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Membrane concentration of antioxidants from *Castanea sativa* leaves aqueous extracts

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ABSTRACT

Aqueous extraction of *Castanea sativa* leaves (CsL) was scaled up and the extract was processed in a series of two UF membranes (5 and 10 kDa) with the aim of concentrating the active phenolic compounds with antioxidant activity. Due to the occurrence of adsorptive fouling of the phenolic compounds found in one membrane's configuration assayed (Configuration I), batch dilution of the retentate was required for optimal performance. However, this strategy did not improve the selectivity of the process and the active compounds were diluted. The best prediction of the permeate flux was provides by the cake layer formation model (CFM) to the 5 kDa membrane and by the standard pore blocking model (SBM) to the 10 kDa membrane when the Configuration II was assayed. Secuencial filtration without dilution of the 5 kDa permeate (Configuration I) provides a selective separation of active compounds on 10 kDa membrane filtration process. The final concentrated extracts presented radical scavenging capacities comparable to Trolox and to BHA. The test with reconstituted human epidermis (Episkin®) confirmed that the extracts were non irritant at 1%. Operation with reutilization of the permeate was feasible and the extraction yield of active compounds was not significantly affected. Due to the non selective retention of phenolic and soluble protein, the separation of this latter by ethanol precipitation was proposed. The ethanol precipitation of concentrates streams increased its purity and activity of the final products by about 15%.

1. Introduction

Castanea sativa leaves (CsL) are a folk remedie to treat cough, rheuma and muscular pain. Recent research has confirmed the interesting properties of aqueous and ethanolic extracts including the free radical scavenging capacity [1–3], antimicrobial activity [4,5], and the potential and safety as ingredient in cosmetic compositions [6,7].

In a previous study an aqueous process for the extraction of antioxidants from *C. sativa* leaves (CsL) was optimized to obtain a mixture of active compounds (gallic acid, protocatechuic acid, 4-hydroxybenzoic acid, vanillic acid, rutin, quercetin and apigenin), which was as potent radical scavenger, comparable to synthetic antioxidants [8]. The purity referred to phenolic compounds was in the range 34–50%. Further refining of the crude extracts obtained could be desirable for some particular applications. Maximal yields and purity were obtained for extractions carried out at 50 °C, however the maximal radical scavenging capacity was found at 25 °C. The performance of an extraction at room temperature was proposed and the possibility of improving the phenolic concentration in the final extract was addressed with membranes.

Despite the advantages of water as a selective agent for the extraction of antioxidants from CsL as well as a biorenewable non toxic solvent for processing nutraceutical and food ingredients, the high residual volumes compromise the environmental benefits and concentration costs. In order to define a process suited for scaling up and with reduced environmental impact both the consumption and the disposal of aqueous streams should be limited. Membrane technology can be used to concentrate and/or selectively fractionate bioactive compounds with antioxidant activity from aqueous and alcoholic processing streams of products, by-products and wastes from agro-food industry, particularly soluble protein and peptides and phenolic compounds. Some successful applications include the concentration [9] and fractionation [10,11] of grape phenolics; the fractionation of phenolics from mushrooms [12], persimmon pulp [13], almond skin extracts [14], mulberry root cortices [15], black [16] and green tea [17], and *Salvia miltiorrhiza* [18].

An important parameter to define a scalable design is the membrane fouling. During a filtration process the membrane properties are affected by the feed characteristics and the operating

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conditions. The fouling mechanisms include membrane–solute–solute interactions (UF fouling) and membrane–solute interactions (adsorptive fouling), influenced both by membrane properties (surface chemistry and surface pore size) and feed characteristics (pH and salt content). Adsorptive fouling was confirmed during the adsorption of polyphenolics from green tea on the surface and inside polyethersulfone (PES) membranes [19]. The adsorptive fouling of polyphenols onto PES membranes was influenced by the effect of pore size, polar interactions (van der Waals, electron donor–acceptor), multiple hydrogen bonds towards the additive PVP in PES, benzene ring interaction by π–π stacking and changes in water structure at the membrane polymer surface [19,20]. Mixtures of polyphenols with other components, such as polysaccharides, could form aggregates having a strong contribution to adsorptive fouling of PES membranes [20]. During UF of cork processing wastewaters, which are a complex mixture of phenolic compounds, the more hydrophobic component (ellagic acid) was almost totally retained forming an adsorbed layer on cellulose acetate membranes independently of the MWCO (6 and 98 kDa) and the hydrodynamic conditions [21].

The objective of this study was to scale up the aqueous extraction process from *C. sativa* leaves (CsL), to assess the performance of a sequence of two ultrafiltration membranes for the concentration of phenolic compounds with antioxidant activity and to verify that these properties of concentrated streams are not lost during membrane processing.

2. Materials and methods

2.1. Materials

Chestnut (*C. sativa*) leaves (CsL) from 2005 harvest were locally collected in Ribeira Sacra (Ourense, Spain). Air-dried and ground CsL (final moisture 9.68% and less than 1 mm) were stored in sealed plastic bags in dry dark place before use. Butylhydroxyanisol (BHA) and butylhydroxytoluene (BHT) (Analema) were used.

2.2. Aqueous extraction

Ground CsL (1 kg) was contacted with acidified water (25 L) under conditions leading to maximal radical scavenging activity (25 °C during 90 min) in a home-made tank with stirring rate and temperature control. Solid:liquid separation was accomplished by vacuum filtration, and the liquid phase was processed in a series of two ultrafiltration membranes.

2.3. Membrane equipment and operation

Membrane fractionation was performed in an UF lab pilot home-made plant, consisting of a feed tank with 2.5 L, a peristaltic Masterflex pump and a membrane module. Pressure was monitored at the entrance and exit of the membrane module, a needle valve located after the module was used for TMP regulation. The pilot plant was equipped with 5 and 10 kDa Omega membranes (Minisette, Pall Filtron) (0.12 mm × 0.10 mm) having an effective surface area of 0.07 m^2. The membrane material is modified polyethersulfone and the maximum operation pressure is 4 bar.

The aqueous phase generated after extraction was ultrafiltered with the 5 kDa membrane, operating in concentration mode, at 20 °C and at a fixed transmembrane pressure of 2 bar, since at lower values no permeation was observed and higher values could not be feasible. Volume permeation fluxes were measured up to a VRF (Volume Reduction Factor) of about 2–6. The VRF can be expressed as the ratio between the initial feed volume and the volume of retentate.

$$\mathrm{VRF} = \frac{\text{Volume of feed}}{\text{Volume of retentate}} = \frac{V_0}{V_R} \quad (1)$$

Instantaneous samples of permeate and retentate were regularly taken and assayed for total solids, total phenolics, total nitrogen, soluble protein, radical scavenging capacity (ABTS, DPPH), and reducing power. Once reached a VRF of 2.3, the retentate was processed in the 10 kDa membrane with simultaneous retentate recycling and permeate removal (Configuration I, Fig. 1).

2.3.1. Batch redilution of retentate

To improve operation in the 10 kDa membrane water dilution (1:2.125) of the retentate from the 5 kDa membrane (R_1) was performed (Configuration II, Fig. 1).

After operation, the fouled membranes were cleaned with a caustic detergent solution (0.5% Ultrasil 11, Henkel Ecolab) at 50 °C for 60 min operating in full recirculation mode, and the system was then operated with fresh water in order to check the flux recovery.

2.4. Analytical methods

The total phenolic content was determined by Folin-Ciocalteau method using gallic acid as standard. Identification analyses were carried out using an Agilent HPLC 1100 instrument equipped with a Waters Spherisorb ODS-2 column and a DAD detector, operating with a flow rate of 1 mL/min. A nonlinear gradient of solvent A (Acetonitrile/5% (v/v) formic acid in water, 10:90) and solvent B (Acetonitrile/5% (v/v) formic acid in water, 90:10) was used: 0 min, 100% A, 0% B; 40 min, 85% A, 15% B; 45 min, 0% A, 100% B; 60 min, 100% A, 0% B. The protein concentration was determined by the Bradford method with bovine serum albumin (Sigma Chem. Co.) as the standard. The total nitrogen was analysed by elemental analysis with a Thermo Finnigan EA 1112 analyzer.

2.5. Antioxidant activity

2.5.1. α,α-Diphenyl-β-picrylhydrazyl (DPPH) radical scavenging activity

Two milliliters of a 3.6×10^{-5} M methanolic solution of DPPH (Fluka), were added to 50 μL of a methanolic solution of the antioxidant. The decrease in absorbance at 515 nm was recorded after 16 min. EC_{50} was calculated as the amount of extracts (redissolved in methanol) causing a 50% inhibition of the DPPH radical. Each study was performed in triplicate.

2.5.2. TEAC (Trolox equivalent antioxidant capacity)

ABTS radical cation ($ABTS^{\bullet+}$) [2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate)] was generated by reacting 7 mM ABTS solution with potassium persulfate (final concentration 2.45 mM) and maintaining the mixture in the dark for 12–16 h before use. $ABTS^{\bullet+}$ solution was diluted with phosphate buffer saline (PBS) (pH 7.4) to an absorbance of 0.70 at 734 nm and equilibrated at 30 °C. One mL of diluted $ABTS^{\bullet+}$ solution was mixed with 10 μL of extract or Trolox standards in ethanol or phosphate buffer saline (PBS). Absorbance readings were taken up to 6 min and the percentage inhibition of absorbance was calculated as a function of the concentration of extracts and Trolox. Each study was performed in triplicate

Ferric reducing antioxidant power (FRAP) was determined with a reactive solution prepared with 25 mL of 300 mmol/L acetate buffer (pH 3.6), 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 ferric chloride in distilled water. An aqueous solution of known Fe (II) concentration was used for calibration. Samples (100 μL) were mixed with 3 mL