



## Open Archive Toulouse Archive Ouverte (OATAO)

OATAO is an open access repository that collects the work of Toulouse researchers and makes it freely available over the web where possible

This is an author's version published in: <http://oatao.univ-toulouse.fr/25143>

**Official URL:** <https://doi.org/10.1016/j.indcrop.2017.08.044>

### To cite this version:

Candy, Laure<sup>ORCID</sup> and Bassil, Sabina<sup>ORCID</sup> and Rigal, Luc<sup>ORCID</sup> and Simon, Valérie<sup>ORCID</sup> and Raynaud, Christine<sup>ORCID</sup> *Thermo-mechano-chemical extraction of hydroxycinnamic acids from industrial hemp by-products using a twin-screw extruder.* (2017) *Industrial Crops and Products*, 109. 335-345. ISSN 0926-6690

Any correspondence concerning this service should be sent to the repository administrator: [tech-oatao@listes-diff.inp-toulouse.fr](mailto:tech-oatao@listes-diff.inp-toulouse.fr)

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

Laure Candy<sup>a,b,\*</sup>, Sabina Bassil<sup>a,\*</sup>, Luc Rigal<sup>a</sup>, Valerie Simon<sup>a</sup>, Christine Raynaud<sup>a,b</sup>

<sup>a</sup> Laboratoire de Chimie Agro-industrielle (LCA), Université de Toulouse, INRA, INPT, Toulouse, France

<sup>b</sup> Centre d'Application et de Traitement des Agroressources (CATAR), INPT, Toulouse, France

## ARTICLE INFO

### Keywords:

*Cannabis sativa*  
Hemp hurds  
Hemp dust  
Twin-screw extrusion  
Ferulic acid  
*p*-Coumaric acid

## 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 Sarmiento, 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 (Mathäus 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 Fornosolv process to produce pulp (De Vega and Ligeró, 2017). Concerning hemp dust, its impact on CO<sub>2</sub> and N<sub>2</sub>O emissions has been recently tested on soils after its transformation into hydrothermally

\* Corresponding authors at: Laboratoire de Chimie Agro-industrielle (LCA), Université de Toulouse, INRA, INPT, Toulouse, France.

E-mail addresses: laure.candy@ensiacet.fr (L. Candy), bassisabiina@hotmail.com (S. Bassil).

carbonized char (Dicke et al., 2015). The pretreatment of hemp dust by supercritical CO<sub>2</sub> 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 C<sub>9</sub> phenylpropanyl units whose precursor monomers are *p* coumarilic, coniferyl and sinapyl 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<sub>2</sub>SO<sub>3</sub>, 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<sub>2</sub>CO<sub>3</sub> (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.

Organic content was calculated as the difference between dry and mineral contents.  $Y_{EXT}$  is the yield in organic matter calculated as the ratio between the organic content of the extract and of the raw organic matter (g/kg ROM).

Total polyphenol content (PP) was determined using adapted Folin Ciocalteu method (Singleton and Rossi, 1965) with gallic acid as a standard. The standards or the samples (1 mL) were mixed with Folin Ciocalteu reagent (0.5 mL) and 20% sodium carbonate solution (1 mL) completed with water (7.5 mL). After 10 min at 70 °C, the absorbance at 700 nm was measured at 25 °C. The result was expressed in gallic acid equivalent (g GAE/kg) and referred to the initial organic matter or to the extracted organic matter. The first represents the polyphenols extraction yield or  $Y_{PP}$  (g GAE/kg ROM) and the second the content in PP of the extract (g GAE/kg EOM).

Qualitative and quantitative analyses of HCA (*p* CA and FA) were achieved as previously described (Simon et al., 2015). They were evaluated at a 0.7 mL min<sup>-1</sup> flow rate via an HPLC DAD reverse phase system (Dionex Ultimate 3000 HPLC) equipped with an Omnispher C<sub>18</sub> column (3 mm × 100 mm; 3 μm) (Varian, France) at 30 °C. Eluents were (A) water (acidified to pH = 2 with H<sub>3</sub>PO<sub>4</sub>) and (B) acetonitrile. The gradient was: 0–5 min 95% A, 5–10 min 90% A, 10–17 min 72% A, 17–22 min 0% A, 22–24 min 0% A, 24–28 min 95% A. Injection volume was 10 μL. HCA were quantified at 280 and 320 nm wavelengths. The concentration of each component was calculated using the calibration curve from the corresponding synthetic solution. The results were expressed as  $Y_{FA}$  and  $Y_{p\ CA}$  which are FA and *p* CA extraction yields calculated as the ratio between the FA and *p* CA contents in the extract and the raw organic matter (mg/kg ROM).

### 2.3. Determination of cell wall ferulic and *p* coumaric acids

The distribution of free and bound to cell wall FA and *p* CA in hemp by products has been evaluated by sequential extractions. The experimental protocols were adapted from literature (Lozovaya et al., 1999; Sun et al., 2002; Xu et al., 2005; Li et al., 2008). Extractions were carried out in triplicate.  $Y_{FA}$  and  $Y_{p\ CA}$  were measured for each fraction by HPLC analysis.

#### 2.3.1. Free FA and *p* CA

2 g of hemp by product has been extracted for 6 h with 200 mL 60/40 ethanol/water (v/v) in a Soxhlet apparatus. The extract was concentrated under vacuum, dissolved in 50/50 acetonitrile/water and analyzed (free HCA).

#### 2.3.2. Free and bound FA and *p* CA

10 g hemp by product were extracted with 300 mL of 4 M NaOH solution (L/S = 30, 500% NaOH/ROM) in a 1 L reactor at room temperature under magnetic stirring for 24 h. Sample was centrifuged and the liquid phase was neutralized to pH = 6 by adding 6 M HCl. Hemicelluloses were precipitated by adding ethanol 95% (3 v/v) and removed by centrifugation. After concentration, the extract was acidified to pH 2 with HCl 6 M and analyzed (free and bound HCA). This extraction will be referred as batch alkaline reference extraction.

#### 2.3.3. Ester linked HCA

20 g of hemp byproducts were extracted with 200 mL toluene ethanol (2:1, v/v) mixture for 6 h in a Soxhlet extractor to remove waxes. Extracts were concentrated then dissolved in 15 mL 50/50 acetonitrile/water (v/v) and analyzed by HPLC. The solid residue was dried at 40 °C until constant weight. 50 mL of NaOH 1 M were added to the solid residue (L/S = 25, 50% NaOH/ROM) in a multi reactors apparatus (STEM Omni OS6250 Series Reaction Stations, Bibby Scientific). After 18 h at 30 °C under nitrogen and stirring (300 rpm), the sample was centrifuged (8000 rpm, 7 °C, 15 min). The solid residue was washed with water until neutral pH and then dried in an oven at 55 °C for 16 h. The combined supernatant solutions were acidified to

pH = 6 with 6 M HCl and concentrated under reduced pressure. The solubilized hemicelluloses were isolated by precipitation with ethanol 95% (3 v/v), separated by centrifugation (9000 rpm, 10 °C, 5 min), washed with ethanol 70% and air dried until constant weight. The ethanolic extract was concentrated and acidified to pH = 1.5 with 6 M HCl. Precipitated soluble lignins were isolated by centrifugation (9000 rpm, 20 °C, 10 min), dried in an oven at 40 °C for 12 h until constant weight. The residual extract was analyzed by HPLC (ester linked HCA).

#### 2.3.4. Ether linked FA and *p* CA

Ether linked HCA were calculated as the difference between free and bound HCA and the sum of free and ester linked HCA.

### 2.4. Discontinuous extractions

#### 2.4.1. Analytical extractions

The water and hydroalcoholic extractives were evaluated in raw materials and extrudates by measuring the mass loss for test samples after 1 h refluxing at a L/S ratio of 100. This method has been adapted according to the standard TAPPI 204 cm 97 on the Fibertec Tecator M1017 apparatus. All extractions were carried out in duplicate. The extracts, after solid/liquid separation were analyzed.  $Y_{EXT}$ ,  $Y_{PP}$ ,  $Y_{FA}$  and  $Y_{p\ CA}$  were determined.

#### 2.4.2. Batch solvent extraction

25 g of hemp by product were extracted with 400 mL water or W/E 40/60 (L/S = 16) in a 1 L reactor at room temperature under mechanical stirring for 24 h. Sample was centrifuged and the liquid was recovered and analyzed. Extractions were triplicated.  $Y_{EXT}$ ,  $Y_{PP}$ ,  $Y_{FA}$  and  $Y_{p\ CA}$  were determined.

### 2.5. Twin screw extrusion

Thermo mechano chemical extractions were achieved with a co penetrating, co rotating twin screw extruder Cleextral BC21 (Firminy, France). The extruder consists in 7 modular barrels, each 100 mm length. It was equipped with different segmental screw elements of 12.5, 25 and 50 mm length (Fig. 1). Modules were thermo regulated by a heater band and cooled by water circulation. A filter section was inserted at module 6 to collect the filtrate. The filters consist of hemispherical dishes with conical holes (1 mm entry, 2 mm exit). Screw rotation speed ( $S_S$ ), hemp by products input flow rate and barrel temperatures were monitored from a control panel (Operator Intouch version 1.00 Software).

Hemp by products were fed into the first module using a volumetric screw feeder. The first part of the solvent or the alkaline solution was introduced through a piston pump at the module 2 level. Two zones of mechanical pressure, each consisting of 5 bilobal paddle screws, were applied in modules 3 and 4 and ensured grinding and mixing of the solid with a first liquid fraction. Another liquid fraction was injected through a piston pump at the module 5 level. It has a washing effect under mechanical and thermal effects. Modules 2–4 corresponded to the liquid/solid extraction zone whereas modules 6–7 were responsible for the solid/liquid separation. The reverse pitch screws in module 7 were prone to create a zone of high mechanical pressure resulting in the formation of a dynamic plug. The liquid phase (raw filtrate) flew through the filter and the solid phase or “extrudate” was freely ejected at the seventh module.

Twin screw extraction was performed 15 min before any sampling to ensure stabilizing the operating conditions. The raw filtrate and the extrudate were then collected during 15 min. Each extraction was performed once. The parameters directly measured during the extrusion were:  $Q_{RM}$  and  $Q_{ROM}$  = raw and organic matter feed rates (kg/h), L/S = liquid to solid ratio (kg/kg), W/E ratio = relative composition of the solvent in water and ethanol (v/v), %NaOH/ROM = NaOH

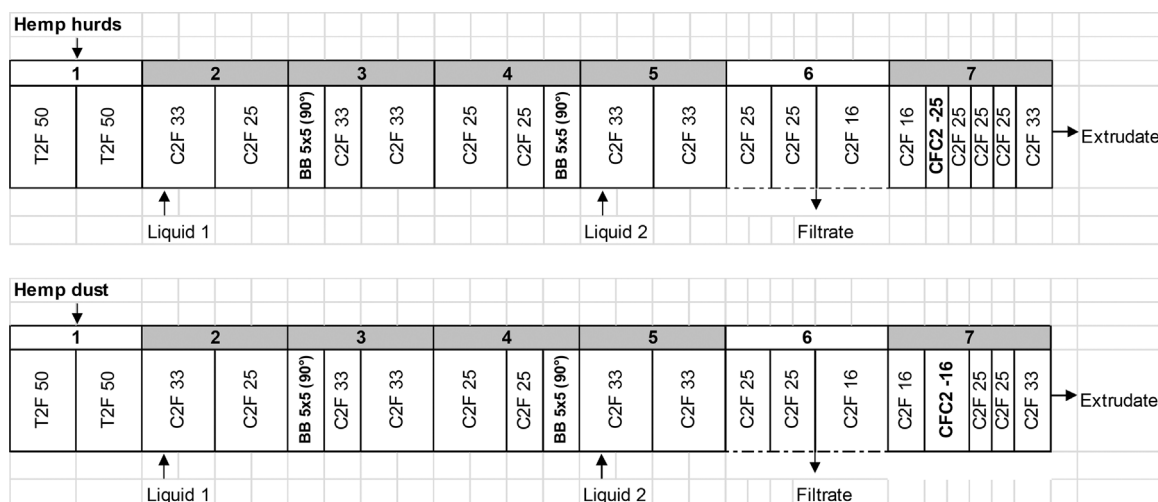


Fig. 1. Screw configurations of the thermo-mechano-chemical extraction of hemp by-products using a twin-screw extruder (BC21, Cleextral).

T2F: Trapezoidal double-thread screws, C2F: Conveying double-thread screws, CF2C: Reversed double-thread screws, BB: Bilobe paddle screws (mixing). The number following the type of element indicates the pitch of the screw for T2F, C2F and CFC2 and the angle between 2 consecutive BB.

quantity related to raw organic matter,  $Q_{L1}$  and  $Q_{L2}$  = inlet flow rates of liquids (kg/h).

The raw filtrate was centrifuged (Sigma 16 K, 9000 rpm, 7 min, 15 °C) to separate the fine solid particles that passed through the filtering barrel. The filtrate was separated and the sediment was weighted. This solid is referred as fine particles (FP).

Filtrates, extrudates and fine particles were weighted and analyzed as described in Section 2.2.

$Q_E$ ,  $Q_{FP}$  and  $Q_F$  are the extrudate, fine particles and filtrate flow rates (kg/h)

DM and OM are the dry and organic matter contents of each fraction (%).

$Y_E$ ,  $Y_{FP}$  and  $Y_F$  are the yields in organic matter, calculated as the ratio between the organic contents of each fraction and of the raw matter (g/kg ROM).

The filtrates were analyzed.  $Y_{PP}$ ,  $Y_{FA}$  and  $Y_{pCA}$  were determined.

The specific mechanical energy (SME) consumed by the motor per unit weight of raw matter was calculated as:

$$SME = P/Q_{RM}$$

$$P = U \times I \times \cos\phi \times (S_s/S_{max})$$

Where  $P$  is the electric power supplied by the motor (W),  $U$  is the motor's operating voltage ( $U = 460$  V),  $I$  is the current feeding the motor (A),  $\cos\phi$  is the theoretical yield of the extruder's motor ( $\cos\phi = 0.95$ ), and  $S_s$  and  $S_{max}$  are the trial speed and maximal speed (682 rpm) of the rotating screws (rpm).

$Q_{RM}$  is the raw matter feed rate (kg/h).

### 3. Results and discussion

#### 3.1. Raw materials characterization

Hemp hurds are constituted of 87% of structural components with a 58/21/21 repartition in cellulose/hemicellulose/lignin. Hemp dust presents a higher content in minerals and 40% of the raw material can

be solubilized under neutral detergent conditions. The cellulose/hemicellulose/lignin repartition is 71/17/12 (Table 1).

The distribution of free and bound (ester and ether links) FA and  $p$  CA was determined for each sample (Table 2). In hemp hurds, the free/ester linked/ether linked distribution is 14/46/40 for  $p$  CA and 9/2/89 for FA whereas for hemp dust these repartitions are 20/70/10 for  $p$  CA and 13/24/63 for FA. The quantified HCA are present at low levels under free form. The main part of FA is etherified for both by products while  $p$  CA is mostly ester linked for hemp dust and equally ester and ether linked for hemp hurds. The alkaline experimental conditions we used to evaluate the bound HCA were harder than the mild alkaline conditions that provided ester linked HCA but softer than the severe alkaline conditions proposed in the literature (Lozovaya et al., 1999) to cleave all of the ester and ether linked HCA. The ether linked HCA were then underestimated. Nevertheless these values were considered as references to evaluate the efficiency of the twin screw extraction process.

#### 3.2. Discontinuous extractions

Two kind of discontinuous extractions have been achieved (Table 3). For analytical extractions, the operating parameters promote the extractability: milling of the material to 0.5 mm, high L/S ratio and high temperature. These conditions minimize the kinetic and diffusional limitations. For solvent batch extractions, also known as macerations, the influence of the polarity in the softest conditions with no heating and long extraction time was evaluated. Both types of extractions used water and hydroalcoholic mixture as green solvents for their known affinity for free phenolic acids. To reach the highest extraction yields in HCA, an alkaline batch extraction is usually used. It implements a large excess of sodium hydroxide (500%/ROM) to ensure a major rupture of ester and ether bonds.

Both hemp by products present a similar behavior versus the operating conditions and equivalent quantities of extracted polyphenols (Fig. 2). Without alkaline reagent,  $Y_{PP}$  were low, less than 10 g GAE/kg ROM, especially in batch conditions. The use of a hydroalcoholic

Table 1  
Chemical composition of hemp by-products (%/DM).

	Dry matter (%)	Minerals	Cellulose (C)	Hemicelluloses (HC)	Lignins (L)	C + H + L	NDF extractives	Proteins	Pectins	Lipids
Hemp hurds	91.7 ± 0.1	2.8 ± 0.1	50.6 ± 3.1	18.2 ± 0.5	18.0 ± 2.8	86.8	13.2 ± 0.1	3.4 ± 0.1	1.8 ± 0.1	2.6
Hemp dust	89.9 ± 0.1	13.2 ± 0.1	43.9 ± 0.4	10.7 ± 0.4	7.0 ± 0.4	61.7	38.3 ± 0.1	8.4 ± 0.1	4.3 ± 0.2	0.6

**Table 2**  
Content of free and bound (ester- and ether-linked) FA & p-CA in hemp by-products.

		p-CA				FA			
		Free + Bound	Free	Ester-	Ether-	Free + Bound	Free	Ester-	Ether-
Hemp hurds	(mg/kg ROM)	3540	507	1613	1420	300	26	6	268
	(Relative%)	100	14	46	40	100	9	2	89
Hemp dust	(mg/kg ROM)	950	202	700	98	160	21	38	101
	(Relative%)	100	20	70	10	100	13	24	63

**Table 3**  
Discontinuous analytical and batch extractions of hemp hurds (trials H) and hemp dust (trials D).

Trial	Operating conditions				Y <sub>EXT</sub> (g/kg ROM)	Y <sub>PP</sub> (g GAE/kg ROM)	Y <sub>FA</sub> (mg/kg ROM)	Y <sub>p-CA</sub> (mg/kg ROM)
	T (°C)	(L/S)	W/E ratio	% NaOH/ROM				
H1	100	100	100/0	0	82	5.0	80	475
H2	78	100	40/60	0	68	6.9	30	207
H3	20	16	100/0	0	19	1.1	3	15
H4	20	16	40/60	0	26	2.4	5	54
H5	20	30	100/0	500	836	12.6	300	3540
D1	100	100	100/0	0	163	8.3	20	64
D2	78	100	40/60	0	113	7.8	30	61
D3	20	16	100/0	0	78	0.8	< 1	< 1
D4	20	16	40/60	0	56	5.1	7	17
D5	20	30	100/0	500	973	16.8	160	950

solvent demonstrated a positive effect on Y<sub>PP</sub>. The polyphenol content of the extract, defined as the ratio between the extracted polyphenols and the global extracted organic matter were higher in the case of hydroalcoholic solvents that were then far more selective for polyphenols extraction. High liquid/solid ratios at boiling temperatures promoted the polyphenols extraction by acting on kinetics and diffusional limits.

Compared to the alkaline hydrolysis, the discontinuous extractions only extract low HCA quantities, corresponding more or less to the free HCA (Fig. 3). The polarity of the solvent did not demonstrate a major influence on yields. Extracting at reflux at high liquid/solid ratios enhances the extraction for identical reasons advocated for polyphenols. Nevertheless, to reach higher extraction yields, the breaking of the chemical bonds in the LCC should be raised.

### 3.3. Twin screw extraction

#### 3.3.1. General strategy

The efficiency of the liquid/solid extraction in the twin screw extruder is the result of the three consecutive defibering steps that

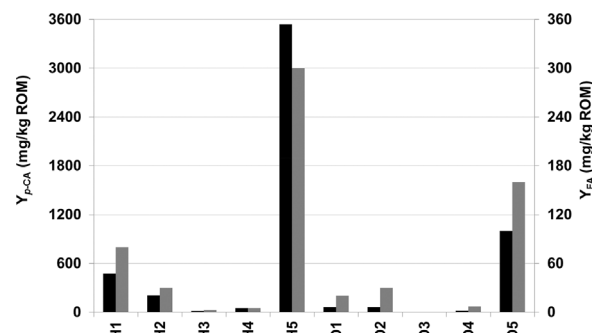


Fig. 3. Yields in p-CA (black) and FA (grey) for the discontinuous extractions of hemp hurds (trials H) and dust (trials D).

increase the exchange surfaces between solid and liquid. It is also the result of the enhanced contact with the injected solvent and of the liquid/solid separation during the pressing and filtration steps.

The HCA extractability in the two raw matters matrices has been evaluated. This consists in the determination of the operating parameters for which the extruder could be forecast as a process for extraction and solid/liquid separation. As the yields of extraction in HCA are highly correlated to the solvent and increased with a polar alkaline solvent, the influence of the following parameters was studied: the temperature (50 or 100 °C) for an aqueous extraction and the solvent for a fixed temperature of 50 °C. Water, 60% ethanol, aqueous alkaline solvent (10 50% NaOH/ROM) and hydroalcoholic alkaline solvent (10 20% NaOH/ROM in ethanol 60%) were chosen. Such mild conditions are compatible with the technological constraints of the extruder but also with environmental specifications.

It is known that alkaline treatments of lignocellulosic matters dissolve lignins, proteins and hemicelluloses with a variable efficiency. They weaken and break inter molecular hydrogen bonds leading to the solubilization of hemicelluloses and the swelling of the crystalline structure of the cellulose. Using a twin screw extruder as a fractionation process, only alkaline conditions (20 35% NaOH/DM) allowed a joint extraction of hemicelluloses and lignins (Vandenbossche et al., 2014).

Regarding the lignocellulosics structure, the release of p CA

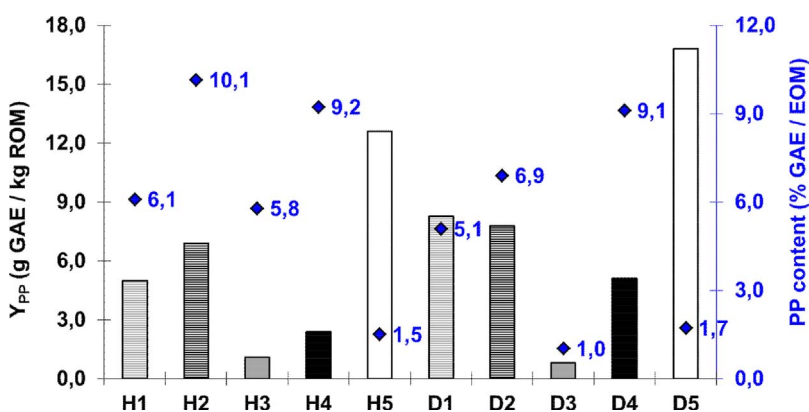


Fig. 2. Yields in polyphenols and polyphenol contents for discontinuous extractions of hemp hurds (trials H) and dust (trials D).

essentially concerns the breaking of the esters bonds with lignins whereas the release of FA concerns ester bonds with hemicelluloses and ether bonds with lignins. Twin screw extraction should aim at the joint extraction of hemicelluloses and lignins. Alkaline extractions were therefore preferred.

Apart from its extractive role, water acts as a natural lubricant during TMC extraction. It helps the progress of raw material through the various stress zones of the screw profile. The main stress zone is represented by the dynamic plug created in the reverse pitch screws. Addition of ethanol to the aqueous extracting phase decreases the lubricating action of the solvent. Therefore, the higher the level of ethanol is, the more difficult is the progress of the material in the stress zone formed by the reverse pitch screws for a same screw profile. On the contrary, the addition of an alkaline reagent such as sodium hydroxide lowers the viscosity of the mixture inside the twin screw reactor and improves the fluidization. From a practical standpoint, the values of the specific mechanical energy (SME) of the motor transcribe these effects.

In severe conditions, the lack of lubrication in the reverse pitch screws (module 7) can lead to an important self heating and to the blocking of the machine. In our case, the choice of a profile with short reverse pitch screws minimizes the risk of self heating in our hydro alcoholic tests (Fig. 1). It also allowed an efficient liquid/solid separation for all tested solvents and assessed the feasibility of the TMC extraction in a twin screw extruder of the highly fibrous hemp by products.

### 3.3.2. Hemp hurds

Apart from the varying temperatures and solvents, fixed parameters for hemp hurds were then defined as: screw profile, screw speed (200 rpm), hemp hurds' feed rate (2.2-2.5 kg/h) and liquid to solid ratios  $(L/S)_1 = 1.5$  and  $(L/S)_2 = 4.5$  (Table 4). The analyses on the fractions allowed concluding on the process efficiency and on the composition of the extracts in polyphenols and HCA (Table 5).

The contents in dry and organic matters in the extrudates reflect the efficiency of the liquid/solid separation. They ranged from 28 to 40% indicating an effective pressing and a significant recovery yield of filtrate for each trial. Higher dry matter contents in the case of W/E 40/60 are indicative of the reduced lubricating effect of ethanol in the reverse pitch screws whereas the lowering of the mechanical strength of the solid in alkaline conditions is consistent with lower organic matter contents. The solid/liquid separation is then more efficient for hydro alcoholic extractions, with or without alkali, and it leads to higher extraction yields in raw filtrate ranging from 75 to 140 g/kg ROM. The decrease in lubrication for hydro alcoholic solvent also results in an upper destructuration in the reverse pitch screws, and thus an increase in the flow of fine insoluble particles in the raw filtrate. This conclusion is also valid for alkaline extraction.

Low yields of extraction (25 g/kg ROM) have been measured without alkaline reagent. Unlike batch extractions, the polarity of the

solvent demonstrates no influence on the extraction yields. It is the same for temperature since an increase to 100 °C does not improve performance. This suggests that the extraction of hemp hurds in twin screw extruder is more subjected to diffusional limitation than to kinetic limitation. The addition of an alkaline reagent under mechanical shear stress conditions (bilobal paddle screws) improves the diffusion of the solvent in the raw material and promotes the chemical action of the reagent. The introduction of the second fraction of solvent, free from chemicals, in a relaxation zone of the solid, is used to extract molecules released by the chemical action of the alkaline reagent prior to their separation by pressing at the reverse screws. Then, adding 13% NaOH/ROM allows tripling the yield in extracted organic matter (70 g/kg ROM). It is slightly improved for a higher NaOH content for which two parameters are concurrent. An increase in NaOH content leads to a superior chemical breakdown of the lignocellulosic structure but also coincides with a slightly less effective liquid/solid separation.

Without alkali, extraction yields in organic matter are equivalent to those obtained by batch extraction. Thus, extracting continuously under mechanical stress at 50 °C, for a L/S ratio of 6 in 1-3 min, is equivalent to a 24 h batch extraction, for a higher L/S ratio of 16 at room temperature. However, these yields only represent the third of the analytical extractives potential obtained by hot water extraction. Referring to alkaline aqueous hydrolysis in batch (20 °C, 24 h, L/S = 30, 500% NaOH/ROM), the yield of twin screw extraction under alkaline conditions is only 10% thereof. Nevertheless, the batch hydrolysis conditions are extreme conditions for which the organic matter is substantially fully dissolved by alkaline attack. These operating conditions are not forecast for the development of an industrial process.

As for total organic matter extraction, the yields in polyphenols or HCA are equivalent at 50 and 100 °C confirming the absence of kinetic limitation.

The selectivity of the hydro alcoholic solvent for polyphenols is confirmed in the case of a twin screw extraction (Fig. 4). The maximum value obtained in the absence of basic chemicals represents 33% of the analytical potential confirming the diffusional limitation in the absence of reactants. The polyphenol content in the extracts is equivalent for both techniques of extraction and represents about 10%/EOM. The efficiency of the hydro alcoholic solvent is also demonstrated in alkaline extractions. For 13% NaOH/ROM, the yield of total polyphenols in the hydroalcoholic solvent is 1.5 times higher than that found in the aqueous solvent. If the mechanical operating conditions allow the solvent to penetrate the structure of hemp hurds, the latter indiscriminately extracts the molecules that are affine to it. As in the case of the total organic matter, the addition of sodium hydroxide triples the extraction yield of polyphenols, while maintaining an identical selectivity to that seen without reagent.

Comparing the yields of polyphenols by aqueous alkaline extraction, it is noted that with 13% NaOH/ROM, 4.0 g GAE/kg ROM are extracted, which is higher than the 2.2 g GAE/kg ROM measured in the case of a higher concentration of 32% NaOH/ROM and to which the global extraction yields are however slightly improved. Phenolic compounds thus appear to be less accessible to the extractant. High NaOH concentrations lead to a soapy effect. The matter slides between the screws and the effect of mechanical stress decreases at the expense of the efficiency in the extraction of polyphenols.

The highest yield of total polyphenols is measured for trial H11 (5.8 g GAE/kg ROM) by combining NaOH and 60% ethanol. The release of NaOH by mechanical action in hemp hurds swells the cell wall, particularly the crystalline structure of cellulose and breaks the intermolecular hydrogen bonds, which results in the release of the phenolic compounds. The latter are then solubilized in the hydro alcoholic solvent. A single pass in the twin screw extractor results in the recovery of half of the polyphenols obtained by batch alkaline hydrolysis in 24 h. Finally, the polyphenol content of the extract is 10%/EOM while for the batch extraction, the purity of the latter collapses to 1.5%/EOM. Assuming further purifying the extracts, this parameter is fundamental.

**Table 4**  
Operating conditions for hemp hurds extraction using a BC21 twin-screw extruder.

T (°C)	Q <sub>RM</sub> (kg/h)	Q <sub>ROM</sub> (kg/h)	(L/S)	Liquid 1			Liquid 2		
				W/E ratio	%NaOH/ ROM	Q <sub>L1</sub> (kg/ h)	W/E ratio	Q <sub>L2</sub> (kg/ h)	
H6	50	2.5	2.3	5.9	100/0	0	3.8	100/0	11.3
H7	100	2.4	2.2	6.2	100/0	0	3.8	100/0	11.3
H8	50	2.4	2.1	6.5	40/60	0	3.8	40/60	11.6
H9	50	2.2	2.0	6.7	100/0	32	3.8	100/0	11.3
H10	50	2.2	1.9	6.9	100/0	13	3.7	100/0	11.3
H11	50	2.2	2.0	6.8	40/60	13	3.8	40/60	11.3

Conditions S<sub>g</sub> = 200 rpm, Profile 1.

**Table 5**

Yields and chemical composition of the fractions from hemp hurds extraction using a BC21 twin-screw extruder.

Trial	Extrudate				Fine particles				Filtrate				SME (W.h./kg)			
	Q <sub>E</sub> (kg/h)	DM <sub>E</sub> (%)	OM <sub>E</sub> (%)	Y <sub>E</sub> (g/kg ROM)	Q <sub>FP</sub> (kg/h)	DM <sub>FP</sub> (%)	OM <sub>FP</sub> (%)	Y <sub>FP</sub> (g/kg ROM)	Q <sub>F</sub> (kg/h)	DM <sub>F</sub> (%)	OM <sub>F</sub> (%)	Y <sub>F</sub> (g/kg ROM)		Y <sub>PP</sub> (g GAE/kg ROM)	Y <sub>FA</sub> (mg/kg ROM)	Y <sub>p CA</sub> (mg/kg ROM)
H6	7.6	28	28	925	0.85	14.3	13.6	51	9.2	0.72	0.58	24	0.6	8	45	225
H7	7.4	27	27	931	0.78	13.5	12.8	46	8.9	0.72	0.55	23	0.7	7	62	200
H8	5.8	33	33	901	1.20	13.9	13.4	76	10.3	0.58	0.47	23	2.3	7	59	241
H9	7.5	30	24	888	0.44	19.9	13.4	30	9.2	5.76	1.79	82	2.2	35	440	0
H10	6.3	30	27	893	0.57	15.4	12.4	37	10.3	3.22	1.31	70	4.0	67	1127	0
H11	4.7	40	36	861	0.82	22.2	17.9	74	12.0	2.38	1.06	65	5.8	99	1814	50

The trend for the yields of HCA is comparable to that observed for total polyphenols (Fig. 5). Increasing the temperature from 50 °C to 100 °C has no significant positive effect. The yields obtained without reagent in twin screw extruder (L/S = 6, 50 °C, 2 min) are similar for both solvents and equivalent to batch extraction by maceration (L/S = 16, 20 °C, 24 h). Nevertheless, these values remain low and between 4 to 10 times less than the reference yields obtained in analytical extraction (L/S = 100, 100 °C, 1 h). Extracting HCA without introducing chemical reagent acting on the raw matter's structure, even under thermo mechanical stress, is not conclusive in terms of extraction yield.

Performance is optimal for alkaline ethanolic solvent that can extract two times more HCA compared to the aqueous solvent. Once spread by mechanical effect in hemp hurds, sodium hydroxide swells the cell wall and breaks part of the chemical bonds holding *p* CA and FA. Phenolic acids are released and extracted by the ethanolic solvent to which affinity is proven. These yields are still lower by a factor 2 to 4 relative to batch alkaline hydrolysis. However, this difference is more than satisfactory since the NaOH concentration is 45 times lower when extracting in a twin screw extruder. Moreover, the low L/S ratio minimizes solvent consumption which represents an economic and environmental advantage. We can notice that the L/S ratio of 6 is even lower than the commercial absorption capacity of 10 L/kg set for hemp hurds. Extraction by extrusion could be seen as an alternative green processing technique (Chemat et al., 2017) as it reduces extraction time, energy, solvent and reagent consumptions. As advantages, it accelerates the mass transfer by enhancing the diffusion of solvent. It also decreases solvent consumption thanks to the liquid/solid in situ separation. The extrudates that can be considered in a first approach as a major by product of the process could be directly forecast as valuable new fibers in fields such as composites or new fractionation processes (Organosolv, Formosolv).

For all trials, *p* CA is the most abundant phenolic acid with a *p* CA/FA ratio ranging from 6 to 18. The latter was estimated at 12 in batch alkaline conditions. Using twin screw extruder, it is possible to guide the selectivity between the two phenolic acids depending on the

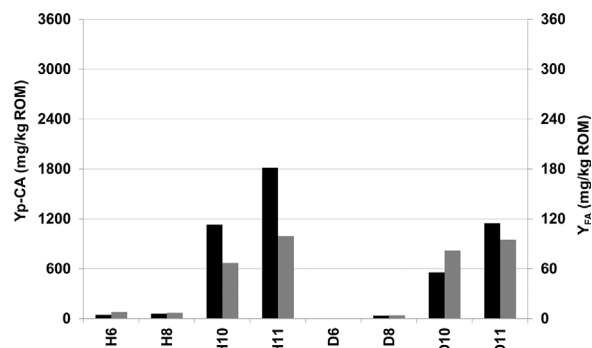


Fig. 5. Yields in *p*-CA (black) and FA (grey) for continuous extractions in TMC extruder of hemp hurds (trials H) and dust (trials D).

operating conditions. In the hemp hurds lignocellulosic structure, FA is essentially linked by hardly hydrolysable ether bonds and *p* CA is equally linked by ether and ester bonds, the latter being more susceptible to chemical attack. In the case of aqueous extraction, working under mechanical stress at low temperature allows extracting 22% and 32% of present FA and *p* CA for a 1 to 3 min residence time in the extruder. The introduction of 13% aqueous NaOH/ROM is sufficient to hydrolyze an equivalent of all FA ester bonds and more than half of *p* CA ester bonds. Employing the alkaline hydro alcoholic solvent increases sharply the amounts of extracted FA and *p* CA. Little NaOH is then sufficient to hydrolyze a large number of chemical bonds as long as the reagent is effectively spread in the raw material. The choice of the extracting solvent is then defined by the affinity of the molecules of interest to it. The alkaline hydro alcoholic extraction respectively leads to 33% and 51% of the free and bound FA and *p* CA. To increase the extraction yields, a preliminary grinding of hemp hurds would increase the surface area of the solid for an effective dissemination of the alkaline reagent. Indeed, without prior grinding, bilobal paddle screws are involved in both the shearing of the raw material and the mixing between the latter and the alkaline reagent. By grinding upstream from

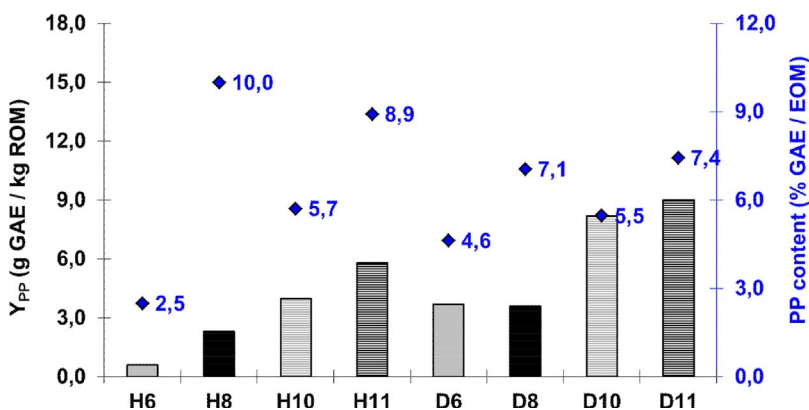


Fig. 4. Yields in polyphenols and polyphenols contents for continuous extractions in TMC extruder of hemp hurds (trials H) and dust (trials D); Grey (W/E 100/0), black (W/E 40/60), Grey stripes (W/E 100/0, NaOH), Black stripes (W/E 40/60, NaOH).



the extrusion, bilobal paddle screws would play most effectively their mixing role thereby promoting chemical action of the reagent and in creasing the diffusion of the solvent in the vegetable matrix.

To assess the destructure of hemp hurds and to study its composition in residual extractable molecules, the extrudates of trials H10 and H11 were analyzed: ADF NDF characterization (Table 8), hot water and hot W/E extractives (Table 9). The contents in hemicelluloses, lignin and cellulose demonstrate a low destructure of the extrudates compared to the initial hemp hurds. Little lignin and hemicellulose were extracted during twin screw extrusion and a low variability in the distribution of the parietal fractions is observed with a slight increase of the cellulosic fraction. The amount of NDF extractives is higher in the case of the hydro alcoholic extrudate confirming the destructuring effect when using a solvent with a limited lubricating capacity.

The analytical extractions of both extrudates are efficient for extracting the free residual molecules. The thermo mechanical action under alkaline conditions has acted on the structure of the raw material, releasing a large amount of organic matter. Although part of these molecules has not been extracted during the extraction sequence by extrusion, they remain in the free form in the structure of the extrudate and are readily extractable by a polar solvent. For trial H10 and H11, 182 and 224 g/kg ROM are recovered by hot water extraction. This is 2.5–3.5 times the extractable yield of organic matter in the filtrates.

The other solid issue of the extrusion process is fine particles. The fraction of remaining liquid trapped in this solid structure may be equivalent to the filtrate in its chemical composition. Washing this solid with a polar solvent may increase the amount of extractable soluble organic matter. Performing a simulated calculation, we concluded that the fraction of free organic matter present in the fine particles is less than 5% of that present in the filtrate. This output of the process may therefore be neglected.

Adding the yields of organic matter from the filtrate and the aqueous fraction issued from extrudate (H11), a global yield of 289 g/kg ROM could be obtained. Even if it is more than 2 times lower than the yield of the batch alkaline hydrolysis, an additional step of extraction of the extrudates could improve our overall extraction yield.

Regarding total polyphenols, all analytical extractions are equivalent. The maximum value of 6.6 g GAE/kg ROM exceeds the yield of polyphenols in the filtrates. For trial 11, adding the PP yields in the filtrate and the extrudate leads to 12.4 g GAE/kg ROM. It is equivalent to the batch alkaline extraction. Mechanical strain, applied in a polar reactive alkaline medium causes disintegration of the hemp hurds. A fraction of the polyphenols is obtained by solid/liquid separation in the extruder. The other fraction released but retained in the extrudate is available for a second extraction step that would be highly favorable to a significant increase in PP yields.

The highest levels of HCA are recovered by combining the two extraction steps: twin screw extraction and analytical extraction of the extrudates. The amount of *p* CA is doubled whereas the cumulated amounts of FA remain low. Thus, the alkaline extraction using twin screw extruder leads to the preferential hydrolysis of the ester bonds. The FA ether bonds would only be partially attacked. The second extraction stage can contribute to the reactive extraction of *p* CA molecules without leading to further hydrolysis of ether bonds for which harsher chemical conditions would be necessary.

### 3.3.3. Hemp dust

Hemp dust is less fibrous than hemp hurds with a lower lignocellulosic content. Zones of conveying and liquid to solid extraction were kept identical to the one implemented for hemp hurds. Profiles differed at the liquid/solid separation zone with adapted reverse pitch screws (Fig. 1). The final reverse pitch screws selected were 25 mm long with a pitch of 16 (Profile 2). The fixed operating conditions were: raw matter feed input of 1.5 kg/h, L/S ratio of 10, and screw speed of 200 rpm (Table 6).

As reflected by the contents of dry or organic matter in the

**Table 6**

Operating conditions for hemp dust extraction using a BC21 twin-screw extruder.

	T (°C)	Q <sub>RM</sub> (kg/h)	Q <sub>ROM</sub> (kg/h)	(L/S)	Liquid 1			Liquid 2	
					W/E ratio	%NaOH/ ROM	Q <sub>L1</sub> (kg/h)	W/E ratio	Q <sub>L2</sub> (kg/h)
D6	50	1.5	1.2	9.7	100/0	0	3.8	100/0	11.3
D7	100	1.6	1.3	9.4	100/0	0	3.8	100/0	11.3
D8	50	1.2	0.9	13.2	40/60	0	3.9	40/60	11.8
D9	50	1.5	1.2	10.0	100/0	54	3.8	100/0	11.3
D10	50	1.6	1.3	9.2	100/0	20	3.8	100/0	11.2
D11	50	1.6	1.3	9.5	40/60	21	4.0	40/60	11.3

Conditions S<sub>S</sub> = 200 rpm, Profile 2.

extrudates, the liquid/solid separation in the twin screw extruder was effective (Table 7). With an alkaline solvent, the pressing efficiency decreases because of the destructuring effect of NaOH on the raw material. An alkaline ethanolic extraction leads to a very efficient liquid/solid separation with an extrudate largely depleted in residual filtrate. As in the case of hemp hurds, adding ethanol to the extraction solvent reduces lubricity. The liquid/solid separation is more difficult to achieve and the specific mechanical energy increases.

Once the raw filtrates centrifuged, the repartition of organic matter between the filtrate and the fine particles was assessed. The hydro alcoholic solvent, alkaline or not, is responsible for driving a large amount of organic matter in the raw filtrate as insoluble fine solid particles.

No temperature effect is observed. This confirms the absence of kinetic limitation for the extraction of the hemp by products in a twin screw extruder.

The maximum yield without alkaline reagent is equivalent to that obtained for a 24 h batch with a L/S ratio of 16 at 20° C. It represents 50% of the analytical extraction (100 °C, 1 h, L/S = 100).

Adding sodium hydroxide allows doubling the amount of extracted organic matter. However, extracting with 20% or 50% NaOH/ROM has little influence on the solubilization of organic matter. This may be due to the very short residence time in the twin screw extruder which smooths the effect of the reagent. The maximum alkaline yield in total organic matter (160 g/kg ROM) in “mild” twin screw extraction conditions represents 16% of the yield in severe alkaline batch extraction (20 °C, 24 h, L/S = 30, 500% NaOH/ROM).

For polyphenols, temperature and extraction solvent have no impact on the extraction and selectivity (Fig. 4). The yield of 4 g GAE/kg ROM is 50% of the analytical extraction. The content in polyphenols of the extracts for the aqueous solvent (4.6%/EOM) and hydro alcoholic (7.1%/EOM) in twin screw are close to the respective analytical values. The absence of solvent selectivity for the polyphenols is confirmed in alkaline conditions. However, the addition of NaOH triples the polyphenols extraction yields through its hydrolytic chemical action and the swelling of the matter which facilitates the diffusion of the solvent. By increasing the NaOH content to 54%/ROM, this dual action is slightly increased. The maximum value in polyphenols (9 g GAE/kg ROM) accounts for 54% of the batch alkaline hydrolysis. Regarding the composition of the extracts, the maximum polyphenols content of 7.4%/EOM is well above the 1.7%/EOM measured by alkaline hydrolysis.

Without alkaline reagent, the extraction yields of HCA are negligible irrespective of the temperature or the solvent (Fig. 5). In aqueous alkaline conditions, yields are equivalent regardless of the NaOH concentration. The combination hydroalcoholic solvent/NaOH is the most efficient. As for hemp hurds, the mechanical effect promotes the hydrolysis of part of the chemical bonds between the phenolic acids and the cell wall. The hydro alcoholic solvent effectively diffused into the plant matrix extracts the released molecules. *p* CA is the most abundant extracted HCA, with 7–12 times more *p* CA than FA. The *p* CA/FA ratio

**Table 7**

Yields and chemical composition of the fractions from hemp dust extraction using a BC21 twin-screw extruder.

Trial	Extrudate				Fine particles				Filtrate						SME (W.h./kg)	
	Q <sub>E</sub> (kg/h)	DM <sub>E</sub> (%)	OM <sub>E</sub> (%)	Y <sub>E</sub> (g/kg ROM)	Q <sub>FP</sub> (kg/h)	DM <sub>FP</sub> (%)	OM <sub>FP</sub> (%)	Y <sub>FP</sub> (g/kg ROM)	Q <sub>F</sub> (kg/h)	DM <sub>F</sub> (%)	OM <sub>F</sub> (%)	Y <sub>F</sub> (g/kg ROM)	Y <sub>PP</sub> (g GAE/kg ROM)	Y <sub>FA</sub> (mg/kg ROM)		Y <sub>p CA</sub> (mg/kg ROM)
D6	1.6	57	53	688	1.7	21	16	231	13.2	0.93	0.73	80	3.7	0	0	190
D7	1.4	68	64	698	1.7	22	17	219	12.9	1.02	0.80	82	3.6	0	0	55
D8	1.2	47	44	555	1.8	26	20	394	13.6	0.40	0.35	51	3.6	4	37	267
D9	3.4	35	23	674	1.4	27	14	167	10.3	6.29	1.80	160	9.2	80	538	0
D10	2.3	41	36	658	1.6	25	15	192	11.3	3.60	1.68	150	8.2	82	558	0
D11	1.6	59	50	645	1.4	33	21	234	13.3	2.33	1.14	121	9.0	95	1150	61

**Table 8**

Chemical composition of extrudates (%/DM) obtained from hemp by-products extraction in a BC21 twin-screw extruder.

Trial	Minerals	Cellulose(C)	Hemicelluloses (HC)	Lignins (L)	C + H + L	NDF extractives
H10	8.7	45.2 ± 0.1	13.3 ± 0.6	15.6 ± 0.1	74.1	25.9 ± 0.7
H11	10.0	42.5 ± 0.2	14.1 ± 0.5	13.6 ± 0.3	70.2	29.8 ± 0.1
D10	13.1	45.6 ± 0.3	8.3 ± 0.5	10.5 ± 0.1	64.4	35.6 ± 0.6
D11	15.4	41.0 ± 0.5	12.8 ± 0.5	6.7 ± 0.4	60.5	39.5 ± 0.4

**Table 9**

Yields of solvent extraction of the extrudates.

Solvent	W/E ratio	Y <sub>F</sub>		Y <sub>PP</sub>		Y <sub>FA</sub>		Y <sub>p CA</sub>	
		(g/kg ROM)		(g GAE/kg ROM)		(mg/kg ROM)		(mg/kg ROM)	
		100/0	40/60	100/0	40/60	100/0	40/60	100/0	40/60
Trial	H10	203	116	5.9	6.6	29	24	1971	2073
	H11	260	176	6.6	6.6	18	19	2373	1944
	D10	213	172	4.9	4.5	17	14	897	807
	D11	292	209	5.4	5.1	17	15	1014	872

is greater than that obtained in batch extraction ( $p\text{ CA}/\text{FA} = 6$ ). The maximum amount of twin screw extracted FA is 2 times lower than in batch alkaline hydrolysis whereas these yields are equivalent for  $p\text{ CA}$ . FA is mainly ether bound to the lignocellulosic structure of hemp dust whereas  $p\text{ CA}$  is essentially ester bound. Working with a 20% NaOH/ROM aqueous solvent, in 1–3 min at 50 °C under mechanical stress extracts more than half of the present FA and  $p\text{ CA}$ . By coupling NaOH and ethanol, the amounts of extracted FA and  $p\text{ CA}$  are doubled. Under such conditions, 59% of the FA potential is reached whereas the  $p\text{ CA}$  potential is overtaken.

An alkaline ethanolic extraction could then be advocated for hemp dust. The operating conditions applied in twin screw extractor are suitable for industrial application: short time, continuous, low NaOH quantities, and low L/S ratios. An experimental L/S ratio of 10 is even much lower than the absorbency of commercial hemp dust (40 kg/L) and may be further optimized.

The extrudates were analyzed. The absence of variation on hemicelluloses, cellulose and lignins contents underlines a weak destruction of hemp dust (Table 8). The analytical extraction of extrudates (trials D10 and D11) confirms the efficiency of the use of alkaline ethanolic solvent and the doubling of the extracted organic matter (Table 9). Summing the filtrates of the two stages, 30% of the raw organic matter is solubilized. For total polyphenols, the maximum value of 5.4 g GAE/kg ROM in the analytical extracts is lower than the polyphenols yield in the filtrates. For trial D11, adding the polyphenols in the filtrate and the extract from the extrudate leads to 14.4 mg GAE/kg ROM. This is equivalent to the yield measured with batch alkaline hydrolysis. The analytical extraction of extrudates also doubles the global  $p\text{ CA}$  yield that exceeds the alkaline batch yield. The hydrolysis of  $p\text{ CA}$  ester bonds are favored by the twin screw extraction. The

liberated molecules become easily extractable in a second extraction stage. FA ether bonds seemed to be weakly attacked under the developed operating conditions.

#### 4. Conclusions

Two industrial hemp by-products, hemp hurds and dust, were forecast as providers of high value added HCA. Analytical hydrolysis protocols determined the content of reference in free and bound FA and  $p\text{ CA}$  and their distribution in the lignocellulosic structure. The global content in HCA was three times higher in hemp hurds than in hemp dust. The free/ester linked/ether linked distribution in hemp hurds was 14/46/40 for  $p\text{ CA}$  and 9/2/89 for FA whereas, for hemp dust, these repartitions were 20/70/10 for  $p\text{ CA}$  and 13/24/63 for FA. The distribution differed with a higher content in free and ester linked HCA in the case of hemp dust.

Twin screw extruder demonstrated its potential as a promising process for the efficient solid/liquid extraction of HCA from hemp by-products. The efficiency of the extraction scheme is based on the continuous operating units of mixing, extracting and separating solid and liquid fractions. Moreover, the extraction of HCA is concluding when working under mild polar alkaline conditions and preferentially with a water/ethanol alkaline solvent. Such conditions allowed reaching yields of 50% and 33% of the potentials of reference in free and bound  $p\text{ CA}$  and FA for hemp hurds. For hemp dust, all of the reference potential in  $p\text{ CA}$  was extracted whereas 60% of the FA potential was recovered. Extracting FA remains more difficult because of its strong chemical interactions with the lignocellulosic structure. To release etherified FA, hydrolysis conditions should be hardened, increasing chemical and energetic cost of the process.

The mechanical action in twin screw extruder favors the mixing of liquid and solid and leads to high extraction yields. The first extraction in twin screw extruder greatly shifts the diffusional limit for a second extraction step. After the reactive destructure and the liquid/solid separation in the extruder, it has been proven that part of the released molecules is still retained in the solid residue. These molecules could be recovered by a second extraction step using polar solvent. The global content of *p* CA of the raw material could then be extracted whereas half of FA is recovered. The process hydrolyzes preferentially ester bonds and partially ether bonds.

Working with an extruder equipped with two zones of filtration would present additional advantages: low cumulated liquid/solid ratios, flexibility for solvents and temperatures and low NaOH/ROM ratios. The development of a solid/liquid extraction process by extrusion takes into account several criteria to reach optimal yields with limited environmental impacts. The energetic cost, extraction times, liquid/solid ratios, quantities of added reactants and number of stages are prone to be minimized.

### Conflict of interest

Authors declare no conflict of interest.

### Acknowledgements

This work was partially funded by the French Ministry of Research and Education which contributed to Sabina Bassil's PhD grant.

### References

- Acosta-Estrada, B.A., Gutiérrez-Urbe, J.A., Serna-Saldívar, S.O., 2014. Bound phenolics in foods, a review. *Food Chem.* 152, 46–55.
- Amaducci, S., Scordia, D., Liu, F.H., Zhang, Q., Guo, H., Testa, G., Cosentino, S.L., 2015. Key cultivation techniques for hemp in Europe and China. *Ind. Crops Prod.* 68, 2–16.
- Barberousse, H., Roiseux, O., Robert, C., Paquot, M., Deroanne, C., Blecker, C., 2008. Analytical methodologies for quantification of ferulic acid and its oligomers. *J. Sci. Food Agric.* 88, 1494–1511.
- Beejmohun, V., Fliniaux, O., Grand, E., Lamblin, F., Bensaddek, L., Christen, P., Kovensky, J., Fliniaux, M.A., Mesnard, F., 2007. Microwave-assisted extraction of the main phenolic compounds in flaxseed. *Phytochem. Anal.* 18, 275–282.
- Boz, H., 2015. *p*-Coumaric acid in cereals: presence, antioxidant and antimicrobial effects. *Int. J. Food Sci. Technol.* 50, 2323–2328.
- Buranov, A.U., Mazza, G., 2009. Extraction and purification of ferulic acid from flax shives: wheat and corn bran by alkaline hydrolysis and pressurised solvents. *Food Chem.* 115, 1542–1548.
- Capriotti, A.L., Cavaliere, C., Foglia, P., Piovesana, S., Ventura, S., 2015. Chromatographic methods coupled to mass spectrometry detection for the determination of phenolic acids in plants and fruits. *J. Liq. Chromatogr. Relat. Technol.* 38, 353–370.
- Carus, M., Sarmento, L., (2016, 02/11/2016). The European Hemp Industry: Cultivation, processing and applications for fibres, shivs, seeds and flowers. from <http://eiha.org/media/2016/05/16-05-17-European-Hemp-Industry-2013.pdf>.
- Celhay, C., Mathieu, C.E., Candy, L., Vilarejo, G., Rigal, L., 2014. Aqueous extraction of polyphenols and antioxidants from wood by-products by a twin-screw extractor: feasibility study. *CR Chim.* 17, 204–211.
- Chemat, F., Rombaut, N., Meullemiestre, A., Turk, M., Perino, S., Fabiano-Tixier, A.S., Abert-Vian, M., 2017. Review on green food processing techniques. Preservation, transformation, and extraction. *Innov. Food Sci. Emerg. Technol.* 41, 357–377.
- Chiremba, C., Rooney, L.W., Beta, T., 2012. Microwave-assisted extraction of bound phenolic acids in bran and flour fractions from sorghum and maize cultivars varying in hardness. *J. Agric. Food Chem.* 60, 4735–4742.
- Colas, D., Doumeng, C., Pontalier, P.Y., Rigal, L., 2013. Twin-screw extrusion technology, an original solution for the extraction of proteins from alfalfa (*Medicago sativa*). *Food Bioprod. Process.* 91, 175–182.
- Cornu, A., Besle, J.M., Mosoni, P., Grenet, E., 1994. Lignin-carbohydrates complexes in forages: structure and consequences in the ruminal degradation of cell-wall carbohydrates. *Reprod. Nutr. Dev.* 34, 385–398.
- De Vega, A., Ligerio, P., 2017. Fomosalv fractionation of hemp hurds. *Ind. Crops Prod.* 97, 252–259.
- Dicke, C., Luhr, C., Ellerbrock, R., Kern, J., 2015. Effect of hydrothermally carbonized hemp dust on the soil emissions of CO<sub>2</sub> and N<sub>2</sub>O. *BioResources* 10, 3210–3223.
- El-Seedi, H.R., El-Said, A.M.A., Khalifa, S.A.M., Göransson, U., Bohlin, L., Borg-Karlson, A.K., Verpoorte, R., 2012. Biosynthesis, natural sources, dietary intake, pharmacokinetic properties, and biological activities of hydroxycinnamic acids. *J. Agric. Food Chem.* 60, 10877–10895.
- Gandolfi, S., Ottolina, G., Riva, S., Fantoni, G.P., Patel, I., 2013. Complete chemical analysis of Carmagnola hemp hurds and structural features of its components. *BioResources* 8, 2641–2656.
- Gandolfi, S., Ottolina, G., Consonni, R., Riva, S., Patel, I., 2014. Fractionation of hemp hurds by Organosolv pretreatment and its effect on production of lignin and sugars. *ChemSusChem* 7, 1991–1999.
- Gandolfi, S., Pistone, L., Ottolina, G., Xu, P., Riva, S., 2015. Hemp hurds biorefining: a path to green L-(+)-lactic acid production. *Bioresour. Technol.* 191, 59–65.
- Gautam, A., Choudhury, G.S., 1999. Screw configuration effects on residence time distribution and mixing in twin-screw extruders during extrusion of rice flour. *J. Food Process Eng.* 22, 263–285.
- Gogoi, B.K., Choudhury, G.S., Oswalt, A.J., 1996. Effects of location and spacing of reverse screw and kneading element combination during twin-screw extrusion of starchy and proteinaceous blends. *Food Res. Int.* 29, 505–512.
- Hunt, A., Attard, T., Bainier, C., Re naud, M., Lanot, A., McQueen-Mason, S., Clark, J., 2015. Hemp waste as a potential source of valuable chemicals. In: 250th ACS National Meeting & Exposition. Boston, MA, United States, American Chemical Society Washington D.C.
- Jacquemin, L., Mogni, A., Zeitoun, R., Guinot, C., Sablayrolles, C., Pontalier, P.Y., 2015. Comparison of different twin-screw extraction conditions for the production of arabinoxylans. *Carbohydr. Polym.* 116, 86–94.
- Karus, M., Vogt, D., 2004. European hemp industry Cultivation, processing and product lines. *Euphytica* 140, 7–12.
- Khan, B.A., Wang, T., Warner, P., Wang, H., 2015. Antibacterial properties of hemp hurd powder against *E. coli*. *J. Appl. Polym. Sci.* 132.
- Kroon, P.A., Williamson, G., 1999. Hydroxycinnamates in plants and food: current and future perspectives. *J. Sci. Food Agric.* 79, 355–361.
- Li, L., Shewry, P.R., Ward, J.L., 2008. Phenolic acids in wheat varieties in the HEALTHGRAIN diversity screen. *J. Agric. Food Chem.* 56, 9732–9739.
- Li, X., Xiao, R., Morrell, J.J., Zhou, X., Du, G., 2017. Improving the performance of hemp hurd/polypropylene composites using pectinase pre-treatments. *Ind. Crops Prod.* 97, 465–468.
- Lozovaya, V.V., Gorshkova, T.A., Yablokova, E.V., Rumyantseva, N.I., Valieva, A., Ulanov, A., Widholm, J.M., 1999. Cold alkali can extract phenolic acids that are tethered to cell wall components in dicotyledonous plants (buckwheat, soybean and flax). *Phytochemistry* 50, 395–400.
- Manach, C., Scalbert, A., Morand, C., Remesy, C., Jimenez, L., 2004. Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.* 79, 727–747.
- Matthäus, B., Brühl, L., 2008. Virgin hemp seed oil: an interesting niche product. *Eur. J. Lipid Sci. Technol.* 110, 655–661.
- Max, B., Salgado, J.M., Cortes, S., Dominguez, J.M., 2010. Extraction of phenolic acids by alkaline hydrolysis from the solid residue obtained after prehydrolysis of trimming vine shoots. *J. Agric. Food Chem.* 58, 1909–1917.
- Mussatto, S.I., Dragone, G., Roberto, I.C., 2007. Ferulic and *p*-coumaric acids extraction by alkaline hydrolysis of brewer's spent grain. *Ind. Crops Prod.* 25, 231–237.
- Ou, S., Luo, Y., Xue, F., Huang, C., Zhang, N., Liu, Z., 2007. Separation and purification of ferulic acid in alkaline-hydrolysate from sugarcane bagasse by activated charcoal adsorption/anion macroporous resin exchange chromatography. *J. Food Eng.* 78, 1298–1304.
- Ou, S.Y., Luo, Y.L., Huang, C.H., Jackson, M., 2009. Production of coumaric acid from sugarcane bagasse. *Innov. Food Sci. Emerg. Technol.* 10, 253–259.
- Pojic, M., Mišan, A., Sakač, M., Dapčević Hadnadev, T., Šarić, B., Milovanović, I., Hadnadev, M., 2014. Characterization of byproducts originating from hemp oil processing. *J. Agric. Food Chem.* 62, 12436–12442.
- Rehman, M.S.U., Rashid, N., Saif, A., Mahmood, T., Han, J.-I., 2013. Potential of bio-energy production from industrial hemp (*Cannabis sativa*): Pakistan perspective. *Renew. Sustain. Energ. Rev.* 18, 154–164.
- Saija, A., Tomaino, A., Trombetta, D., De Pasquale, A., Uccella, N., Barbuzzi, T., Paolino, D., Bonina, F., 2000. *In vitro* and *in vivo* evaluation of caffeic and ferulic acids as topical photoprotective agents. *Int. J. Pharm.* 199, 39–47.
- Savoire, R., Lanoisellé, J.L., Vorobiev, E., 2013. Mechanical continuous oil expression from oilseeds: a review. *Food Bioprocess. Technol.* 6, 1–16.
- Shea, A., Lawrence, M., Walker, P., 2012. Hygrothermal performance of an experimental hemp-lime building. *Constr. Build. Mater.* 36, 270–275.
- Simon, V., Thuret, D.N., Candy, L., Bassil, S., Duthen, S., Raynaud, C., Masseron, A., 2015. Recovery of hydroxycinnamic acids from renewable resources by adsorption on zeolites. *Chem. Eng. J.* 280, 748–754.
- Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. *Am. J. Enol. Vitic.* 16, 144–158.
- Struik, P.C., Amaducci, S., Bullard, M.J., Stutterheim, N.C., Venturi, G., Cromack, H.T.H., 2000. Agronomy of fibre hemp (*Cannabis sativa* L.) in Europe. *Ind. Crops Prod.* 11, 107–118.
- Sun, R.C., Sun, X.F., Wang, S.Q., Zhu, W., Wang, X.Y., 2002. Ester and ether linkages between hydroxycinnamic acids and lignins from wheat rice, rye, and barley straws, maize stems, and fast-growing poplar wood. *Ind. Crops Prod.* 15, 179–188.
- Tang, P.-L., Hassan, O., Md-Jahim, J., Mustapha, W.A.W., Maskat, M.Y., 2014. Fibrous agricultural biomass as a potential source for bioconversion to vanillin acid. *Int. J. Polym. Sci.* 1–8 (509035/1-509035/9).
- Terzopoulou, Z.N., Papageorgiou, G.Z., Papadopoulou, E., Athanassiadou, E., Reinders, M., Bikiaris, D.N., 2016. Development and study of fully biodegradable composite materials based on poly(butylene succinate) and hemp fibers or hemp shives. *Polym. Compos.* 37, 407–421.
- Tilay, A., Bule, M., Kishenkumar, J., Annapure, U., 2008. Preparation of ferulic acid from agricultural wastes: its improved extraction and purification. *J. Agric. Food Chem.* 56, 7644–7648.
- Torre, P., Aliakbarian, B., Rivas, B., Dominguez, J.M., Converti, A., 2008. Release of ferulic acid from corn cobs by alkaline hydrolysis. *Biochem. Eng. J.* 40, 500–506.

- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583–3597.
- Vandenbossche, V., Doumeng, C., Rigal, L., 2014. Thermomechanical and thermo-mechano-chemical pretreatment of wheat straw using a twin-screw extruder. *BioResources* 9, 1519–1538.
- Xu, F., Sun, R.-C., Sun, J.-X., Liu, C.-F., He, B.-H., Fan, J.-S., 2005. Determination of cell wall ferulic and *p*-coumaric acids in sugarcane bagasse. *Anal. Chim. Acta* 552, 207–217.
- Zakzeski, J., Bruijninx, P.C.A., Jongerius, A.L., Weckhuysen, B.M., 2010. The catalytic valorization of lignin for the production of renewable chemicals. *Chem. Rev.* 110, 3552–3599.
- Zamzuri, N.A., Abd-Aziz, S., 2013. Biovanillin from agro wastes as an alternative food flavour. *J. Sci. Food Agric.* 93, 429–438.
- Zhao, S., Yao, S., Ou, S., Lin, J., Wang, Y., Peng, X., Li, A., Yu, B., 2014. Preparation of ferulic acid from corn bran: its improved extraction and purification by membrane separation. *Food Bioprod. Process.* 92, 309–313.
- Zielinski, H., Kozłowska, A., Lewczuk, B., 2001. Bioactive compounds in the cereal grains before and after hydrothermal processing. *Innov. Food Sci. Emerg. Technol.* 2, 159–169.