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Isolation of bioactive compounds from spruce bark waste using sub- and supercritical fluids

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ABSTRACT

The aim of this study was to investigate the possibility of isolating bioactive phenolics compounds from spruce bark (*Picea abies*), using sub- and supercritical fluids. In order to improve the yields and to assure a higher recovery of the phenolic compounds, in the first part of this study, extraction of lipophilic compounds was performed using only supercritical (SC) CO_2 as solvent. Amount of obtained lipophilic compounds was quantified. In the second step, to the $SCCO_2$ a cosolvent was added, namely 70% (v/v) aqueous ethanol, in order to isolate the bioactive phenolic compounds more efficiently. Effect of temperature, pressure and cosolvent flow rate on the yield of phenolic compounds was observed, at 40–60 °C, 100–200 bar and at 1.2 mL/min and 2.5 mL/min, respectively. Obtained extracts were analysed for their total phenolics (TPC), tannins (TTC) and flavonoids (TFC) content, as well as their antioxidant activity using UV–vis spectrophotometric methods. The maximum extraction yield of phenolic compounds (30.46 ± 1.20)% was achieved at 100 bar pressure and 40 °C and the determined total phenolics content was 314.49 mg/g dry extract, the total flavonoids content was 100.67 mg/g dry extract and the total tannins content was 26.38 mg/g dry extract. Additionally, the content of different phenolic compounds, typically found in woody biomass was assessed using high pressure liquid chromatography, ferulic acid and *p*-coumaric acid being the two major quantifiable phenolic compounds identified. Lastly, the consumption of solvents for production of kg of product with highest possible purity was determined and discussed.

1. Introduction

The isolation of bioactive compounds from different vegetal materials especially wastes using novel or improved methods is a current trend in fields, such as waste management and product recovery, since annually waste biomass on global scale is produced in millions of tons [1]. Among the many possible extraction techniques available, extractions using high pressure fluids i.e. sub- and supercritical fluids (sub- and supercritical fluid extraction) are considered as an emerging alternative to the conventional extraction techniques. A fluid is considered to be in its supercritical state when forced to a pressure and temperature above its critical point. Above these conditions the properties of a fluid are between that of a gas and a liquid [2]. Subcritical fluids on the other hand are compressed (liquefied) fluids at pressures that enable them to stay in their liquid state when heated above their normal boiling point. The use of supercritical fluids (SCF) as solvents in chemical processes offers important environmental benefits (by replacing the conventional organic solvents and its low energy consumption during operation), health and safety benefits (taking into account that the most important SCFs are non-cancerogenic, non-toxic, non-mutagenic, non-flammable and thermodynamically stable) but also chemical benefits derived from thermo-physical proprieties of SCFs (high diffusivity, low viscosity, etc.), [3].

Among all the supercritical fluids, supercritical CO_2 is the most widely used solvent in high pressures processes [4,5]. The biggest advantages of supercritical CO_2 is its non-toxicity, non-flammable and non-corrosive character, its availability and cost and its easily achievable critical point. Although depending on the solubility of the wanted compounds in the CO_2, addition of co-solvents such as methanol, ethanol or water is recommended for increasing the extraction efficiency [6].

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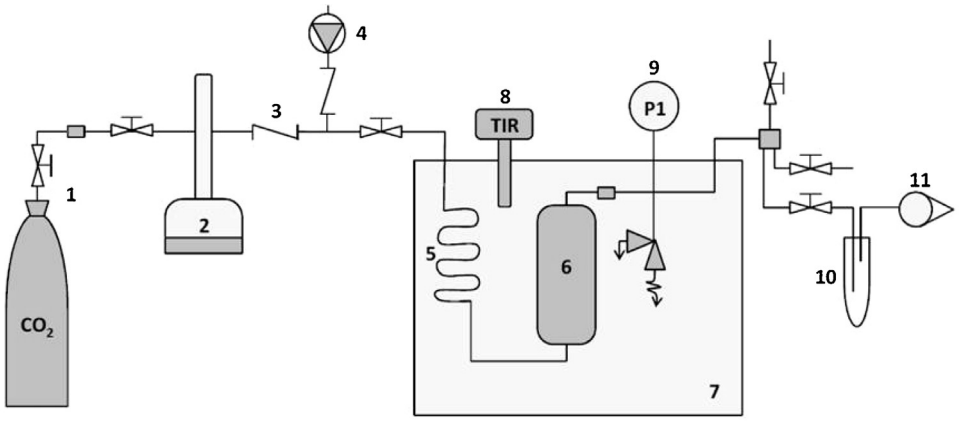

Fig. 1. Supercritical fluid extractor: 1 – Liquid CO_2 cylinder (liquid); 2 – high pressure pump; 3 – one-way valve; 4 – high pressure pump for co-solvent; 5 – preheating coil; 6 – extractor; 7 – water bath; 8 – heater; 9 – manometer; 10–sample trap; 11 – gas meter.

SFE is considered as a cleaner alternative for extraction a large class of bioactive compounds (volatiles oils, triterpenoids, triterpenes, lipids) [7–11], being environmentally friendly compared to conventional extraction techniques, since it does not require large amounts of organic solvents, is shorter and offers the possibility to recycle and reuse the supercritical fluid [12,13].

Several studies regarding the phenolic compounds extraction from different types of the trees bark, recommend SFE as a highly convenient extraction method, which produces purer extracts with higher concentrations in tannins, flavonoids, anthocyanins and phenolic acids, compared to conventional extraction methods [14–16].

Knowing that spruce bark is a rich source of phenolic compounds [17,18] and the fact that it could be provided in large amounts representing 10–20% of the tree trunk [19], since it is usually considered a waste in the wood processing and forestry industry, gives it the potential of representing a valuable resource material for other fields of industries.

The main objective of this work was to study the effect of working parameters (temperature, pressure and flow rate of solvent) required to obtain the highest purity of spruce bark extracts possible. For this purpose, semi-continuous extraction applying sub- and supercritical fluids was performed in two steps. Firstly only pure CO_2 was used, in order to remove present waxes and volatile oils, such as essential oils from the bark. In the second step, the material was exposed to a CO_2–EtOH/water (70/30 v/v) mixture, which at the observed conditions was present in two phases. The addition of water and ethanol enhances the extraction of phenolic compounds. Using the two step procedure, it is enabled to separate the lipophilic extractives from the phenolic compounds. The effects of extraction pressure, temperature and amount of co-solvent added to the CO_2 were studied in the second step and the extraction yields were determined. The composition profiles of the total phenolics, flavonoids and tannins content were observed and the extracts antioxidant activities were assessed. Also the influence of process parameters on the chromatographic profiles of the obtained final products was studied. Furthermore, solvent consumption was monitored, in order to determine the mass of solvents needed for production of kg of product.

2. Materials and methods

2.1. Plant material

The raw material was provided as a waste from the timber company Alpine LTD (Vatra Dornei, Romania). The spruce bark was quickly washed with water, in order to remove dirt particles, dried at room temperature under normal aeration conditions and grinded. The main particle size distribution (30% of particles with size <0.25 mm, 55% of particles with size between 0.25–1 mm and 15% of particles with size between 1–2 mm) was determined using a set of standard sieves. Humidity of the material (10.44 ± 0.18)% was determined by drying ~1.5 g of spruce bark at 120 °C for 10–15 min until complete water removal, using a RADWARG MAX 5011 thermo-balance.

2.2. Chemicals

All reagents, standards and solvents were of analytical grade and were purchased from Sigma–Aldrich, Fluka (Germany), Merck (Germany), Acros Organics (Belgium), and Kemika (Croatia). The CO_2 of purity 2.5 (99.5% (v/v)) was obtained from Messer (Ruše, Slovenia).

2.3. Methods: sub- and supercritical fluid extraction (SFE)

Extractions were performed in duplicate using a semi continuous apparatus (Fig. 1). Approximately 20 g of spruce bark was loaded into the extractor ($V = 60$ mL). The extractor was placed in a water bath preheated to the desired operating temperature. Liquefied CO_2 was pumped with a high-pressure pump (ISCO syringe pump, model 260D, Lincoln, Nebrasca, USA) through a preheating coil submerged in the water bath, and in to the extractor. An additional high pressure pump (LDC Analytical, ConstaMetric 3000 solvent delivery system, Riviera Beach, Florida, USA) was connected with apparatus allowing the addition of co-solvent (70% aqueous ethanol or EtOH-70) to the CO_2. CO_2 and EtOH-70 which was pumped simultaneously into the extractor and the flow rate was regulated using a thermostatic micrometric valve. CO_2 consumption was monitored using a gas meter.

The first extraction step was performed in static conditions with supercritical CO_2 ($SCCO_2$) for a selected time of 150 min studied at pressures of 100 bar, 150 bar, 200 bar and temperatures of 40 °C, 50 °C and 60 °C. After this defined extraction time period (established from preliminary tests), subsequently EtOH-70 was pumped together with the CO_2 into the extractor and the lipophilic extract was collected in a separator at ambient conditions. After the first drop of liquid ethanol extract, the extraction was halted and the second extraction step was initiated.

The second step was performed in dynamic conditions for extraction of phenolic compounds using CO_2 and EtOH-70, under the same pressure and temperature conditions as stated above. The

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