeads 700; TEAC, Trolox equivalent antioxidant capacity; TOTOX, total oxidative value; TPTZ, 2,4,6-tripyridyl-*s*-triazine; WO, water-in-oil; w/w, weight/weight.

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