Complete Chemical Analysis of Carmagnola Hemp Hurds and Structural Features of Its Components

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As interest in lignocellulosic biomass as a feedstock for conversion into biofuels is steadily growing, analysis of its components becomes ever more important. The complete chemical composition of waste hemp hurds from the industrial variety "Carmagnola" has been determined to optimize its utilization as a raw material. The results from chemical analysis show that hemp hurds contain 44.0% alpha-cellulose, 25.0% hemicellulose, and 23.0% lignin as major components, along with 4.0% extractives (oil, proteins, amino acids, pectin) and 1.2% ash. Structural and physicochemical properties of hurds components were analysed by FTIR or GC/MS. The data revealed that isolated components are pure and comparable to standard components. Acetone extractives show higher total phenolic content and antioxidant capacity compared with lignin and dichloromethane extractives. Water extractive shows the presence of proteins (1.6%), free amino acids (0.02%), and pectin (0.6%). The degree of esterification of pectin was estimated to be 46.0% by FTIR and enzymatic hydrolysis. The results of this study show that Carmagnola hurds contain low amounts of ash and high amounts of carbohydrates compared with other varieties of hemp hurds; therefore they can be considered as a potential feedstock for biorefinery.

Keywords: Hemp hurds; Lignocellulosics; Biorefinery; Cellulose; Holocellulose

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INTRODUCTION

Nowadays, the use of renewable biomass to replace non-renewable fossil fuels is becoming a priority in energy policy and management. The major production of biofuels originates from energy crops. These can be lignocellulosic materials, such as agricultural by-products, herbaceous crops, or forestry residues (Kim and Dale 2004). In a biore-finery, this biomass is converted into a variety of high value-added products and biofuels. Lignocellulosic materials, with a high content of carbohydrates, are abundant, inexpensive, and largely unused. The main chemical components of lignocellulosic materials are: cellulose, hemicelluloses, and lignin, with minor amounts of other compounds such as ash, proteins, lipids, waxes, and various extractives. Lignocellulose structure and composition vary greatly, according to plant species, plant parts, growth conditions, *etc*. (Ding and Himmel 2006; Zhang and Lynd 2004).

Hemp is one of the fastest-growing plants in the world and it comprises a number of varieties of *Cannabis sativa* L. that are traditionally grown for fibers and seeds.

Compared with other crops, industrial hemp is very high yielding in biomass (~30 tons/hectare) and requires a low level of irrigation and fertilizers after its establishment (Struik *et al.* 2000; Cappelletto *et al.* 2001; van der Werf 2004; Amaducci *et al.* 2008). Industrial hemp is characterized by low (less than 0.20%) tetrahydrocannabinol (THC) content, and many countries are represented on the list of approved cultivars. The European Union permits the cultivation of 54 different varieties of industrial hemp, and among them Carmagnola is one of the oldest approved varieties. Fibers are a valued product of hemp and are mainly used for textile applications. In a typical process, fibers are separated from the hemp stalk through retting and scutching. The residual biomass (containing mainly a woody core, dust, and small amounts of short fibers, known as core fibers) is considered a by-product of fiber production. These woody core parts constitute 70% of the stalk (Dang and Nguyen 2006) and have minor applications, such as for animal bedding (95%), garden mulch, or as a component of lightweight concrete (~5%).

The use of hemp hurds as a feedstock for a modern biorefinery facility could supply a variety of market sectors (*e.g.*, chemistry, energy, transportation). The hemp biomass used in the present study is a by-product from the textile industry, and it is locally available as a waste material and considered a potential source of lignocellulose. As a prerequisite to add value to this waste biomass, an accurate compositional analysis is important in order to evaluate the conversion yields and the efficiency of the proposed process.

Industrial interest in hemp is increasing because it is eco-friendly and due to its possible applications such as in pulp and paper (González-García *et al.* 2010), bio-composite (Boutin *et al.* 2006; Carus *et al.* 2008; Magnani 2010), and as raw material for biofuel production (Sipos *et al.* 2010; Kreuger *et al.* 2011). For instance, an accurate measurement of biomass carbohydrate content is essential because it is directly related to ethanol yield in biochemical conversion processes (Aden *et al.* 2002). Furthermore, the minor components of a biomass can include proteins, ash, organic acids, and other nonstructural materials.

Although these individual components may make up only a small fraction of the feedstock, their presence can have a significant effect on the running of an industrial-scale biorefinery. Therefore, the objectives of this study are to quantify both the major (*e.g.*, cellulose, hemicellulose, lignin) and minor components of Carmagnola hemp hurds, with the aim to utilize them as raw materials for biorefinery.

EXPERIMENTAL

Materials

The residual biomass of Carmagnola hemp was supplied by Assocanapa-Coordinamento Nazionale per la Canapicoltura (Carmagnola, Italy) as chopped pieces with a length of 5 cm or less.

Three morphological portions of the biomass—woody cores, short fibres, and dust—were separated using a sieve (screen size 2 mm). Sieving was used to separate the dust from woody cores and short fibres. The latter were manually separated from woody cores.

The recombinant enzyme pectate lyase from *Aspergillus* sp. (EC 4.2.2.2) was obtained from Megazyme (E-PCLYAN2). All chemicals used in this study were commercially available authentic samples and purchased from Sigma-Aldrich.

Methods

Sample preparation

For chemical analysis, woody cores, called hurds, were disintegrated into powder by using an IKA MF 10 knife mill and sieve (screen size 0.5mm). The moisture content of the milled samples was analysed according to TAPPI T 264 cm-97. Figure 1 shows the scheme used for the chemical analysis.

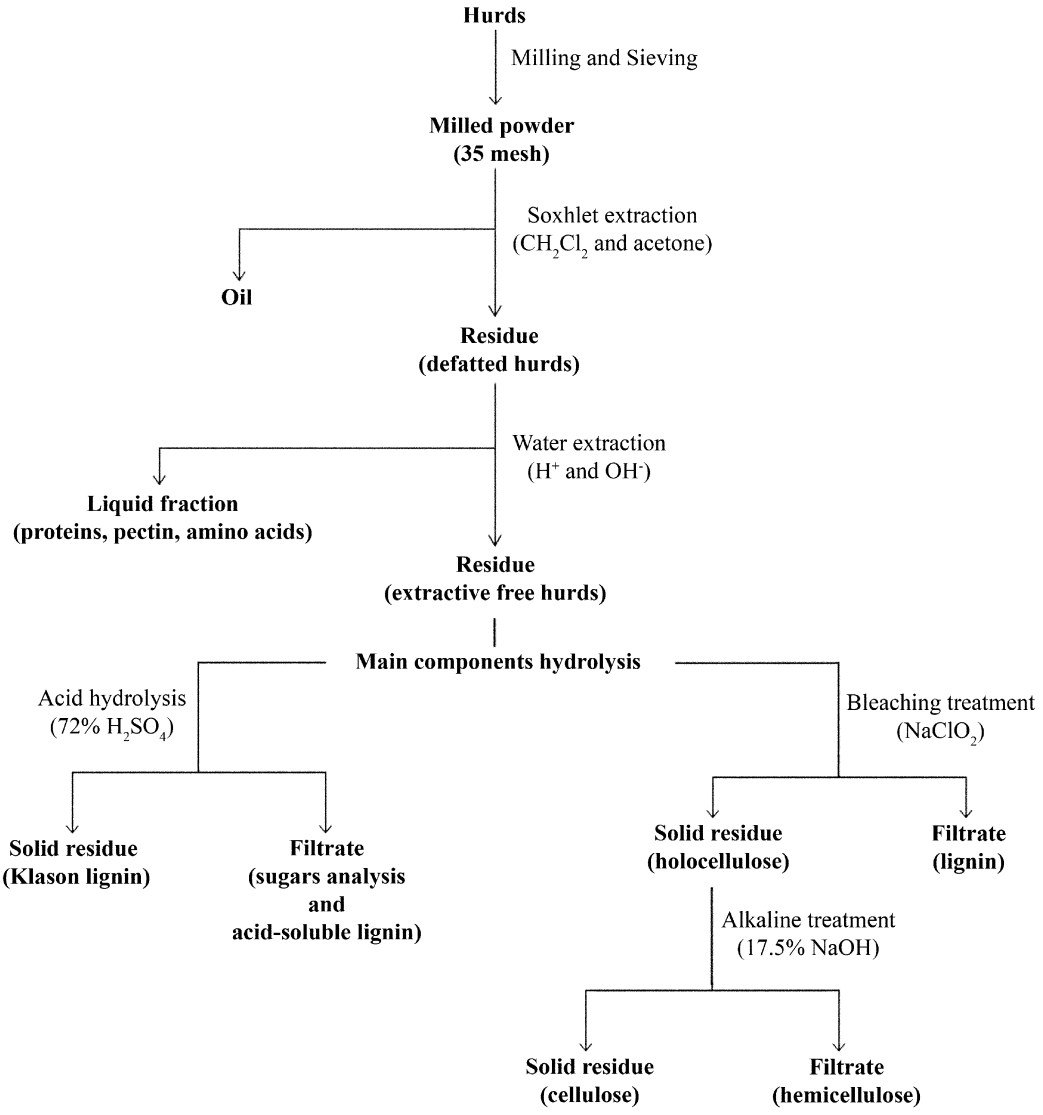

Fig. 1. Scheme for chemical analysis of hurds

Extractive analyses

A milled and oven-dried sample was used for isolation of extractives (solvent and water extractives). The oil was extracted with CH_2Cl_2 and with acetone by using a Soxhlet apparatus for 8 h at 90 ºC. The defatted hurds were used for further analysis. Proteins were quantified in a dry milled sample by total nitrogen determination using the Kjeldahl method (AOAC 1999). Isolation of water extractives was performed under basic and acidic conditions. Basic conditions were applied to obtain free amino acids and proteins. The extraction was done under continuous stirring, at pH 10 for 24 h at 40 °C. The solid/liquid ratio was 1:20. The liquid portion collected by filtration was adjusted to pH 7 and centrifuged in order to separate insoluble protein aggregates from free amino acids. The protein fraction was analysed by SDS-PAGE, while the free amino acids compositions were analysed by HPLC using the DABS-Cl pre-column derivatization method. Pectin extraction was performed under acid conditions and continuous stirring at pH 1.7 for 24 h at 85 °C using a solid/liquid ratio of 1:20. The pH of the filtrate portion was adjusted to 3.5, and pectins were precipitated by adding 3 volumes of 2-PrOH at 4 °C. The resulting gel was recovered by centrifugation, washed three times with 2-PrOH, and dried at 50 °C. Pectin identification was performed according to the JECFA method (Hansen *et al.* 2001).

Ash and lignin content analyses

Total ash content was measured according to TAPPI T 211 om-02. The content of acid-insoluble (Klason) and acid-soluble lignin (ASL) was determined from biomass samples according to TAPPI T 222 om-06 and TAPPI UM250, respectively. Isolation of ASL was also done by liquid–liquid extraction using $CHCl_3$.

Isolation of holocellulose and cellulose

The preparation of holocellulose and α-cellulose was carried out according to the literature (Yokoyama *et al.* 2002).

Chemical characterization of extractives

The oil (solvent extractive) was analysed by using GC/FID or GC/MS. Injector and detector temperatures were set at 300 °C and 350 ºC, respectively. Hydrogen was used as a gas carrier for GC analysis and helium for GC/MS analysis. The capillary column used was Agilent Technology DB-5HT (15 m × 0.1 mm × 0.25 mm film) for FID and DB-5MS (30 m × 0.25 mm × 0.25 mm film) for MS.

The quantification of galacturonic acid present in the pectin fraction was determined in accordance to the meta-hydroxydiphenyl colorimetric assay (Filisetti-Cozzi and Carpita 1991) using known concentrations of galacturonate as standard (in a range from 0 to 200 mg/L).

The degree of esterification (DE) of pectin was determined by Fourier transfer Infrared (FTIR) spectrometry (Gnanasambandam and Proctor 2000) and enzymatically using pectate lyase. For the enzymatic determination of DE, pectin samples or standards (from citrus fruit, Sigma P9561, P9436, P9311; esterification ≥85%, 55-70%, 20-34% respectively) were solubilized (2 mg/mL) in 50 mM Tris-HCl buffer, pH 8, and the pectin solutions were mixed with 790 µL of 50 mM Tris-HCl buffer, 1 mM $CaCl_2$, pH 8, and 10

µL of enzyme (0.01 U in 50 mM Tris-HCl buffer, 1 mM $CaCl_2$, pH 8). The reaction and blanks were conducted at 40 °C for 30 min (end point) and monitored at 235 nm. The amount of product (unsaturated oligogalacturonides) was calculated using the $\varepsilon 235$ = 4600 M cm^{-1} (Hansen *et al.* 2001). The degree of esterification was calculated from the calibration curve of the pectins standards (end points *vs*. DE). All measurements were performed in triplicate.

Free amino acid composition from basic water extraction was performed using HPLC on an Agilent Eclipse XDB-C18 5 µm (4.6 × 150 mm) column with the UV-Vis detector at 436 nm. The mobile phase consisted of two eluents: 50 mM acetate buffer (pH 4.1) (solvent A) and acetonitrile (solvent B), and the gradient was from 20% A to 70% B in 25 min (linear). The flow rate was 1.3 mL/min.

Spectroscopic characterization (FTIR)

FTIR spectra were obtained using a KBr disc containing 1% finely ground samples. Thirty-two scans were taken for each sample recorded from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}.

Total phenol content and antioxidant capacity

The total phenol content in Klason lignin and in solvent extractives was determined by the Folin-Ciocalteau colorimetric method (Vázquez *et al.* 2008) using gallic acid as a standard phenolic compound. The antioxidant capacity of the same samples was determined by the radical scavenging activity method using ABTS radical (Re *et al.* 1999). This method was modified as follows: the ABTS·+ solution was diluted with ethanol to an absorbance of 0.70 (±0.05) at 734 nm (ϵ = 1.6 × 104 $mol^{-1}L\ cm^{-1}$). The reaction was performed by addition of 1.0 mL of $ABTS^{\cdot+}$ solution to 100 µL of sample, or standard (Trolox). The mixture was stirred for 30 s and the absorbance was recorded until the end point (~30 min) at 30 ºC.

HPLC analysis of monosaccharides

The composition of monosaccharides from hydrolyzed liquid fractions of hurds was performed by derivatization of sugars with PMP (1-phenyl-3-methyl-5-pyrazolone) according to Dai *et al.* (2010) and analysed by HPLC on an Agilent Eclipse XDB-C18 5 µm (4.6 × 150 mm) column with the UV-Vis detector at 245 nm. The mobile phase was 0.1 M phosphate buffer (pH 6.7) and acetonitile (83:17 v/v, %) at a flow of 1 mL/min.

Nitrobenzene oxidation

Nitrobenzene oxidation of hurds for syringylpropane to guaiacylpropane units (S/G) ratio determination was performed according to Sun *et al.* (1995). The major components were identified by addition of authentic samples to the reaction mixture.

RESULTS AND DISCUSSION

The purpose of this work was to study the chemical composition of Carmagnola hemp hurds to optimize its utilization in high-value applications, such as the production

of biodegradable products, chemicals, and biofuels. Results of the hemp hurds characterization are reported in Table 1. The main components of this biomass are: cellulose, hemicellulose, lignin, lipids, proteins, pectin, water, and ash.

Extractives Yield

Extractive components (oil, waxes, pectin, proteins, and tannin) were isolated by increasing solvent polarity. For the extraction of oil and waxes, the well-known Soxhelt method was used with CH_2Cl_2 and acetone (instead of the usual hazardous extraction solvent, an ethanol–benzene mixture). The yield of total extractives was ~4.0% (Table 1), of which the oil and waxes content, estimated to be ~1.8% (sum of CH_2Cl_2, 1.1%, and acetone, 0.8%), was low, but comparable with those of other hemp varieties (Vignon *et al.* 1995). Water extraction was performed either under acidic or basic conditions, to obtain pectin (0.6%) and proteins (1.6%, including free amino acids ~0.02%). Extractives were removed before sample hydrolysis to avoid incorrect determination of Klason lignin. Extractive-free samples were used for all chemical analysis.

Table 1. Chemical Composition of Carmagnola Hemp Hurds Weight Percentage on a Dry Basis and Comparison with Other Referenced Values

Components	This Work [a]	Published [b]
Moisture	7.0 ± 0.1	
Extractives (solvent and water)		
Oil – CH_2Cl_2	1.1 ± 0.1	
Oil – Acetone	0.8 ± 0.1	
Pectin – Acidic water	0.6 ± 0.1	1
Protein and amino acid – Basic water	1.6 ± 0.1	
Ash		
Acid-insoluble ash	1.0 ± 0.1	2-4
Total ash	1.2 ± 0.1	
Lignin		
Klason lignin	21.0 ± 1.0 [c]	
Acid-soluble lignin (by UV)	2.4 ± 0.1	
Acid-soluble lignin (by extraction)	3.2 ± 0.1	
Total lignin	23.0 ± 1.0	16-23
Carbohydrates		
Holocellulose	75.0 ± 1.0	
α-Cellulose	44.0 ± 1.0	39-49
Hemicellulose	25.0 ± 1.0	16-23

[a] Standard deviations were calculated from triplicates
[b] Vignon *et al.* 1995; Hurter 2006; Barta *et al.* 2010
[c] After correction of acid-insoluble ash

Ash and Lignin Content

Ash constitutes an extensively studied component of biomass, which is nevertheless poorly understood. Ash is defined as the inorganic and the mineral matter of a biomass. For industrial biomass application, it is important to know the amount of ash that is present. The ash content of the sample was 1.2%, a very low amount when

compared with other varieties of hemp (Vignon *et al.* 1995), a feature that can be considered a positive point.

Lignin isolation was carried out by using a strong acid hydrolysis treatment (72% H_2SO_4): The solid residue, called acid-insoluble or Klason lignin (22%), contains 1.0% of acid-insoluble ash (Table 1). The acid-insoluble lignin content of hemp hurds is in line with that reported by Barta *et al.* (2010). During hurds hydrolysis, a portion of lignin was solubilized and called acid-soluble lignin (ASL, 2% to 3%). In this study, two different methods were used to define the percentage of ASL, namely the commonly used TAPPI method, by measuring the absorbance at 205 nm with a spectrophotometer, or by extraction with chloroform, to isolate ASL from the aqueous solution. This extraction method gives a slightly higher value compared with the UV measurement, probably due to the presence of lignin carbohydrate complexes (LCC).

Holocellulose and Cellulose Yield

The major component of hurds is holocellulose, a polysaccharide obtained by a bleaching process with sodium chlorite. The yield of holocellulose was 75% (Table 1), which is a little higher than reported by Barberà *et al.* (2011), but comparable with values obtained with hardwoods. To obtain α-cellulose from holocellulose, a 17.5% sodium hydroxide solution was used as the reagent. The α-cellulose content was 44% of the dry biomass, which is in good agreement with values reported for other varieties of hemp (Vignon *et al.* 1995). The value of hemicellulose (~25%) was calculated by subtraction of α- and β-cellulose from holocellulose.

Characterization of Extractives

The total lipid extractives (with CH_2Cl_2 and acetone) of Carmagnola hemp hurds accounted for 1.7% of the starting material. They were analyzed by GC and GC/MS. The chromatogram reported in Fig. 2 (A-CH_2Cl_2, B-acetone) shows the lipid extractive composition, which consists mainly of fatty acids, alkanes, aldehydes, and sterols; among them phenols, clionasterol, phytosterol, and coumarin were identified. Results from hurds oil were similar, except for waxes, to the composition of fibers oil (Gutiérrez *et al.* 2006). The protein content of defatted hurds isolated from basic water extraction was 1.6% (Table 1). The characterization of the isolated proteins was carried out by SDS-PAGE analysis. The results did not show the presence of predominant proteins, in contrast to what was observed in the hemp seeds' isolated protein profile (Tang *et al.* 2006).

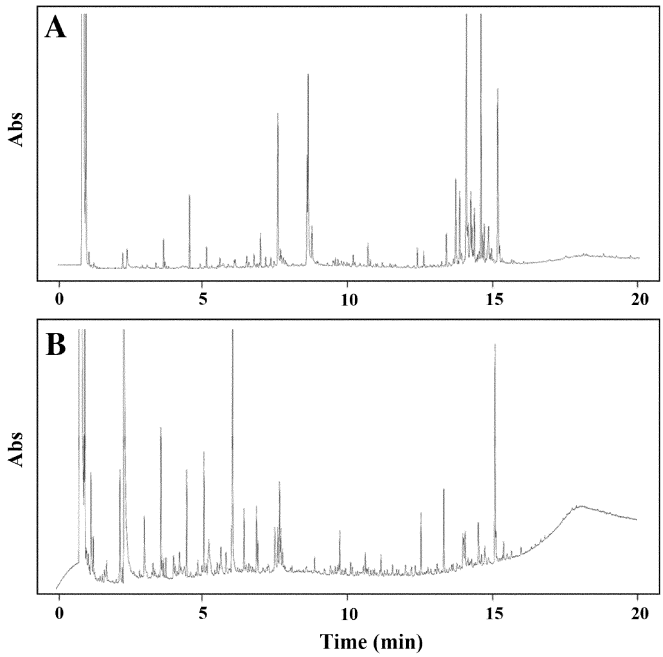

Fig. 2. GC/MS chromatograms of the lipid extracts from hemp hurds (A-CH_2Cl_2, B-acetone). Peak eluted between 4 and 6 min, fatty acids; 7–12 min, aldehydes; 13–15 min, aldehydes and sterols

After removal of proteins from the liquid fraction, the free amino acids content was evaluated to be 0.02%. In order to identify and quantify the free amino acids composition, HPLC analysis was carried out (Fig. 3A). The chromatogram shows the presence of at least nine different free amino acids; four of them were essential amino acids. The more abundant amino acids from the liquid fraction were proline and valine (24 and 18%, respectively). Pectin extraction from different sources may give different yields, according to process parameters (pH, time, temperature) and sample features. The yield of isolated pectin from hemp hurds was 0.6% on a dry matter basis, a lower value compared with those reported from major sources of pectic substances such as citrus fruits and even to what was reported for hemp straw (Vignon *et al.* 1995), probably due to the retting process to which the starting material was subjected. Galacturonic acid is the main component of pectin and was found to be 70% in the samples. The degree of esterification (DE) is an important industrial parameter for the gelling propriety of pectin. The DE of extracted pectin was determined using the enzyme pectate lyase. This enzyme splits the glycosidic bonds of a galacturonic chain, with a preference for glycosidic bond next to a free carboxyl group, by trans-elimination of hydrogen from the 4 and 5 carbon position of the galacturonosyl moiety to form a double bond, thus giving an increase in absorbance at 235 nm. Taking advantage of this peculiarity, the enzymatic hydrolysis of pectin standard (with different DE) and polygalacturonic acid were tested, showing a good linear response as a function of the DE (Tardy *et al.* 1997). By this approach, the DE of the pectin sample was estimated to be 46%, a result in accordance with the data obtained by FTIR.

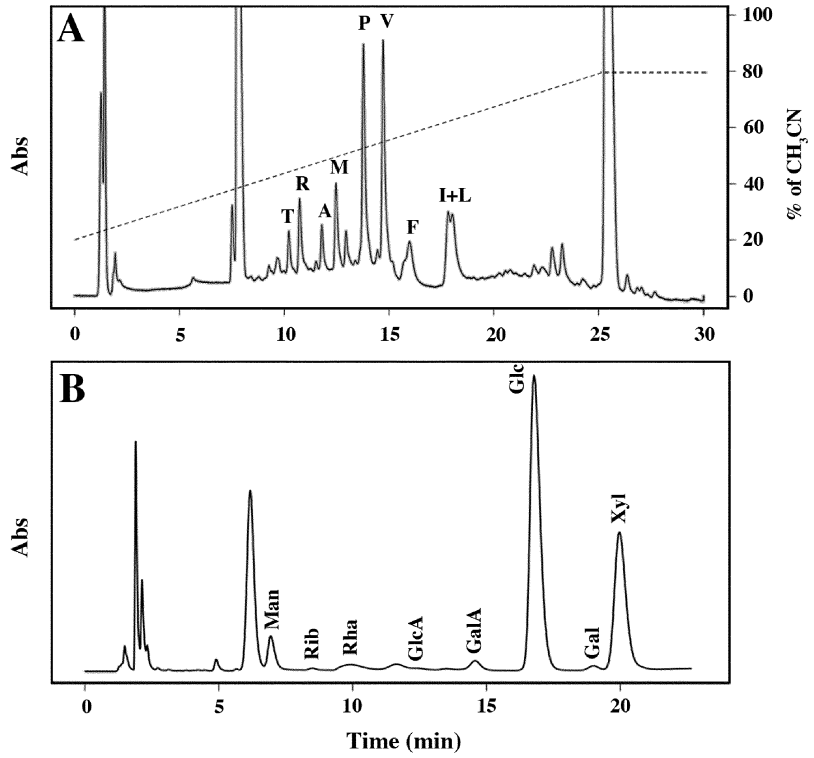

Fig. 3. HPLC chromatograms of the mixture of extracted free amino acids (A) and the monosaccharides mixture (B) obtained by acid hydrolysis of hurds. Dotted line: gradient of acetonitrile. Abbreviations used: T, threonine; R, arginine; A, alanine; M, methionine; P, proline; V, valine; F, phenylalanine; I, isoleucine; L, leucine; Man, mannose; Rib, ribose; Rha, rhamnose, GlcA, glucoronic acid; GalA, galacturonic acid; Glc, glucose; Gal, galactose, Xyl, xylose.

FTIR Spectra Analysis

FTIR spectroscopy was used as a simple technique to obtain rapid information regarding the structure and physicochemical properties of hurds and their components (*i.e.*, cellulose, lignin, holocellulose, and pectin) in comparison with standard materials. FTIR spectra of all samples are shown in Fig. 4. All samples were found to have different absorption in the range 3400 to 2900 cm^{-1}, a strong hydrogen bond O-H stretching absorption around 3400 cm^{-1}, and a prominent C-H stretching absorption around 2900 cm^{-1}. The area between 1800 to 900 cm^{-1}, called the finger print area of spectra, has many sharp and discrete absorption bands due to the various functional groups present in each component. Based on previous literature data, the bands at around 1740 cm^{-1} (hemicellulose), 1500 cm^{-1} (lignin), and 897 cm^{-1} (cellulose) are typical for characterization of pure samples. Spectra from hurds samples, following removal of extractives, show no difference compared with the starting material (data not shown).

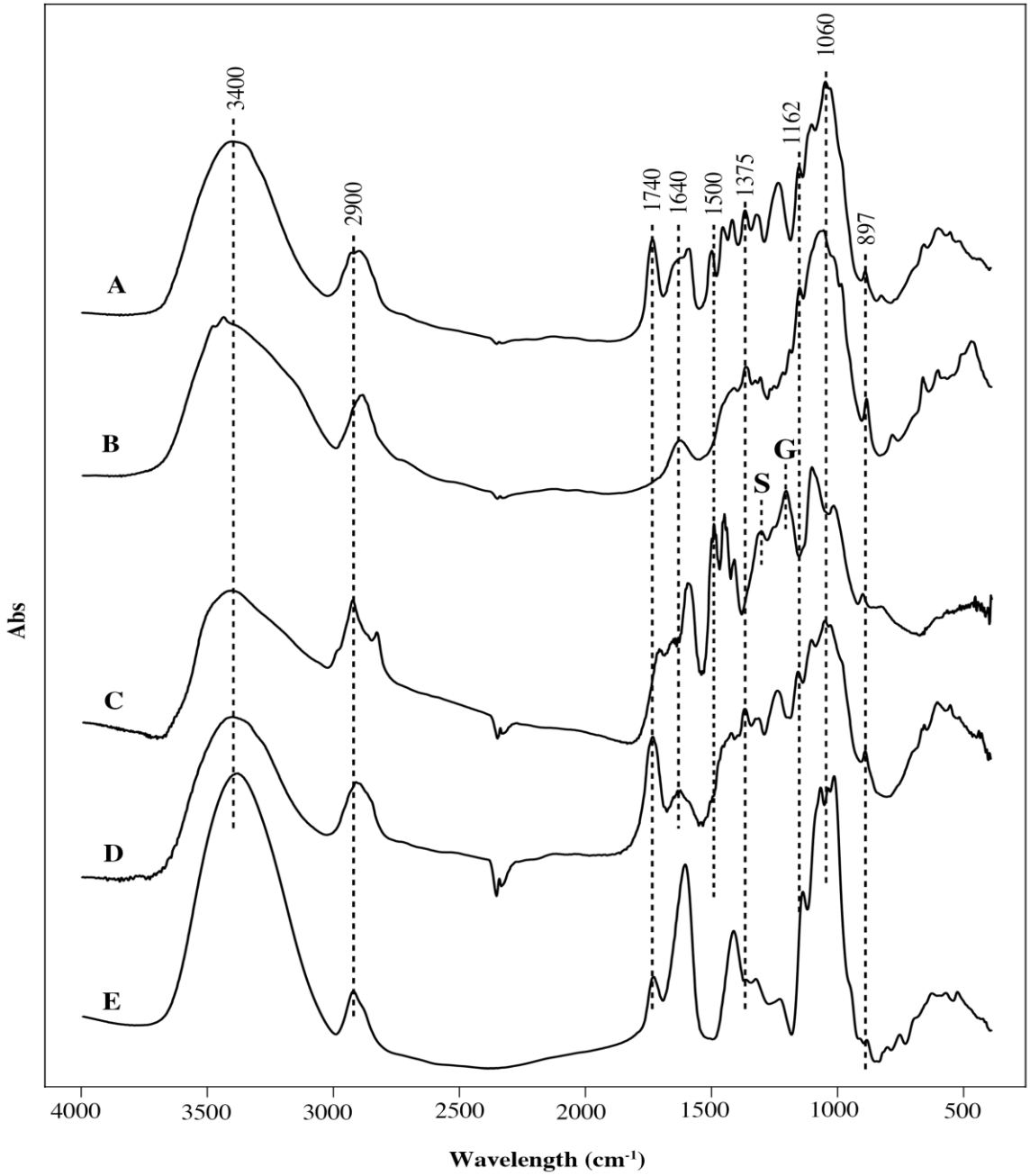

Fig. 4. FTIR spectra of hurds (A), and of cellulose (B), lignin: S, syringyl and G, guaiacyl units (C), holocellulose (D), and pectin (E) isolated from hurds

The absorption bands at 1462, 1423, 1311, 1214, and 1112 cm^{-1} arise mostly from lignin, while the bands around 1376, 1162, 1060, and 897 cm^{-1} are mainly due to carbohydrates and have no significant contributions from lignin (Pandey 1999, Pandy and Pitman 2003; Sun *et al.* 2004; Peng *et al.* 2009). Significant changes have been observed in the fingerprint region of the IR spectra due to various vibration modes in all samples. In two spectra (spectrum B and spectrum D), the absorbance around 1640, 1375, 1060,

and 897 cm^{-1} are attributed to native cellulose. The bands at 1740, 1245, and 1162 cm^{-1} present in spectrum D are due to hemicellulose in holocellulose samples. The band intensity at 1740 cm^{-1} was observed to be higher in the spectra of holocellulose compared with the hurds spectrum because of the C=O stretching vibration of carboxyl groups due to the acetyl moiety presence in hemicellulose (xyloglucan) (Popescu *et al.* 2011). The absence of the band at 1740 cm^{-1}, for a carbonyl group in spectrum B, suggests that the cellulose isolated from hurds with 17.5% NaOH is free of acetyl groups. The band at 1640 cm^{-1} is associated with the bending mode of absorbed water. The higher absorbance at 1375 cm^{-1} arises from C-H symmetric deformation in cellulose and holocellulose. The two bands at 1162 and 985 cm^{-1} are typical of arabinoxylans (Peng *et al.* 2009). The presence of arabinosyl side chains is suggested by weak shoulders at 1162 cm^{-1} (spectrum D). The change of intensity for this band suggests a contribution from arabinosyl substituents. The C-O-C pyranose ring skeletal vibration gives a prominent band around 1060 cm^{-1} in spectra B, D, and E. The region between 950 and 700 cm^{-1}, called the anomeric region, has bands at 897 cm^{-1} in spectra A, B, D, and E and not C, because of the C-1 group frequency or ring frequency, which is indicative of β-glycosidic linkages. The absence of this band in spectrum C reveals that isolated lignin was almost pure without sugar moieties.

The band around 1500 cm^{-1} is assigned to benzene ring vibration and can be used as an internal standard for the lignin sample. Hemp hurd lignin, called guaiacyl–syringyl (hardwood) lignin, is composed of coniferyl and sinapyl-alcohol–derived units, where guaiacyl-type lignin has a weak 1267 cm^{-1} band and a strong band at 1214 cm^{-1}, while syringyl-type lignin has a band near 1315 cm^{-1}. In the samples, a 1267 cm^{-1} band (Pandey 1999) was not detected. The band at 1460 cm^{-1} arises from methyl and methylene deformation, with very high intensity in lignin samples compared with hurds (spectrum A and C). The absorption band at 1715 cm^{-1} for C-O stretching shows the presence of hydroxycinnamates, such as p-coumarate and ferulate (Sun *et al.* 2000). The intensity of this band increases in spectrum C, indicating a higher content of hydroxyl-cinnamates in the isolated lignin sample.

In the case of a pectin sample (spectrum E), absorption in the O-H region is due to the inter- and intra-molecular hydrogen bonding of the galacturonic acid. Bands around 2950 cm^{-1} include CH, CH_2, and CH_3 stretching bending vibrations. Bands occurring at 1740 cm^{-1} and 1615 cm^{-1} indicate an ester carbonyl (C=O) group and carboxylate ion stretching band (COO-), respectively. A carboxylate group shows two bands, an asymmetrical stretching band near 1615 cm^{-1}, and a weaker symmetric stretching band near 1421 cm^{-1}. Bands at 1740 and 1615 cm^{-1} are important for the identification and quantification of the degree of esterification (DE) in pectin samples (Gnanasambandam and Proctor 2000). Three standard pectins with known DE were used to find the linear relationship between the area of the ester carbonyl band and the DE values (R=0.98, n=3), giving a ~46% of esterification for the sample.

Data from FTIR analysis revealed that isolated components are structurally comparable to the standard commercial samples (data not shown).

Phenol Content and Antioxidant Capacity of Solvent Extractives and Lignin

Total phenol content is expressed as gallic acid equivalent (GAE, g/100 g of sample). Acetone extracts showed the highest value of about 6.5 GAE, while Klason lignin and CH_2Cl_2 extracts gave a value of 4.0 and 3.4 GAE, respectively. The highest value of phenol content was obtained from acetone extracts due to the presence of tannins.

To test the radical scavenging ability of solvent extractives (CH_2Cl_2 and acetone) and Klason lignin from hurds, an ABTS test was chosen. The results, reported as Trolox equivalent antioxidant capacity (TEAC), gave 4%, 4%, and 3% for Klason lignin, acetone, and CH_2Cl_2 extracts, respectively.

HPLC Analysis of Monosaccharides

The sugar composition from the hydrolyzed liquid fraction of hurds was obtained by HPLC analysis. The HPLC profile of PMP-sugars (Fig. 3B) shows the presence of eight different monosaccharides, and among them glucose (56.7%), xylose (31.2%), and mannose (4.9%) were the most abundant. Minor amounts of rhamnose (2.1%), galactose (0.9%), and a trace amount of ribose (0.3%), but an absence of arabinose were observed in the samples. Uronic acid, including glucuronic acid (0.2%) and galacturonic acid (2.0%), also appeared in minor quantities. Since xylose and mannose were found in good percentage, we suggest that the hemicellulose fraction would be composed mainly of glucuronoxylan and glucomannan. This agrees with the classification of hemp as a hardwood. Glucose accounted for ~57% of monosaccharides, which correspond to 51% of glucan, this is in good agreement with cellulose found from isolation with NaOH solution. The percentage of glucan found in Carmagnola hemp hurds is higher than reported for other varieties (Moxley *et al.* 2008; Barta *et al.* 2010).

Nitrobenzene Oxidation of Hurds

The eight phenolic components obtained by alkaline nitrobenzene oxidation of hurds were identified by HPLC in comparison with authentic samples. Major components were found to be vanillin (45.1%) and syringaldehyde (35.1%). Minor amounts of gallic acid (0.5%), *p*-hydroxybenzaldehyde (8.5%), vanillic acid (0.9%), syringic acid (6.3%), *p*-coumaric acid (2.9%), and acetosyringone (0.6%) were also identified. The syringylpropane to guaiacylpropane units (S/G ratio) was estimated to be 1.42 and determined according to the method described by Santos *et al.* (2012). The monolignols composition is in accordance with FTIR spectra since the intensity of the syringylpropane units is rather weak, compared to the guaiacylpropane units (see Fig. 4).

CONCLUSIONS

1. The chemical analysis of hemp hurds from the industrial variety "Carmagnola" was performed using standard methods, and the isolated components were fully characterized in order to obtain the chemical composition and the main structural features of its components.

2. Cellulose, holocellulose, and lignin were assessed by hydrolysis. Polysaccharides (cellulose, 44.0%, and hemicelluloses, 25.0%) were the most abundant components of hurds followed by lignin (23.0%), extractives (oil, proteins, amino acids, pectin *etc*., in total 4%), and ash (1.2%).
3. Compared to other hemp varieties, Carmagnola hurds contain very small amounts of ash with high amounts of polysaccharides underlying potential benefits for biofuels production. Nitrobenzene oxidation and FTIR analysis confirmed the presence of guaiacyl and syringyl units in hemp hurds lignin. High yield of vanillin was observed suggesting high availability of guaiacyl units.
4. This study is useful as base line data for agro-economic evaluation of the Carmagnola hemp as a feedstock for an integrated biorefinery, because the valorization of hemp hurds is still overlooked and not fully exploited.
5. To fully valorize the hemp hurds, further investigation on the optimization of pretreatment technique is required.

ACKNOWLEDGMENTS

The authors wish to thank Regione Lombardia for their support through the project "VeLiCa – From ancient crops, materials, and products for the future" (protocol no. 14840/RCC).

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Article submitted: January 29, 2013; Peer review completed: March 18, 2013; Revised version received and accepted: April 7, 2013; Published: April 10, 2013.