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Short communication

Douglas bark dry fractionation for polyphenols isolation: From forestry waste to added value products

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ABSTRACT

In order to isolate polyphenols from Douglas fir outer bark, this latter was mechanically fractionated by means of knife and ball millings. The sieving of the resulting powder gave rise to a set of fractions with different particle sizes and shapes. The particles ranging from 0.56 to 0.16 mm size were mainly composed of tannins (46%) while, taxifolin was located in the coarser fraction (83%) with particles size above 1 mm. The isolated phenolic compounds are suitable of upgrading to higher value products.

1. Introduction

Douglas fir (*Pseudotsuga menziesii Franco*) is one of the premier timber trees in the world. Originated from North America, it was introduced in France before 1850 (Ponette et al., 2001), and now it covers more than 400,000 ha, positioning this country as the foremost European producer. In the last decade, the sawmill industry has gradually increased its demand for this new resource, which on a national scale caters for nearly 800,000 cubic metres of sawing timber, i.e. 12% of the overall softwood production (Ferron, 2014). As a result, large quantities of wood residues, such as barks, are produced every year. Mostly exploited for heating purpose, these side-products are combusted. Nevertheless, It was well reported that Douglas fir bark is relatively rich in flavonoids, including condensed tannins and taxifolin (Kiehlmann and Li, 1995). This barks' richness in phenolics make them an economically interesting raw material for pharmaceutical and bioactive compounds, green polymers and biobased materials.

Taxifolin, also known as dihydroquercetin is a powerful antioxidant, widely used in, pharmaceutical preparations, health care products and as additive in food and beverage industries. (Ponette et al., 2001). On the marketplace of phytochemical products, taxifolin is found in several complex preparations such as sylimarin (which is advocated for liver disease), Pycnogenol®, and venoruton® (Weidmann, 2012). However, the scarcity of pure taxifolin combined to its high cost (800–1000$/g) impedes its wide application.

Condensed tannins are polyphenols generally based on flavan-3-ol monomers. Through past centuries (and still today), they were mainly used in leather production. However, their phenolic nature allowed them to replace fossil-based phenol in many applications such as insulating foams, adhesives, polyurethanes and epoxy resins (Benyahya et al., 2014; Drovou et al., 2015). For this, tannins industry continually seeks new inexpensive feedstocks to produce tannin extracts of various qualities for the expending markets.

A plethora of methods describing the taxifolin and tannins extraction from biomass have been disclosed. The dominant process is based on refluxing extraction with solvents such as water, ethanol or acetone (El-Adawi et al., 2011; Holmbom et al., 2009; Ponette et al., 2001). Unfortunately, these extraction, and sometimes, purification methods involve long and tedious procedures employing large quantities of expensive and toxic organic solvents and could result on flavonoids alteration. Achieving high taxifolin and tannins yields without using a high quantity of chemicals and without generating wastewater is of major importance for developing flavonoids extraction at pilot or industrial scale. Dry fractionation processes based on the mechanical deconstruction of lignocellulosic biomass are designed to meet those requirements. (Barakat et al., 2013). Indeed, according to the mechanical solic-

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itations induced by the dry milling techniques, tissues could be dissociated into particles of different sizes, shapes, densities and/or compositions which are subsequently separated by an appropriate sorting step (Hemery et al., 2011). In the present study, dry fractionation processes, based on the milling of Douglas bark (by means of knife and ball mills), followed by the sieving of the resulting powder, were assessed for their potential to obtain taxifolin and tannins enriched fractions.

2. Material and methods

The raw material used for this study was supplied by Brassac Industries sawmill (Tarn region, France). It was a heterogeneous size material (chips of 5–20 cm length and 1 mm–3 cm thickness) produced from the debarking of 50 years old Douglas fir trees harvested in April 2013.

2.1. Dry fractionation of barks

Douglas fir bark (**DFB**) chips have been dried in MEMMERT oven at 40 °C over night to reach a moisture <10%.

First fractionation process: knife milling was performed in a Retsch SM 100 system with 6 mm size screen, operating at room temperature at a speed of 1500 rpm, to give fraction F0. This latter was sieved in a sieving column (ROTEX), equipped with three sieves with 1 mm, 0.56 mm and 0.16 mm size screens respectively. The sieving was carried out during 20 min by a vibrational movement. Fractions F1 (>1 mm), F2, (1–0.56 mm), F3 (0.56–0.16 mm) and F4 (<0.16 mm) were generated.

Second fractionation Process: Fraction F0 (obtained previously) was milled once more in a 5 L Faure type ball mill. This mill was equipped with 2 kg of ceramic balls (1/3 of balls with 1.2 cm diameter, 1/3 of balls with 1.6 cm diameter and 1/3 of balls with 2.6 cm diameter) at a speed of 120 rpm for two residence times of respectively 75 min and 12 h. This milling process aimed to enhance the dissociation of the more friable tissues through impact and compression stresses generated by the milling device. After milling, the sorting of particles was performed through the same sieving device, described previously. Fractions F5 (>1 mm), F6, (1–0.56 mm), F7 (0.56–0.16 mm) and F8 (<0.16 mm) were collected.

2.2. Tannins and taxifolin characterisation

The depolymerisation reaction of tannins was realized by thioglycolysis reaction. Thus, **DFB** fraction (200 mg) was suspended in 2 mL of methanol (HPLC grade), then, 2 mL of thioglycolic acid (>98%, Sigma) was added (0.8% v/v in 0.2 M HCl in methanol) in a sealed glassware. The mixture was heated at 90 °C for 6 min. The depolymerisation reaction of **DFB** condensed tannins led to the releasing of the extension units as thioether derivatives along with terminal units as neutral monomeric flavonoids. During the depolymerisation reaction, taxifolin, which is not included in the tannins polymeric chain, does not undergo any structural modification.

The Flavonoid derivatives identification and quantification were realized by UPLC–MS analyses. The LC-DAD-ESI/MS consisted of an Acquity UPLC (Waters, Milford, MA) equipped with a photodiode array detector. The column (HSS T3, 100 × 2.1 mm, 1.8 mm) was a Nucleosil 120–3C18 endcapped (Macherey-Nagel, Sweden). The flow rate was 0.55 mL min−1, and the gradient conditions were as follows: solvent A (H_2O–HCOOH, 99/1, v/v), solvent B (CH_3CN–H_2O–HCOOH, 80/19/1, v/v/v); initial 0.1% B; 0–5 min, 60% B linear; 5–7 min, 99% B linear; 7–8 min, 99% B isocratic; and 8–9 min, 0.1% B linear. The DAD was set at 280 nm and the molar relative response factor of each monomer was determined at this wave length. The Acquity UPLC system was coupled online with an amaZon X ESI Trap mass spectrometer (Bruker Daltonics, Bremen, Germany). In the source, the nebulizer pressure was 44 psi, the temperature of dry gas was set at 200 °C with a flow of 12 L min^{-1} and the capillary voltage was set at 4 kV. The mass spectra were acquired over a mass range of 90–1500 Th in the positive ionization mode. The speed of mass spectrum acquisition was set at 8.1 m/z min^{-1}.

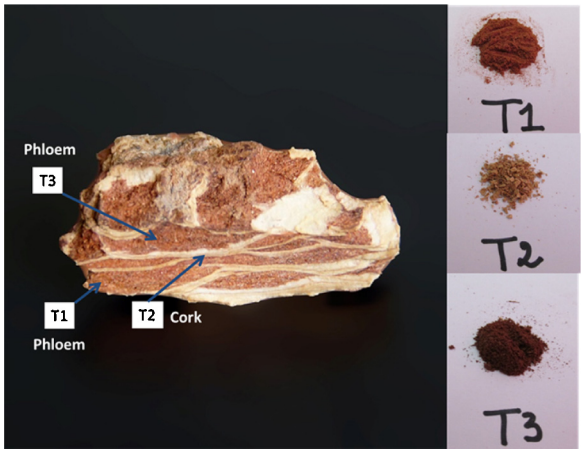

Fig. 1. a cross sectional picture depicting the main tissues composing **DFB** sample. Tissues T1–T3 after manual dissection and liquid nitrogen assisted grinding.

3. Results and discussion

A cross sectional picture of a Douglas fir bark (**DFB**) specimen is shown in Fig. 1. As described by Ferreira et al. (2015), the outer bark (also called rhytidome) is consisting of a sequential layers of cork (T2) interspersed by phloemic tissues. The darkest one (T3) is the oldest phloem and it is located on the external part. The youngest phloem (T1), with a slightly lighter colour, is close to the living inner bark.

The three parts T1–T3 were separated by manual dissection. Then each of them was milled in liquid nitrogen precooled Dangoumau grinder (Longjumeau, France). This operation showed that tissues T1 and T3 are brittle and crumbly while tissue T2 is harder, which was reflected in the differences of particle sizes and shapes (Fig. 1). This observation is in accordance with Miranda et al. studies which showed that the cork is a closed-cell material with a large elastic compression and a dimensional recovery (Sen et al., 2010), while the phloem is mainly constituted of fragile and thin walled parenchyma cells (Miranda et al., 2013).

In order to determine the phenolic composition of the three fractions cited above, taxifolin and the monomeric units (extension and terminal units), generated by the tannins depolymerization were quantified (Table 1).

As clearly depicted in Table 1, taxifolin is synthesized and/or stored in the cork T2 and tannins are located in the internal part of phloem, close to the sapwood (T1). However, the external phloem (T3) is rather low in flavonoid compounds.

Based on these preliminary results, the two fractionation processes described in the material and methods section were performed in the aim to obtain enriched fractions in taxifolin and tannins (Fig. 2). Fractions F1–F4 were generated from the first process while the second one gave rise to fractions F5–F8.

The phenolic composition of the different fractions resulting from both protocols was summarized in Table 2 (see supporting information for taxifolin and tannins yields).

As shown in Table 2, fraction F0 is composed of 5.1% of flavonoid derivatives including 3.0% of condensed tannins (as terminal and extension units) and 2.1% of taxifolin (Fig. 3).