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Thermo-mechano-chemical extraction of hydroxycinnamic acids from industrial hemp by-products using a twin-screw extruder

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ABSTRACT

This work aims at developing a continuous intensified green process to extract high-value added molecules from industrial hemp by-products. Hemp hurds and hemp dust were studied as potential sources for the production of two hydroxycinnamic acids (HCA): ferulic (FA) and *p*-coumaric acids (*p*-CA). Prior to pilot scale extraction, FA and *p*-CA analytical contents were evaluated to 0.3 and 3.5 g/kg dry matter (DM) for hemp hurds and 0.1 and 0.8 g/kg DM for hemp dust as potentials of reference. The continuous pilot scale extraction was then carried out using twin-screw extrusion. Mild conditions were developed: 50 °C, alkaline aqueous or hydroalcoholic solvent (less than 0.5 M NaOH) and low liquid to solid ratios. The mechanical effect helps the diffusion of the solvent, promotes the hydrolysis of the ester and ether bonds and favors the extraction of HCA in a short time. Yields in *p*-CA and FA reached 50% and 33% of the free and bound contents for hemp hurds. For hemp dust, all of *p*-CA was extracted whereas 60% of FA was recovered. The solid residue may be submitted to a second extraction stage with a polar solvent in order to increase HCA recovery. Extraction by extrusion could be seen as an alternative green processing technique as it is responsible for a reduction of extraction time and energy and a decrease in solvent and reagent consumptions.

1. Introduction

Hemp or *Cannabis sativa* L. is one of the oldest non-food crops that has been cultivated for centuries. After a severe decline in the 19th century, this crop has been reintroduced recently thanks to its numerous advantages (Struik et al., 2000; Amaducci et al., 2015). This non-food crop can be grown with high yielding under a wide variety of agro-ecological conditions. It is a valuable annual preceding crop in rotations with a deep rooting system which has a favorable influence on the soil structure. Compared to the major crops maize and stem, hemp requires little fertilizing and does not suffer from any pest or disease. The processing of hemp into its main products and by-products requires no additives and no water. It allows producing different useful components from biomass, from seeds to fibers and other by-products that make it an excellent multi-output system. It is currently cultivated in China, Canada and Europe. According to the European Industrial Hemp Association, France is the 2015 European leader in terms of production or transformation with more than half of the European hemp cultivated areas (Carus and Sarmento, 2016).

Farming of hemp provides seeds or fibers. Hemp for seeds application is farmed 40 days after hemp straw. 35% of hemp crops are then grown for hempseed farming. Among hempseeds applications, alimentary uses for human nutrition (Matthäus and Brühl, 2008) and animal feeding are the most relevant thanks to high omega 3 and 6 contents in the oil and high levels in nutritional compounds in the residual meal (Pojic et al., 2014). Concerning hemp straw, the mechanical farming of industrial hemp provides fibers as the main product of interest and two by-products: hemp hurds or shives (55%/straw) and hemp dust (10–20%/straw). The principal non energetics uses of fibers are: special paper manufacturing, insulation in building, materials added in biocomposites (Karus and Vogt, 2004). It can also be used for bioenergy production (Rehman et al., 2013). The hemp by-products are both used as animal bedding as main application thanks to their high absorption capacity (Karus and Vogt, 2004). Hemp hurds could also be integrated as filler in plastic composites (Terzopoulou et al., 2016; Li et al., 2017), used in buildings (Shea et al., 2012) or in antibacterial applications (Khan et al., 2015). It can also be considered as raw material for a fractionation process into Organosolv lignin (Gandolfi et al., 2014), sugar streams to produce L-lactic acid (Gandolfi et al., 2015) or for a Formosolv process to produce pulp (De Vega and Ligero, 2017). Concerning hemp dust, its impact on CO_2 and N_2O emissions has been recently tested on soils after its transformation into hydrothermally

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carbonized char (Dicke et al., 2015). The pretreatment of hemp dust by supercritical CO_2 extraction could represent a first step in a biorefinery process and could provide crude waxes rich in high value added lipophilic molecules (Hunt et al., 2015).

As all of lignocellulosic materials, hemp by-products can be considered as a Lignin-Carbohydrates Complex (LCC) (Buranov and Mazza, 2009). Lignins are tridimensional amorph polymers acting as a resin to strengthen the cellulosic matrix (Zakzeski et al., 2010). They are constituted of C9 phenylpropenyl units whose precursor monomers are *p*-coumarylic, coniferylic and sinapylic alcohols. Constitutive units of these monolignols in the polymer are respectively *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units that are linked through carbon–-carbon and carbon-oxygen bonds. The proportion in phenylpropane units depends on the vegetal material. For hemp hurds, the S/G ratio has been evaluated to 1.42 (Gandolfi et al., 2013). Lignin is associated with polysaccharides and especially hemicelluloses through covalent links resulting in LCC (Acosta-Estrada et al., 2014). In addition to polysaccharides and lignins, LCC complexes contain phenolic acids and mostly hydroxycinnamic acids (HCA) such as ferulic acid (FA) and *p*-coumaric acid (*p*-CA). These HCA are mainly ester-bound to hemicellulose and ester- and ether-bound to lignin. A small part can also be found under free form in the LCC structure (Acosta-Estrada et al., 2014). It has been proven that FA is principally ether-bound to lignin and that *p*-CA is rather ester-bound to lignin (Cornu et al., 1994). FA can also be found as dimers between two hemicelluloses chains (Manach et al., 2004).

HCA are naturally used in both chemical and structural plant defense strategies. Thanks to their antioxidant and/or antimicrobial character, they are currently used in food as preservatives (Kroon and Williamson, 1999) or in cosmetics as photoprotectives (Saija et al., 2000). They have also demonstrated several pharmacokinetic properties and have been shown to present beneficial effects in various human diseases (El-Seedi et al., 2012; Boz, 2015). FA may also be useful as a precursor for the biosynthesis of high value-added molecules such as vanillin (Zamzuri and Abd-Aziz, 2013) or vanillic acid (Tang et al., 2014).

The extraction processes of phenolic acids have been essentially developed at analytical scale to evaluate the global content in phenolic acids in plants. These extraction and analysis conditions were reviewed (Barberousse et al., 2008; Acosta-Estrada et al., 2014; Capriotti et al., 2015). Focusing on FA and *p*-CA that are mainly bound to the structural components of plants, extracting them involves hydrolyzing chemical bonds and recovering the released molecules in a polar solvent. Alkaline hydrolysis is preferred as acid hydrolysis breaks glycosidic bonds and solubilizes sugars but leaves ester-bonds intact (Barberousse et al., 2008). Mild to harsh alkaline operating conditions were then developed with varying concentrations of sodium hydroxide (0.5–4 mol/L), liquid to solid ratio (10–30), temperature (room temperature to 170 °C) and time (few minutes to several hours) (Mussatto et al., 2007; Tilay et al., 2008; Torre et al., 2008; Buranov and Mazza, 2009; Max et al., 2010). The concentrations in sodium hydroxide expressed in mol/L could also be reported to the raw materials weight. In average, the alkaline hydrolysis uses high quantities of sodium hydroxide, between half to more than twice the mass of raw material. Such conditions are highly reagent consuming and lead to brown alkaline extracts difficult to purify. Further studies were achieved to reconcile high yields of extraction with milder operating conditions, more suitable to a potential scale up. The batch hydrolysis was improved by ultrasound- or microwave-assistance. Ultrasound-assistance decreases reaction times since the surface contact area between solid and liquid is increased by particle disruption and microwave-assistance increases extraction yields with reduced solvent consumption and reaction times by enabling the rupture of plant tissues (Beejmohun et al., 2007; Chiremba et al., 2012).

As alternatives to alkaline extraction, working with pressurized polar solvents (water and ethanol) promotes the one-step production of vanillin from FA in flax shives, corn bran and wheat bran (Buranov and Mazza, 2009). Weak nucleophiles are efficient for the selective cleavage of the aliphatic double bond in FA unit and the ether bond between lignin and FA in the lignin/phenolics-carbohydrates complexes at high temperatures (180–220 °C) and pressures (5.2–8 MPa).

The extraction and purification of FA and *p*-CA at pilot scale were studied on 2 kg samples of sugarcane bagasse (Ou et al., 2007, 2009) in a 30 L batch reactor. Mild alkaline experimental conditions were developed: a liquid/solid ratio of 10, 10–20 g NaOH/L and 0.1 g/L Na_2SO_3, 40–50 °C, 4 h. These conditions were slightly modified for corn bran extraction. An alkaline hydro-alcoholic extraction (10 g NaOH/L; 50/50 ethanol/water) for 2 h at 75 °C released 83% of bound FA (Zhao et al., 2014). No continuous, pilot extraction process was detected.

Some food pretreatments enhance the release of bound phenolics. Thermomechanical process such as extrusion-cooking leads to a large increase in free FA in cereals by the breaking of conjugated moieties (Zielinski et al., 2001). Apart from extrusion cooking, twin-screw technology has been developed for the continuous extraction of different substances from vegetable raw materials. For example, it is possible to extract vegetable oil from various seeds (Savoire et al., 2013), proteins from alfalfa (Colas et al., 2013), hemicelluloses from straw and bran (Jacquemin et al., 2015), polyphenols from wood bark (Celhay et al., 2014). This process needs an accurate optimization of its operating parameters according to the treated raw material. A twin-screw extruder designed as an extractor combines several actions in a single operation: conveying, heating and cooling, shearing, mixing, solid/liquid extraction and separation, drying. The screw profile, the barrel temperature and the solvent (type, liquid/solid ratio) are the parameters influencing the performance of the extraction process that can be quantified by extraction yields, residence time in the extractor and mechanical energy input (Gogoi et al., 1996; Gautam and Choudhury, 1999).

The development of a continuous mild process for the extraction of hemp by-products with an optimal recovery of free and bound FA and *p*-CA has been studied. Thermo-mechano-chemical extraction by twin screw extrusion was chosen with a focus on the solvent effect.

2. Material and methods

2.1. Raw materials

Hemp hurds and hemp dust were provided by Agrofibre (Cazères, France). They were used as received for twin-screw extraction and milled to 0.5 mm (Pulverisette Fritsch Bro 181) for all other experiments.

Sodium hydroxide (99%), gallic acid (99%), *p*-coumaric acid (98%), ferulic acid (98%), ethanol (96%, technical grade), acetonitrile, dioxane and toluene (all HPLC grade) were purchased from Sigma Aldrich (L'Isle d'Abeau, France). Folin-Ciocalteu reagent and Na_2CO_3 (99%) were from VWR (Fontenay-sous-Bois, France). Hydrochloric acid (37%) was purchased from Fisher Scientific (Illkirch, France).

2.2. Analytical evaluation of raw materials and products

2.2.1. Characterization of hemp by-products and extrudates

Dry content was evaluated by drying the sample at 103 °C for 24 h. Mineral content was measured by the calcination of the dry sample during 6 h at 550 °C. Organic content was calculated as the difference between dry and mineral contents.

The lipid and protein contents of the hemp by-products were evaluated according to the French standards NF V03-908 and NF V18-100.

The Van Soest method (Van Soest et al., 1991) was adapted and used to estimate the cell wall constituents (cellulose, hemicelluloses and lignins) in raw materials and extrudates after twin-screw extraction.

2.2.2. Analyses of the extracts and filtrates

Dry and mineral contents were evaluated as in the 2.2.1. section.