J. of Supercritical Fluids 62 (2012) 135–148

Contents lists available at SciVerse ScienceDirect

The Journal of Supercritical Fluids

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

High pressure solvent extraction of maritime pine bark: Study of fractionation, solvent flow rate and solvent composition

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ARTICLE INFO

Article history:
Received 25 August 2011
Received in revised form 26 October 2011
Accepted 27 October 2011

Keywords:
Maritime pine bark
High pressure extraction
Fractionation
Phenolic compounds
Procyanidins

ABSTRACT

Maritime pine bark, an abundant Portuguese residue rich in high-value phenolic compounds, was subjected to fractionated and non-fractionated high pressure extractions (F-HPE and NF-HPE, respectively). Supercritical carbon dioxide (scCO_2) was the chosen solvent to extract the pine bark low-polarity fraction and ethanol (EtOH) was added to scCO_2 to recover the phenolic fraction. The effect of the solvent flow rate was studied on first step (scCO_2) and second step (CO_2:EtOH 90:10, v/v) F-HPE kinetics. Due to the low first step yield (0.6–1.0%, d.b.) HPE was further performed with no fractionation at 303 K and ~25 MPa. The flow rate that achieved the highest global yield (7.6×10^{-5} kg/s) was chosen to carry out NF-HPE with different EtOH compositions (30–90%, v/v). The HPE results were compared with hydrodistillation and Soxhlet extraction results in terms of global yields, extracts compositions and extracts antioxidant activities. The results showed that fractionation, solvent flow rate and solvent composition affected extraction kinetics and the characteristics of the extracts. In particular, the solvent composition CO_2:EtOH (30:70) led to the extract with the highest contents of total phenolic compounds and of procyanidins (25.6% and 19.8%, respectively), being similar to the ones achieved by Soxhlet extraction (26.0% and 18.2%, respectively). The HPE methodology takes advantage over the conventional methodology due to the reduced EtOH consumption, lower solvent-to-solid ratio, lower extraction temperature, and oxygen-free medium in which it occurs.

1. Introduction

Maritime pine (*Pinus pinaster* Ait.) is a conifer native to south-western Europe and north-western Africa, with major forestry developments on the Atlantic coast of southern France, Spain and Portugal. The Portuguese pine forest area reached its highest value in 1974 (1388×10^3 ha) and has reduced since then, being 710.6×10^3 ha in 2006 [1,2]. Despite the crisis that has been affecting the Portuguese pine sector, it still represents an important component of the total forest economic value (around 17%), being the third most important species after eucalyptus and cork oak [3]. The main industrial activities are related to the usage of its wood, and include sawmills, wood panels, pulp and paper, carpentry, packing and furniture [4]. Pine bark is an abundant residue of these industries, since it represents 10–20% of the pine tree trunk. It presents several favorable features such as some important phytochemical constituents, low price and long-term stability that together make the usage of this waste highly attractive [5]. Nevertheless, at present, it is almost exclusively used as fuel, being also subjected to composting, utilized to cover public gardens, or simply thrown away on landscapes [6].

There are quite a few possible applications of pine bark widely reported in scientific literature. Most of them are related with its rich phenolic composition, mainly with procyanidins. Typical phenolic compounds present in pine bark are (+)-catechin, (−)-epicatechin, dihydroquercetin, as well as phenolic acids. Most of these compounds are procyanidin dimers, trimers, oligomers and polymers [7,8]. Procyanidins from different pine species have received considerable attention in the fields of nutrition, health, and medicine owing to their physiological and biological activities, namely antibacterial, antiviral, anticarcinogenic, anti-inflammatory and cardiovascular system diseases prevention [8–11]. There is even a patented aqueous ethanolic extract from the bark of French maritime pine, Pycnogenol®, which is marketed worldwide as a food supplement or as an herbal-based medication [12–15]. Maimoona et al. [16] have recently published a review on the biological, nutraceutical and clinical aspects of maritime pine extracts.

The choice of the extraction process, solvents and operational conditions is always conditioned by the required extract quality and by other particular specifications, like extraction yield

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doi:10.1016/j.supflu.2011.10.016

and presence of undesired compounds. Conventional solid–liquid extraction with water, alcohols and/or acidified alcohols is usually employed for extraction of procyanidins. However, and because natural products usually contain a wide variety of low and high molecular weight phenolic compounds (and their complexes), natural extracts from these materials will always contain a mixture of different classes of these phenolic substances, depending on the chosen extraction solvent and on the particular employed operational conditions. Usually, additional steps are then required in order to purify/concentrate the desired compounds and to remove the undesired phenolic and other non-phenolic substances.

For food and pharmaceutical applications, high pressure solvent extraction (HPE) represents an attractive option to conventional solid–liquid extraction methods and, in some cases, supercritical fluid extraction can also be applied, offering several advantages in terms of selectivity, separation conditions and on the use of environmental friendly technology and solvents. HPE involves the use of H_2O or organic solvents at considerable elevated temperatures (313–473 K) and pressures (3.3–20.3 MPa), offering the possibility to perform efficient extractions due to its improved characteristics in terms of mass transfer and solvating properties. If high temperatures are applied, the liquid solvent viscosity diminishes, the solvent diffusivity through the plant matrix is improved and consequently extraction kinetics is accelerated. Moreover, high pressure forces the solvent into the matrix pores and hence should facilitate extraction of target compounds [17]. Therefore, this extraction technique takes advantage of the beneficial combination between typical liquids solvation properties and the advantageous transport properties of supercritical fluids [18]. However, a liquid separation step is additionally required in the post-extraction preparation steps, which represents a disadvantage relatively to the SFE methodology.

Water and organic solvents usually applied in HPE are able to establish intermolecular interactions with plant compounds with hydroxyl, amino and nitro functional groups that are not soluble in supercritical CO_2, even with small quantities of a polar cosolvent. In particular, the OH groups of phenolic compounds interact favorably with alcoholic solvents, since they provide polarity and a site for accepting and donating hydrogen bonds. Several types of intermolecular interactions between solute molecules, between solvent molecules and between solute and solvent molecules will compete with each other and will determine solubility. Therefore, it may be necessary to try several solvents to achieve the maximum specificity for a given system.

If CO_2 is used in combination with H_2O or with an organic solvent, a gas-expanded liquid is formed. A unique and potentially useful property of CO_2-aqueous and CO_2-alcohol gas-expanded liquids is the *in situ* generation of carbonic acid and alkyl carbonic acid, respectively [19,20]. The pH decrease that follows the formation of these acids may be beneficial or detrimental to the extraction of the desired compounds and should be taken into consideration.

There are also some other aspects that should be considered in an HPE process, such as the thermodynamic properties of the individual solvent or solvent mixture applied, like density and viscosity. However, due to the typical difficulties of performing thermodynamic measurements at high pressures, the amount of equilibrium data available in the literature is reduced. Moreover, if a solvent mixture is applied, like an aqueous-alcoholic one, the phase-equilibrium diagram at the operation conditions of pressure and temperature should be verified. Experimental conditions of pressure, temperature and solvent mixture composition may be chosen so as to avoid a two- or three-phase equilibrium, since the presence of a gaseous phase in the extraction cell may be detrimental to process dynamics and target compounds solubility.

As opposed to SFE, fractionation in a HPE process does not rely mostly on solvent density which, in turn, depends on the experimental conditions of pressure and temperature chosen. Actualy, since selectivity in the HPE process is based on the capacity of the solvent to establish molecular interactions with solutes possessing functional groups, the usage of different solvents or solvent mixtures (having different compositions) at consecutive steps may be the proper choice to achieve fractionation. In this case, a first CO_2 extraction step may be performed to separate low polarity compounds. In some cases this strategy may render the remaining vegetable material compounds more available for the consecutive extraction steps [21].

The main purpose of this study was to extract phenolic compounds from pine bark using high pressure extraction methodologies and to compare these procedures to Soxhlet extraction (SoE) and to hydrodistillation (HD). Supercritical carbon dioxide ($scCO_2$) was the chosen solvent to extract the pine bark low-polarity fraction, and ethanol (EtOH) was added to $scCO_2$ in order to obtain phenolic-rich fractions. The effects of solvent flow rate and of solvent mixture composition were studied on the extraction kinetics results as well as on the CO_2:EtOH extracts composition.

2. Materials and methods

2.1. Raw material

Comminuted maritime pine bark was provided by a wood processing company from Beira Litoral, Portugal. Particles presenting a size distribution between 60 and 18 mesh were separated using sieves under mechanical stirring (Retsch, Germany). Light scattering experiments were performed using a Laser Malvern Mastersizer (Hydro 2000 MU, Worcestershire, UK) so as to determine the particle size distribution. Finally, the raw material humidity was determined by the xylol distillation method of Jacobs [22] with triplicate assays.

2.2. Chemicals

Extraction experiments were performed using carbon dioxide (99.998%, Praxair, Spain), ethanol (99.5%, Panreac Quimica S.A., Spain) and distilled water. For TLC and spectrophotometric analyses of extracts, the following analytical grade chemicals and solvents were used: Folin–Ciocalteu's phenol reagent (Merck, Germany), sodium carbonate (99.9%, Pronalab, Portugal), vanillin (José M. Vaz Pereira S.A., Portugal), ethanol (99.5%), ethyl acetate (p.a.) and *n*-hexane (99%) from Panreac Quimica S.A. (Spain), methanol (puriss. p.a.), formic acid (~98%), glacial acetic acid (p.a.), p-anisaldehyde, hydrochloric acid (37%) and sulfuric acid (95–98%) from Sigma–Aldrich Inc. (Germany), o-xylene (97%), anhydrous methanol (99.8%), 2-aminoethyl diphenylborinate (97%) from Fluka (Germany), and distilled water. Chemicals used for HPLC analysis were formic acid (98–100%, Sigma–Aldrich Inc., Germany), water (HPLC grade, Carlo Erba, Italy), and acetonitrile (HPLC grade, Fisher Scientific, UK).

Standards used for TLC and spectrophotometric analyses were quercetin dehydrate (≥98%, HPLC grade), rutin hydrate (≥95%, HPLC grade), D-(+)-catechin hydrate (98%), p-caffeic acid (99%) and gallic acid (≥98%) from Sigma–Aldrich Inc. (Germany), and (−)-epicatechin (≥90%, HPLC grade) and taxifolin (≥85%, HPLC grade) from Fluka (Switzerland). Standards used for the GC analysis were alkane standard solutions C_8–C_{20} and C_{21}–C_{40} (Fluka, Switzerland).

2.3. Experimental procedure for high pressure extractions

Fractionated and non-fractionated high pressure extractions (F-HPE and NF-HPE, respectively) were performed using a supercritical fluid extraction apparatus previously described by Braga et al. [23]. In short, a stainless steel extraction cell