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Aqueous extraction of polyphenols and antiradicals from wood by-products by a twin-screw extractor: Feasibility study

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ABSTRACT

An efficient and eco-friendly extracting process of wood by-products (maritime pine knots and stumps and aspen knots and barks) using a twin-screw extruder has been developed. This continuous device allowed a thermo-mechanical treatment of the woody material to recover fractions containing hydrophilic polyphenols and antiradical compounds. The breakdown of woody materials and the high level of pressing of the solid/liquid mix were mainly influenced by the solvent-to-solid ratio for maritime pine knots and stumps and aspen knots, and by the solvent-to-solid ratio and the barrel temperature in the case of aspen barks. Compared to the extractives content of each by-product determined at laboratory scale, twin-screw extractions were promising for the valorisation of wood by-products and the recovery of bioactive substances.

Every year, 3.8 billions cubic meters of wood harvested worldwide are dedicated to the building or furniture manufacturing, for particle-board and paper pulp production and also energetic applications [1]. Such exploitation generates large quantities of by-products such as bark, knots and stumps [2]. These residual plant materials are discarded because of their high content in lignins, polyphenols and resins, detrimental regarding the production of paper pulp and also because of their poor mechanical properties [3–6]. Barks discarded from paper pulp industries per year amounts, in average, to 125 000–250 000 t and knots represent around 5000–10 000 t [7,8]. Since several years, a global concern of the depletion of resources led to the urge of rethinking sustainable processes including wastes recycling. Currently, various agricultural industries already applied such a valorisation scheme, e.g. wine industry with grape pomace recovery [9], or orange industry with limonene and pectin extraction from peel [10], driven by the volumes of wastes to treat, and the value-added of the products generated. By-products from paper pulp industry present advantages such as availability throughout the year, low price, long-term chemical stability and ease of management [11].

Until now, wood by-products were used for the production of steam and energy [7]. However, these residues constitute a rich source of polyphenols and antiradical compounds, efficient against cancers, cardiovascular troubles and neurodegenerative diseases [12,13]. For example, tree knotwood extracts act as strong antioxidants [14,15], also showing antimicrobial and antitumoural activities [16,17]. Extracts from *Pinus* species, due to their high content in stilbenes, turned out to be particularly active. In the other hand, tree bark extracts are also well-known for their benefits for human health such as maritime pine bark extract, commercialized under the name Pycnogenol®, rich in proanthocyanidins and used

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against venous insufficiencies, hypertension, inflammation and erythma [18]. Aspen bark extract is an ingredient of a mix called Phytodolor®, for the treatment of rheumatic diseases [19].

Given the amounts of such by-products, an extracting process in a continuous mode at industrial-scale should be designed, to obtain bioactive products from wood industry waste. Twin-screw technology allows the extraction of substances from plant matrix such as oil and pectins from sunflower [20,21], oil from rapeseed [22,23] and from coriander [24], hemicelluloses from aspen [25]. This powerful tool in terms of transformation of the starting materials needs a fine optimization of its operating parameters according to the treated vegetal matter. A twin-screw extractor is based on successive elements allowing several actions in a single step: conveying, heating and cooling, shearing, mixing, solid/liquid extraction, separation and drying [26]. Forward pitch screws allow the conveying combined to a low mixing and shearing of the plant material. Bilobe paddles carry out a radial compression on the matter, shearing and mixing of the solid and the liquid phases. Reversed pitch screws also exert an intense radial compression and a strong shearing combined with mixing of the material and the extracting liquid. The sequence of the screw elements along the profile, the barrel temperature and the solvent-to-solid ratio are the parameters influencing the performance of the extracting process indicated by extraction yields, residence time in the extractor or mechanical energy input [27–30].

So far, no study has been undertaken concerning the extraction of compounds from tree knots, barks and stumps at industrial scale. For the first time, this study set out to evaluate the new application of twin-screw extractor for the recovery of polyphenols and antiradicals and fibres from wood by-products, in a continuous mode. Two representative species of French coniferous and broad-leaves trees exploited in paper pulp industries were studied: maritime pine (*Pinus pinaster*) and aspen (*Populus tremula*). The characterization of the extraction performance and the effects of the operating parameters such as the barrel temperature and solvent-to-solid ratio were observed by the determination of plant material breakdown (extrudate and particles yields), solid/liquid separation (dry matter of the extrudate and filtrate recovery rate), the polyphenols yield and the antiradical activity of the extracts.

1. Materials and methods

1.1. Materials

All trials were carried out using a single homogenous batch of wood by-products: maritime pine (*Pinus pinaster*) knots, supplied by Smurfit Kappa (France), maritime pine stumps, supplied by Tembec (France), aspen knots, and barks, supplied by Tembec/Asian Pulp and Paper (France). After drying in an oven and grounding in a hammer mill (Electra), the powdered material was sieved to select particles smaller than 6 mm. All solvents and chemicals were obtained from Sigma-Aldrich and VWR.

1.2. Characterization of the wood

Dry matter was determined according to the French standard NF V 03-903. The protein content was determined by the Kjeldahl method [31], according to the French standard NF V18-100, applying a multiplying factor of 6.25 to the nitrogen content [32]. The mineral content was determined according to the French standard NF V 03-322. The fibres content (cellulose, hemicelluloses and lignins) was determined according to the ADF–NDF method [33]. The hydrophilic extractives content was determined with aqueous extractions performed with a Tecator Fibertec M1017 (FOSS), during 1 h at 100 °C. Lipophilic extractives content was determined with cyclohexane extractions performed with a Soxhlet, during 6 h at boiling point. Extractions and analyses were performed in triplicate.

1.3. Extracts analysis

The amount of total phenolics was assayed by means of the Folin–Ciocalteu method adapted from Singleton and Rossi (1965) by mixing Folin–Ciocalteu reagent (0.5 mL), sodium carbonate 20% (1 mL), completed with purified water (7.5 mL) and the extracts solution (1 mL) at 70 °C for 10 min and measuring the absorbance of the mixtures at 700 nm at 25 °C [34]. The results are expressed in grams GAE in the initial dry matter.

The hydrogen-donating ability of a crude extract was performed with a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay adapted from the procedure described by Brand-Williams, Cuvelier and Berset (1995), by mixing DPPH reagent with the extracts and measuring the absorbance of the mixtures at 516 nm [35]. A reaction curve was plotted as follows:

$$f(C_e) = 1 - \frac{A}{A_0}$$

where C_e is the dry matter of the extract in the reaction mixture (mg/L), A and A_0 are, respectively, the absorbance of the extract and the absorbance of the blank after 40 min of incubation in the dark. The antiradical activity was expressed in the half maximal inhibitory concentration (IC_{50}), which is the concentration required to inhibit 50% of the signal of the DPPH radical, expressed in milligram of extract per litre of mixture (mg/L). The IC_{50} was calculated as follows:

$$IC_{50} = 0.5 - \frac{\text{origin} \quad \text{ordinate}_{f(C_e)}}{\text{slot}_{f(C_e)}}$$

Analyses were performed in triplicate.

1.4. Twin-screw extractions

Experiments were conducted with a co-rotating and co-penetrating Clextral BC 45 (Firminy, France) twin-screw extruder made of seven modular barrels. Plant material (milled knots, barks and stumps) was fed into the twin-screw extractor inlet port on module 1 by a volumetric