Industrial Crops & Products 109 (2017) 558–569

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Modelling the hydrophilic extraction of the bark of *Eucalyptus nitens* and *Eucalyptus globulus:* Adsorption isotherm and thermodynamic studies

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ARTICLE INFO

Keywords:
Flavonoid
Eucalyptus bark
Phytoextraction
Isotherm model
Adsorption isotherm
Solute-solid interactions

ABSTRACT

Eucalyptus species have shown to have polyphenolic compounds with proven antioxidant activity. To obtain these active compounds, the basic aspects of the extractive process need to be studied. In this work, the adsorption process of polyphenols obtained from *Eucalyptus nitens* and *Eucalyptus globulus* bark was evaluated, expressed as adsorption isotherms to describe solute/solid interactions. Also, the thermodynamic parameters of the extraction process were calculated. Firstly, the extractive process was optimized by a Box-Benhken experimental design for each *Eucalyptus* species, maximizing the extraction yield, the total phenols content and its antioxidant capacity. Subsequently, the solute/solid interactions of the phenolic/bark system were determined at various concentrations, and temperatures; allowing even later, to model multistage extractions of phenolic compounds from bark powder. The extracted and non-extracted bark powders were morphologically (SEM) and physically (BET adsorption) characterized. The extractive process led surface changes for both species, being the BET areas similar (55 m^2/g) and with porous in the mesoporous range. The equilibrium data were best adjusted to Freundlich isotherm, indicating a simple modelling type, which can be used for global studies of the large-scale extractive process for the modelling of phenolic multi-extractions. Thermodynamic parameters ΔH^o, ΔG^o and ΔS^o demonstrated an exothermic and spontaneous process with restricted movement of adsorbate molecules during adsorption, and low values of ΔG^o and free adsorption energy, E, calculated from D-R model indicated a physical adsorption type for both eucalyptus species. The validation of the isotherm models presented results that are consistent with the experimental data obtained, through a multi-extraction of five stages for both species of eucalyptus.

1. Introduction

Eucalyptus species are the most important source of fiber for pulp and paper production in southwestern Europe (Portugal and Spain), southern Africa, Japan and South America (Brazil and Chile) (Domingues et al., 2011). According to FAO (2017) (Food and agriculture organization of the United Nations), in terms of species distribution, *Pinus* and *Eucalyptus* are dominant species worldwide, with 37.4 and 17.9 million ha in 2000. Forest industries generate large amounts of residual biomass, where the bark is highlighted. For *E. globulus*, for instance, bark represents about 11% of the dry weight of the trunk (Santos et al., 2011). This residue is normally burned for energy production, which is used in the same forest plant. However, this process has a low efficiency, due to the high content of resins contained in the bark, being harmful to the equipment used for its combustion (Freire et al., 2002). This characteristic justifies the research for new bark uses, seeking to get valued by-products.

Phenolic compounds obtained from plants, such as flavonoids, have proven to have therapeutic properties (Vázquez et al., 2012). Specifically, eucalyptus species have been shown to have polyphenolic and terpenic compounds with antioxidant activity, among others bioactivities (Domingues et al., 2011; González, 2016; Mota et al., 2012; Santos et al., 2011; Vázquez et al., 2008). However, a fundamental step in the production processes for obtaining active substances is the extraction from the source material, also called phytoextraction (in the field of plant origin). This involves contacting the material containing the substance of interest (or solute) with a specific solvent, occurring a transfer of the active compound from one phase to another, described by a desorption process (Dreisewerd et al., 2015). Therefore, although bark can be used as raw material to obtain extracts of high value, from the industrial point of view, the economic sustainability of the extraction requires the study of the process, in order to achieve a high efficiency. In this context the isotherms, curves at constant temperature that describe the retention (or release) of the chemicals of a solid at various concentrations appears to be in equilibrium. This equilibrium at the surface of the solid material determines a solute/solid interaction

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http://dx.doi.org/10.1016/j.indcrop.2017.08.059
Received 31 May 2017; Received in revised form 18 July 2017; Accepted 31 August 2017

and the study of these interactions is important in the design of an extraction process (e.g. in the choice of a solvent) (Limousin et al., 2007). Moreover, there are mathematical models for extraction design where solute/solid interactions are included as parameters. Thus, a proper determination of the isotherm is inevitable to ensure adequate results in the design of the extraction process. In this context, Dreisewerd et al. (2015) suggest to study these interactions independently of the extraction itself, and instead, by using the opposite process: the adsorption. This is in order to avoid overlapping effects of the mass transfer phenomena inherent to the vegetal extractive nature. The above is produced because the equilibrium at the surface is just one part of a sequence of different mass transfer steps during extraction, and in the special case in which the solid naturally possesses the solute of interest, i.e. a phytoextraction, the complex structure of the plant material may generate various steps in the mass transfer and equilibrium process, e.g. diffusion through it (which may cause delay effects in reaching equilibrium), indicating that there may be overlapping effects during phytoextraction that prevent a direct measurement of solute/solid interactions.

Hence, to generate a market-valued product from eucalyptus bark and to provide information regarding the fundamental retention process of polyphenolic compounds in the eucalyptus solid matrix, the desorption/adsorption phenomenon of this system need to be studied. For this, two eucalyptus species, that concentrate a high percentage of the surface planted in Chile (INFOR, 2014), were used: *Eucalyptus globulus* and *Eucalyptus nitens*. The different adsorption isotherms of phenols contained in the bark powder of each species were constructed from the adsorption (and not extraction) phenomenon, and then, the possibility of modelling was studied, with the previous isotherms, the behaviour of the multi-extractive process (desorption). Also, the thermodynamic parameters of the extraction process were calculated. In this way, this study is aimed to expand the phenomenological knowledge regarding these species, facilitating the future design of phytoextractions from this by-product of the forest sector.

2. Materials and methods

2.1. Reagents and standards

The bark of six *Eucalyptus globulus* and *nitens* trees were obtained from Comaco Forest Company (Concepción, Chile), which were collected on December 17, 2015 from Los Castaños, Concepción, Biobío Region, Chile. The trees aged between 10–12 years. Folin & Ciocalteu's phenol and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) reagents were purchased from Sigma Aldrich (St. Louis, MO, USA). Sodium carbonate was purchased from Winkler and Zawadzky (Santiago, Chile). Methanol of analytical grade was purchased from Merck (Darmstadt, Germany). The bidistilled water used in all solutions was purified to HPLC grade using a Millipore Milli-Q (MQ) as a fast water system (Bedford, MA, USA).

2.2. Bark pretreatment

The barks were dried and grounded in a mill to a diameter < 2 mm. A preliminary study was carried out to determine the influence of the particle size on kinetic of extraction. Four ranges of particle diameter were used: 0.08–0.35 mm, 0.35–0.59 mm, 0.59–0.85 mm and 0.85–1.00 mm. The equilibrium concentrations and the rates to reach them were compared and the range of 0.08–0.59 mm was selected, where no difference was observed on the equilibrium concentrations, confirming that phenomena of retention of solutes inside the solid matrix (within it, generating a concentration gradient inside the solid material (Hostettmann et al., 2014); or by material trapped inside the solid walls) were minimized, and also the length of diffusive pathways (so that the velocity to reach the equilibrium was maximized). Finally, all the bark powders used in this study were sieved to that diameter range.

2.3. Bark powder characterization

The bark pore size, pore distribution and BET surface area were measured by N_2 adsorption at 77 K (Sample Degas System, Micromeritics, Flowprep 060) for both eucalyptus species. The powder was previously multi-extracted and vacuum cleaned. The semi-quantitative analysis based on the t-graphic was used for the study of micropore distribution. The effect of the extraction on the bark's morphology was studied by scanning electron microscopy (SEM) (JSM-6380LV, JEOL, USA) at 100× and 1000× magnifications.

2.4. Hydrophilic batch extraction

The extraction was performed in conical flasks well sealed with plug and Parafilm paper following the experimental levels described above. Methanol/water and the bark powder of each species were introduced into the flasks, which were wrapped with aluminium foil to minimize flavonoid oxidation by light. The flasks were shaken in an orbital shaker (Bench Top Incubator NBL-205) at 200 rpm for 2 h. This equilibrium time was determined in a previous one-stage extraction experiment with its corresponding kinetic measurement, not shown here, where for both species, the equilibrium was reached in 2 h. After extraction, the flasks were withdrawn and put into an ice bath to stop the extraction. Samples were vacuum filtered and the organic solvent methanol was eliminated by vacuum evaporation using a rotating evaporator (303 K and 6.5 rpm). Finally, the extract was frozen at 269 K and lyophilized using a lyophilizer (Heto Drywinner, Denmark) for extraction yield determination. The dry sample was stored at 275–281 K until further use.

2.5. Experimental design

A Box–Behnken statistical screening design was used to statistically optimize the parameters of the bark extraction for both species of eucalyptus. The main effects, interaction effects and quadratic effects on the responses yield of extraction (calculated as the mass of solid extract obtained from the extraction process, with respect to the mass of initial bark powder used), total phenols concentration of the extracts and its antioxidant capacity were considered, since they are crucial for the process design.

A three-factor, three-level design is suitable for exploring quadratic response surfaces and constructing second-order polynomial models with Statgraphics Centurion XVII (State-Ease, Inc., Minneapolis, MN, USA). This cubic design is characterized by a set of points lying at the midpoint of each edge of a multidimensional cube and centre point replicates (n = 3), while the 'missing corners' help the experimenter to avoid the combined factor extremes. This property prevents a potential loss of data in those cases (Box and Behnken, 1960).

A total of fifteen batches of bark extraction were prepared varying three independent variables: solid/liquid ratio (RS/L), the temperature and methanol concentration in water (% m/w). The bottom, centre and upper levels for the variables were: RS/L, 1:10, 1:35 and 1:60 g/mL; temperature: 303, 318 and 333 K and for % m/w: 33.3, 50 and 66.6%, respectively. The predicted models were developed applying multiple regression analysis on the experimental data. The statistical significance of quadratic models was studied by using the analysis of variance (ANOVA).

The experimental results were adjusted according to the second order polynomial show in Eq. (1). There, Y_n represent the dependent variable, β_o is the model constant, β_i, β_{ij} y β_{ii} are the model coefficients for the lineal interaction and the quadratic effects of independents variables, respectively. x_i and x_j are the independents variables, encoded between −1 and 1, and ε is the experimental error.