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Supercritical CO_2 fractionation of bio-oil produced from wheat–hemlock biomass

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ABSTRACT

The biomass i.e. wheat–hemlock used in this study was first characterized for its composition. The physical and chemical characterization of biomass was estimated using proximate analysis, calorific value, crystallinity, devolatilization behaviour, ultimate analysis, ICP-MS of ash, FT-IR, XRD, CHNS, and HPLC analysis. For commercial purpose the same biomass was used for conversion to bio-oil by fast pyrolysis process. Therefore, in order to investigate its composition, the bio-oil was also characterized using proximate analysis, calorific value, whereas the chemical composition of the bio-oil was estimated using CHNS, ^{1}H NMR, GC-FID and GC/MS. The bio-oil obtained from wheat–hemlock biomass was supplied by Advanced Biorefinery Co. and after the analysis, its composition has been determined. It contains a mixture of hydrocarbons, pyranoids, furanoids, benzenoids and fatty acids/alcohols with 45% of water, which forms azeotrope with organic polar compounds. The supercritical CO_2 (SC-CO_2) is an advanced method for selective extraction of valuable chemicals from bio-oil without solvent residue. The organic fraction of the bio-oil was isolated by SC-CO_2. It was observed that SC-CO_2 fractions collected at 10 and 25 MPa pressure were enriched with furanoids, pyranoids and bezenoids. Similarly the bio-oil was also fractionated by conventional column chromatographic method and the yields and chemical compositions were compared with fractionated bio-oil obtained using SC-CO_2.

1. Introduction

Alternative and renewable energy resources have recently become a high priority for many countries and will play a large role in the chemical industry in the near future. These renewable energy resources have become popularized due to their low environmental risks and pollution and are favorable alternatives to fossil fuels and their derivatives. Biomass can be considered as a promising renewable energy source and is nearly carbon neutral and considered for various liquid fuels and chemicals (Kumar, 2009). In addition to their sustainability, they are, in general, more evenly distributed over earth's surface than fossil fuels or uranium and may be exploited using less capital-intensive technologies. Hence it increases the scope for diversification and decentralization of energy supplies and the achievement of energy self-sufficiency at a local, regional, and national level (Jones, 1989). Cellulosic biomass represents a renewable and largely untapped source of raw feedstock for conversion into liquid fuels, thermochemical products and other energy-related end products (Sanderson et al., 1996).

The worldwide availability of biomass is estimated to be 220 billion oven-dry ton (odt) per year or 4500 EJ (10^{18} J) (Anon, 2004). It is world's largest and most sustainable energy resource. One can reduce the fossil fuel consumption by increasing the capacity of energy produced from biomass. The advantages of this process are to reduce the green house gases compared to fossil fuels and also solve the difficulties of the dependence of imported fossil fuels for many countries (Anon, 2004).

Biomass refers to plant derived organic matter that is available on a renewable basis. It consists of cellulose, hemicellulose and lignin. Lignocellulosic biomass are one of the promising renewable feed stocks for production of bio-fuels and chemicals due to petroleum oil shortage, fluctuating price of the crude oil and environmental problems. The use of renewable resources for fuel, such as bio-oil derived from lignocellulosic biomass has great potential to replace petroleum fuel.

The selected biomasses such as wheat straw and hemlock wood are abundant in Canada and may be used as feed stock for production of bio-oil. In the prairie provinces of Canada, Saskatchewan produces 7.6 millions ton of wheat annually based on the average taken over the production of the past 10 years (Information of Saskatchewan, 2008). In Canada average annual wood cut has been estimated at 167.5 million m^3 creating over 60 million tonnes of residues (Anon, 2004; Mohan et al., 2006). Hemlock (*Tsuga canadensis*) is one of the wood biomass in Canada. It is also commonly called *Canada Hemlock* and *Hemlock Spruce* is native to Asia and Western America (Cowles, 2009). It mostly occurs in USA and southern border of Canada from southern Ontario to Cape Breton Island, Nova Scotia. Hemlock occurs as a dominant or codominant in coniferous and mixed-hardwood forests. Hemlock wood is of

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low value because of brittleness and abundant knots. It is generally used for pulp, light framing, sheathing, roofing, subflooring and boxes.

A fundamental characterization of biomass as a feedstock is required for bio-fuel and chemical production, which exhibit very different properties with respect to traditional fossil fuels and their derivatives (Biagini et al., 2006). In particular, lingo-cellulosic materials are more reactive and have a higher volatility than coals. However, all biomass differs greatly in volatile matter concentration, where even the same type of biomass can show change in composition based on the climatic conditions and seasonal variation. Furthermore, characterization of biomass is imperative as the chemical composition of biomass affects the conversion processes differently. For example, high nitrogen and ash concentration reduces hydrocarbon yield during thermochemical conversion (Sanderson et al., 1996), whereas lignin concentration, one of the three basic components of lingo-cellulosic material (cellulose, hemicellulose and lignin) does not have detrimental effect on thermochemical product yield. Biomass feedstock composition determines the theoretical yield from a biochemical conversion, and can thereby have a significant impact on conversion process economics. As a part of our ongoing project we have studied the characterization of some Canadian biomass samples which are abundantly available (Naik et al., 2010). Pyrolysis is a thermochemical process applying high heat to lignocellulosic materials in the absence of air or in reduced air that converts organic materials into usable fuels (Demirbas, 2007).

The bio-oil which is a dark aqueous polar liquid, is produced using flash pyrolysis (Mohan et al., 2006). It is used as a substitute for fuel oil in boiler and engine applications and also has the potential for blending with petroleum. Bio-oil which is composed of almost 300 chemicals such as pyranoids, furanoids, benzenoids and fatty acids/alcohols can be directly used as fuel or pyrolysed to produce hydrogen/syngas. However, in order to make the whole process of producing and using bio-oil more efficient and economical, some of the valuable chemicals can be extracted from bio-oil and the rest of the bio-oil can be used as bio-fuel. Thus after removal of pyranoids, furanoids and benzenoids, the quality of the bio-oil is improved with higher percentage of fatty acids/alcohols, which is suitable for fuel application. These chemicals can be extracted using a fractionation or solvent extraction process. However, reports on use of supercritical fluids (SCF) such as supercritical carbon dioxide (SC-CO_2) for the extraction of chemicals from bio-oil are scarce. This technology has advantage over traditional technology by eliminating the use of polluting organic solvents. The SC-CO_2 extraction is commonly used for extraction of natural materials because of the non-toxic, non-flammable characteristics of CO_2 and its availability in high purity with low cost. Since, CO_2 has low critical temperature (31.1 °C) and low critical pressure (73.8 bar), it can be treated as an ideal solvent for extraction of natural products. It offers attractive extraction characteristics, owing to its favorable diffusivity, viscosity, surface tension and other physical properties. Its diffusivity is one or two orders of magnitude higher than those of other liquids, which facilitates rapid mass transfer and faster completion of extraction in compared to liquid solvents (Mukhopadhyay, 2000). Reverchon and De Marco (2006) have reviewed the numerous work carried out on the application of SC-CO_2 in food processing, pharmaceuticals and neutraceuticals. Recently our work on extraction of flax seed with SC-CO_2 has shown improvement in yield along with superior quality in compared to the conventional solvent extraction process (Pradhan et al., 2010). Esquivel and Bernardo-Gil (1993) concluded that SCF extraction explores the solvent power of fluids at temperatures and pressures higher than its critical value. Guo (1995) studied the separation of ethanol from aqueous solution by using liquid CO_2. Due to its low latent heat of vaporisation, low energy input is required for the extraction system. Further, the energy required for SC-CO_2 treatment is often less than the energy associated with distillation of conventional organic solvents. However, the capital costs are higher and total energy consumption depends on the pressure used and on the solute-to-solvent ratios. The solvent capacity of SC-CO_2 is density dependent and it is the sharp variability of density with pressure and temperature (Bijhm et al., 1989; Mukhopadhyay, 2000). The SC-CO_2 fractionation of bio-oil is an advanced process to produce moisture free extract and is an attractive alternative to extract the valuable compounds from bio-oil. SC-CO_2 can be treated as an ideal solvent for extraction of natural products. It offers attractive extraction characteristics due to its favorable diffusivity, viscosity, surface tension and other physical properties. Its density and solvent properties increase with increasing CO_2 pressure. The advantage of this process is that different types of compounds can be selectively extracted/separated at particular CO_2 pressure. For example, at low pressure easily extractable compounds (low molecular weight) are recovered in compared to high molecular weight compounds. Another advantage of SC-CO_2, the water is very soluble (>2.5 mol fraction), therefore it is an attractive alternative for separation of water from bio-oil by using this advanced process. To the best of our knowledge, there is no report available in the literature on separation of organic components from bio-oil using SC-CO_2. Therefore, in this study attempt has been made to characterize the wheat–hemlock biomass and its bio-oil and furthermore fractionate the bio-oil using SC-CO_2 to get value added chemicals, which was compared with that obtained using conventional column chromatographic process.

2. Methods

2.1. Characterization of biomass

Wheat–hemlock biomass and its bio-oil were supplied by Advanced Biorefinery, Ottawa, Canada. The solvents such as pentane, carbon tetrachloride (CCl_4), benzene, chloroform ($CHCl_3$), diethyl ether (Et_2O) used in the experiments were reagent grade (Sigma Aldrich) and distilled in laboratory before use. The analysis of biomass, bio-oil, and supercritical carbon dioxide fractioned fraction were carried out by following standard NREL and ASTM methods. The experimental results obtained are the average of at least three sets of data presented.

2.1.1. Proximate analysis of biomass

All the experiment results were average of three runs and data are presented with relative standard deviation. The moisture content of the biomass was determined using the procedure given in ASTM D 3173-87 (2003). Pulverized sample (1.0 g) was used and oven dried for 1 h at 130 °C. The ash content was determined in laboratory muffle furnace (Holpack, USA) as per ASTM 3174-04 (2004). The biomass sample (1.0 g) was taken in crucible and placed in muffle furnace which was maintained at 575 ± 10 °C for 4 h. The volatile matter in the biomass sample was determined by the procedure given in ASTM D 3175-07 (2007). In that case, 1.0 g biomass sample was taken in a crucible and placed in muffle furnace which was maintained at 950 ± 10 °C for 7 min. Then the crucible was removed from the furnace and placed in the desiccator. The amount of volatile matter of the biomass was determined by taking the difference in weight.

2.1.2. Physical properties of the biomass

The calorific value was determined in a static bomb calorimeter, a sealed Parr 1108, following the procedure described by Hubbard

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et al. (1956). The detailed experimental conditions are given in our earlier publication (Naik et al., 2010).

The XRD analysis of the biomass was performed using Rigaku diffractometer (Rigaku, Tokyo, Japan) using Cu Kα radiation at 40 kV and 130 mA in the scanning angle of 0–45° at a scanning speed of 0.05° min^{-1}. The XRD analysis of cellulose and lignin was carried out for comparative purpose. The pure laboratory grade cotton was taken for cellulose analysis. Lignin was derived from pine wood in our laboratory and was used for thermo-gravimetric analysis. The procedure was given in an earlier publication (Naik et al., 2010).

Thermo-gravimetric (TG) analyses of the biomass, cellulose and lignin were performed by using PerkinElmer, Pyris Diamond TG/DTA instrument in order to determine the devolatilization characteristics of the biomass samples with temperature. The sample (0.5 mg) was subjected to a temperature program 25–835 °C (10 °C/min) with purge gas (Argon) flow of 10 mL/min. The loss of weight and rate of weight loss with temperature were recorded.

2.2. Chemical analysis of biomass

The chemical analysis of biomass was studied by ICP-MS, CHNS, FT-IR and HPLC. The analysis of some common elements present in the ash was determined by ICP-MS and common organic elements i.e. C, H, N and S were analysed in PerkinElmer Elementar CHNS analyzer. The FT-IR spectra of biomass were obtained by KBr pelleting method using PerkinElmer, FT-IR spectrum GX in the IR range of 500–4000 cm^{-1}.

2.2.1. Determination of sugars in biomass

The biomass was acid hydrolyzed for conversion of hemicellulose and cellulose to water soluble sugars using the standard NREL method (2005). Then after water soluble sugars were analysed by Hewlett–Packard HPLC equipped with an RI detector. The analysis was performed by an Aminex HPX 87P column (BioRad, Hercules, CA) equipped with a de-ashing guard cartridge (BioRad). Degassed HPLC grade water was used as the mobile phase at 0.6 mL/min at column temperature of 80 °C. The injection volume used was 20 μL with a run time of 20 min. Mixed sugar standards viz. cellobiose, glucose, xylose, galactose, mannose and arabinose were used for qualitative analysis. The weight percentages of these sugars in the samples were determined by comparing with respective standards. The acid insoluble part of the biomass was treated as lignin.

2.3. Supercritical CO_2 (SC-CO_2) fractions of bio-oil

The bio-oil used in this study was produced from wheat–hemlock biomass by fast pyrolysis process for commercial purpose by Advanced Biorefinery, Ottawa. The bio-oil was fractionated using SC-CO_2 unit supplied by Thar Technologies. The bio-oil (50 g) was mixed with clean 2 mm glass beads and then after placed in the extractor to half of its volume. The SC-CO_2 extraction was carried out at 40 °C with CO_2 flow rate of 40 g/min. Three fractions were collected at three different pressures i.e. 10, 25 and 30 MPa, respectively, with the extraction time of 2 h in each experiment keeping all the other conditions identical. Initially, extraction process was started at 10 MPa pressure and was continued for 2 h. The first fraction was collected at 10 MPa pressure, then after the pressure was raised to 25 MPa and extraction was continued for another 2 h keeping all other conditions identical. After collecting the second fraction, the remaining material with glass beads was again treated in the same way at 30 MPa to collect the third bio-oil fraction. It was observed that the bio-oil used for the study was blackish in colour, whereas SC-CO_2 fractions derived from the bio-oil were reddish in colour.

2.3.1. Physical properties

The moisture content of bio-oil and SC-CO_2 fractions derived from bio-oil was determined by Karl–Fischer titrator, whereas ash content and calorific values were determined by methods described above.

The Karl–Fischer titration was carried out by Mettler Toledo Titrator DL32, Provided by Mettler Toledo, USA. Sample (0.1 g) was injected into the titration cell (Anolyte) connected to a balance to measure the amount of sample injected. Anolyte was provided by Merck. Samples with water content more than 5 wt% were diluted with methanol before the injection. Iodine produced by iodide available in the Anolyte reacts with water. As soon as all the water reacted, the free iodine was detected in the Anolyte and the Karl–Fischer titration was terminated by the equipment (Scholz, 1984).

2.3.2. Chemical properties

The chemical properties of the bio-oil and SC-CO_2 fractions were determined by CHNS, FT-IR, ^{1}H NMR, GC-FID and GC/MS. ^{1}H NMR was recorded on DPX-500 Bruker machine at 500 MHz at 25 °C. For the study, 0.2 g of bio-oil was dissolved in CD_3OD and 0.2 g of SC-CO_2 fractions were dissolved in $CDCl_3$ for spectral analysis. GC analysis was carried out on a Varian CP-3800 Gas Chromatograph equipped with a flame ionization detector (FID) and a 30 m × 0.25 mm WCOT column coated with 0.25 μm film thickness (DB-5). Helium was used as the carrier gas at a flow rate of 1.2 mL/min at a column pressure of 22 kPa. Each sample (0.2 μL) was injected into the injection port of the GC using a split ratio of 50:1. Compound separation was achieved following a linear temperature program of 50–250 °C (5 °C/min), 250 °C (20 min). Percentage composition was calculated using peak normalization method. Each sample was analysed twice in GC; thus a total four GC analysis were performed for extracts from each of the process. The GC/MS analysis was carried out on a Varian Saturn 2200 GC/MS fitted with the same column and temperature programmed as above. Peak identification was carried out by comparison of the mass spectra with mass spectra available on NIST-1 and NIST-II libraries. The compound identification was finally confirmed by comparison of their relative retention indices (RRI) with literature values (Yuping et al., 2008; Vichi et al., 2007). The RRI of the peaks was calculated by standard sample mixture of normal saturated hydrocarbons C-7 to C-22 and was injected into the GC column under the same conditions of sample analysis (Rout et al., 2007).

2.4. Bio-oil fractionation using column chromatography

During this study crude bio-oil (30 g) was extracted with 50% $CHCl_3$ in Et_2O. For the extraction purpose 50 mL mixture of solvent (i.e. $CHCl_3$ and Et_2O) was taken each time and the process was repeated three times to ensure that maximum extracts have been recovered. Thereafter the solvent soluble material was concentrated in rotary evaporator under *vacuo* and $CHCl_3$–Et_2O extracted bio-oil was used for column chromatography.

The $CHCl_3$–Et_2O extracted bio-oil was fractionated in a glass column (80 cm × 2 cm) using 60 g of 100–200 mesh column chromatographic silica gel. First the column was eluated with 500 mL pentane to obtain the pentane soluble fraction. After the pentane fractionation the same column was eluated with 500 mL of CCl_4, benzene, Et_2O and $CHCl_3$ followed by one after another and at the end the column was washed with methanol. All the collected fractions were concentrated in rotary evaporator under *vacuo* and stored at refrigerated condition prior to the analysis. The fractions were analysed by GC-FID and GC/MS.

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Table 1
Proximate analysis, ultimate analysis, calorific value and pH of wheat–hemlock biomass, bio-oil and supercritical CO_2 fractions of bio-oil.

Samples	Proximate analysis (%)				Ultimate analysis (%)							Calorific value (MJ/kg)	pH
	Moisture	Ash	Volatile matter	Fixed carbon[a]	C	H	N	S	O[b]	H/C molar ratio	O/C molar ratio	Empirical formulae[c]	
Wheat–hemlock biomass	8.3 ± 0.2	1.8 ± 0.1	83.0 ± 0.2	6.9 ± 0.2	46.7 ± 0.2	6.4 ± 0.2	0.05 ± 0.02	0.01	46.8 ± 0.3	1.64	0.75	$CH_{1.64}O_{0.75}$	18.6 ± 0.2
Crude bio-oil	45.0 ± 0.9	0.03 ± 0.1	–	–	19.3 ± 0.1	9.2 ± 0.1	0.74 ± 0.02	0.17	70.6 ± 0.2	5.72	2.74	$CH_{5.72}O_{2.74}$	nd
10 MPa fraction	0.5 ± 0.1	–	–	–	75.8 ± 0.2	11.9 ± 0.1	0.01	nd	12.3 ± 0.2	1.88	0.12	$CH_{1.88}O_{0.12}$	41.0 ± 0.4
25 MPa fraction	1.5 ± 0.2	–	–	–	74.6 ± 0.3	11.5 ± 0.2	0.02 ± 0.01	0.01	13.8 ± 0.3	1.85	0.14	$CH_{1.85}O_{0.14}$	40.2 ± 0.2
30 MPa fraction	2.5 ± 0.3	–	–	–	76.0 ± 0.2	11.7 ± 0.1	0.09 ± 0.01	0.02	11.9 ± 0.4	1.85	0.12	$CH_{1.85}O_{0.12}$	43.2 ± 0.5

nd: not detected.
[a] % of fixed carbon calculated from difference of moisture, ash and volatile matter content.
[b] % of O calculated from the difference of C, H, N and S.
[c] N and S not taken into consideration.

3. Results and discussion

3.1. Biomass

The proximate analysis, ultimate analysis, calorific value and pH of the biomass are presented in Table 1. It can be observed that low amounts of N (0.05%) and S (0.01%) are present in the biomass. The elemental analysis of the ash shows that following elements are present in the ash sample (in ppm) i.e. Mg (16,465), Al (2707), Ca (57,059), P (7374), Cr (73), Mn (2651), Fe (9123), Co (63), Cu (339), Zn (4956), Sr (351), Sb (8), Ba (842), Pb (464). The biomass contained high amounts of Mg, Ca, Fe and P, whereas low amounts of Ba and Pb. However, the elements like Sc, Ti, V, Ni, As and W were not detected during the analysis. The XRD analysis of the wheat–hemlock biomass, cellulose and lignin is presented in Fig. 1. The plate count of the biomass was 974,015 and 1,551,389 at 12–14° and 20–22°, respectively, whereas the plate count for the cellulose was 189,122 and 690,750 at 12–14° and 20–22°, respectively, and for lignin it was 2,083,195 at around 20–22°. The plate count relates to the crystallinity of the sample. Similar crystallinity was reported for five types of Canadian biomass (Naik et al., 2010).

The devolatilization behaviour of biomass (mixed) shows the chemical nature as well as the percentage of cellulose, hemicellulose and lignin (Fisher et al., 2002; Raveendran et al., 1996). The devolatilization behaviour of the biomass, cellulose and lignin is shown in Fig. 2. The mass loss and rate of mass loss observed during our study for biomass, cellulose and lignin is shown in Fig 2. The mass loss at temperature less than 100 °C was mainly due to easily volatile matters, 100–130 °C for water, 130–250 °C for

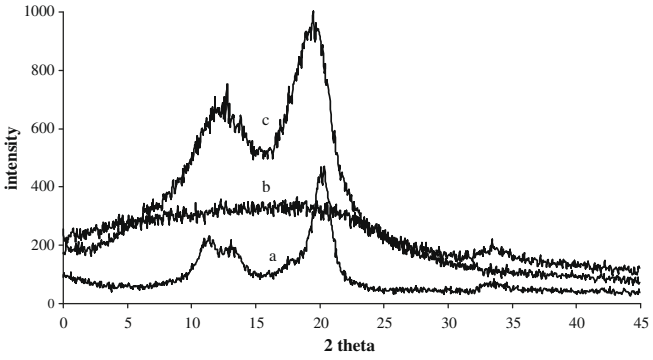

Fig. 1. XRD analysis: (a) cellulose, (b) lignin, (c) wheat–hemlock biomass.

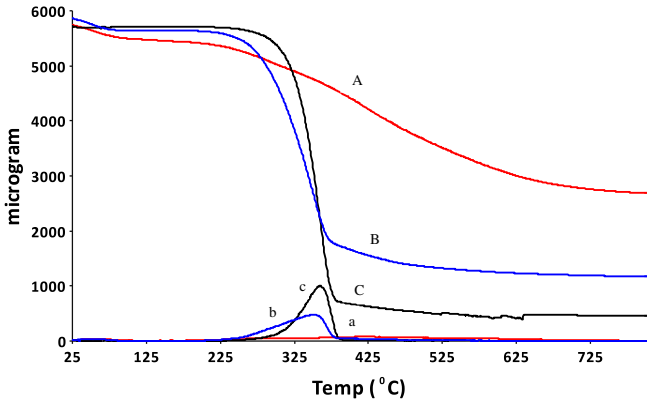

Fig. 2. TGA and DTG of (A, a) lignin, (B, b) wheat–hemlock biomass, (C, c) cellulose, respectively.

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volatile compounds, 250–350 °C for hemicellulose, 350–500 °C for cellulose and lignin and 500–800 °C for lignin. The rate of mass loss was maximum at 300–400 °C for biomass and cellulose, however the lignin followed a wide temperature range i.e. 200–700 °C.

The FT-IR studies (Fig. 3) revealed that the most prominent peaks in the spectrum originate from –O–H stretching vibration (3370–3420 cm^{-1}) and CH_2 and CH_3 asymmetrical and symmetrical stretching vibrations (2935–2915 cm^{-1}). These vibrations were expected from hemicellulose, cellulose and lignin. Very intense peaks in the region 1742–1620 cm^{-1} originate from the stretching mode of carbonyls mainly ketones and esters (Moore and Owen, 2002). Mainly these bands are expected from waxes such as fatty acids, fatty esters, high molecular mass aldehydes/ketones.

To determine the total sugar content in the raffinate biomass, it was treated with dilute H_2SO_4. In the process of acid hydrolysis the oligomers break down into its monomers detected by HPLC, it is considered as the important step before sugar analysis. The percentage of sugars detected in the biomass was found to be glucose (56.4 wt%), xylose (6.3 wt%), galactose (1.9 wt%), arabinose (31.8 wt%) and mannose (3.6 wt%). The cellobiose was not detected in the mixed biomass sugar analysis, which confirms with our previous report, that wheat straw does not produce cellobiose in acid treatment (Naik et al., 2010). A sample of HPLC spectrum is shown in Fig. 4. The figure indicates the peaks for monosaccharides such as glucose, xylose, galactose, mannose, and arabinose (from left to right), respectively. The acid soluble fraction shows cellulose and hemicellulose in the major quantities. As per the sugar analysis, the ratio of hemicellulose: cellulose in the biomass was found to be 1:1.3. However, the acid insoluble portion which is known as lignin shows the yield of 21.5 wt%. The biomass also contained 2–3 wt% wax and other non-detectable polar extractive compounds.

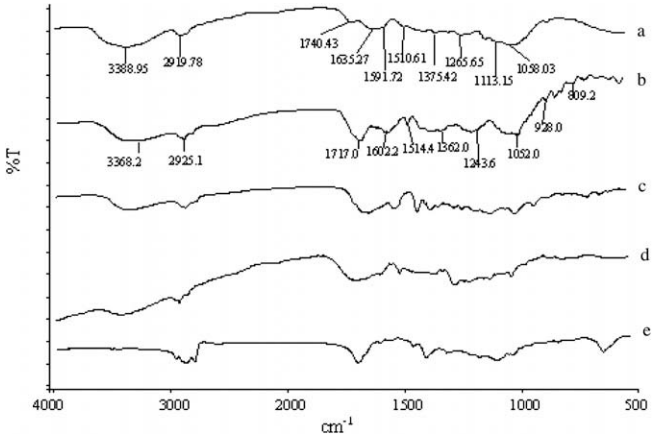

Fig. 3. FT-IR spectra: (a) biomass, (b) bio-oil, (c) CO_2 fraction (10 MPa), (d) CO_2 fraction (25 MPa), (e) CO_2 fraction (30 MPa).

3.2. Bio-oil and SC-CO_2 fractions

The bio-oil produced from wheat–hemlock shows high percentage of water compared to the bio-oil produced from other biomass (Mullen and Boateng, 2008; Sensoz and Angin, 2008; Meire and Faix, 1999). The complexity of the bio-oil indicates its possible potential for extracting value added chemicals. The major challenge was the separation of value added chemicals and liquid fuel in an economical way from the crude bio-oil. It was observed that the crude bio-oil becomes polymerised within few weeks after its production at room temperature due to high percentage of oxygen and water in it. Therefore, the removal of water from the bio-oil is essential to improve its stability, storage and calorific value of the liquid fuel.

Experiments were carried out to remove water from azeotropic mixture. In this case, different techniques such as distillation under reduced pressure and addition of dehydrated Na_2SO_4 to bio-oil were used. During these experiments it was observed that distillation was not effective for removal of water under reduced pressure. Similarly, the use of dehydrated agents for removal of water was also not effective even at high molar ratio (3:1) of Na_2SO_4 to bio-oil. However, the dehydration study shows that Na_2SO_4 only partially (~25 wt%) removes the water from the bio-oil, but the ultimate analysis of Na_2SO_4 treated bio-oil contained higher amounts of sulphur (1.6 wt%) due to possible leaching of sulphur from Na_2SO_4. Therefore, taking all these into consideration SC-CO_2 separation of valuable compounds from crude bio-oil was attempted.

The proximate and ultimate analysis, calorific value and pH of the bio-oil and SC-CO_2 fractions are presented in Table 1. The bio-oil has low percentage of carbon (19.3 wt%) and high percentage of oxygen (70.6 wt%). The empirical formulae (in Table 1) suggested that the bio-oil sample contained more oxygen compared to biomass due to its high percentage of water, which makes it unsuitable for direct application as fuel. High percentage of water in bio-oil is due to prevalent dehydration of polysaccharides and lignin producing more water (Meire and Faix, 1999). The calorific value of crude bio-oil was low. The yields of the SC-CO_2 extracts were 6.8, 18.2 and 20.7 wt% corresponding to 10, 25 and 30 MPa pressure, respectively. The empirical formulae suggest that the SC-CO_2 fractions were superior in quality with less percentage of oxygen. It was also observed that the calorific value of the SC-CO_2 fractions was higher compared to the biomass. The ultimate analysis of CO_2 fractions indicated that the carbon percentage increased due to decrease in the percentage of oxygen as shown in empirical formulae, which ultimately increased the calorific value

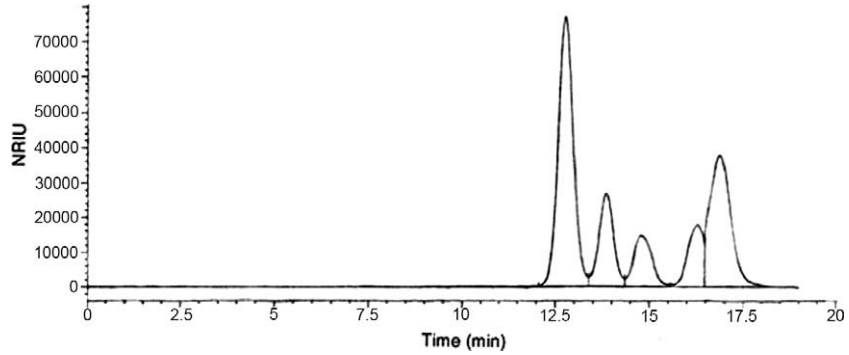

Fig. 4. High performance liquid chromatography (HPLC) spectrum for the monosaccharides.

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of the SC-CO_2 fractions. From the empirical formulae it was also observed that 25 MPa fraction was enriched with oxygen, which may be due to improved percentage of polar compounds and oxygenated benzenoids.

FT-IR study was used to determine the functional groups present in the bio-oil sample and SC-CO_2 extracted fractions. The O–H stretching vibrations 3200–3500 cm^{-1} (see Fig. 3) indicates the presence of phenols and alcohols. The broad band in the bio-oil was due to the formation of hydrogen bond with water. The peak intensity 3200–3500 cm^{-1} was less for SC-CO_2 fractions which are due to the presence of trace amounts of water. The appearance of peak at 3200–3500 cm^{-1} for low pressure fractions (10–25 MPa) is due to the enrichment of alcohols and oxygenated benzenoids, whereas the peak has low intensity for 30 MPa fraction, which is due to less amounts of these polar compounds. The peak between 1700 and 1750 cm^{-1} indicates the presence of ketone and aldehyde groups. The presence of both O–H and C=O stretching vibrations also indicates the presence of acid groups. The symmetrical and asymmetrical C–H stretching vibrations of aliphatic CH_3 and CH_2 groups (2850–2950 cm^{-1}) and C–H bending vibrations between 1350 and 1450 cm^{-1} show the presence of alkane groups, where as the peak at 1380 cm^{-1} represents the presence of CH_3 group (Silverstein and Webster, 2004). The peaks between 1450 and 1550 cm^{-1} represent C=C stretching vibrations, which are due to the presence of alkenes and aromatics compounds.

The hydrogen distributions of ^1H NMR of crude bio-oil and SC-CO_2 fractions of bio-oil are given in Table 2. The hydrogen distribution indicates that the crude bio-oil contained nearly 50% water and a broad peak was observed at 5.1 ppm. The SC-CO_2 fractions contained aromatics (0.1–1.0%), phenolic (OH) or olefinic proton (6.5–8.2%), ring join methylene (3.8–4.4%), CH_3, CH_2 and CH to an aromatic ring (18.6–19.2%), CH_2 and CH β to an aromatic ring (6.2–6.3%), β-CH_3, CH_2 and CH γ or further from an aromatic ring (52.7–54.1%) and CH_3 γ or further from an aromatic ring (8.8–9.1%). In case of SC-CO_2 fractions, an increase in aromatic content was observed with an increase in pressure from 10 to 25 MPa during extraction process, whereas, reverse trend was observed for aliphatic content. The work on ^1H NMR analysis of bio-oil obtained by fast pyrolysis and steam pyrolysis of cotton seed cake was reported by Ozbay et al. (2001). The percentage of aromatics (2.95–5.45%), phenolic (OH) or olefinic proton (1.72–3.17%), ring join methylene (1.29–1.59%), CH_3, CH_2 and CH to an aromatic ring (8.25–19.66%), CH_2 and CH β to an aromatic ring (4.40–6.22%), β-CH_3, CH_2 and CH γ or further from an aromatic ring (63.85–65.69%) and CH_3 γ or further from an aromatic ring (9.63–10.53%). The FT-IR analysis (Fig. 3) for the 30 MPa fraction did not show significant presence of the phenolic-OH at 3200–3500 cm^{-1}. In ^1H NMR analysis, the region between 5.0 and 6.5 ppm is for phenolic proton as well as olefinic proton (see Table 2). In 30 MPa fraction the percentage of fatty acids was improved (see Table 3) and this class of compounds may have major contribution in δ 5.0–6.5 ppm in ^1H NMR analysis. The SC-CO_2 fractions contained more percentage of oxygenated benzenoids in compared to bio-oil composition of cotton seed cake. So the SC-CO_2 fractions may be considered as a source of valuable chemicals.

The yields of bio-oil fractions by SC-CO_2 and conventional column chromatographic method are given in Table 3 along with the comparative compositions of chemical classes. The SC-CO_2 fractions (10–25 MPa) contained improved percentage of furanoids, pyranoids and oxygenated benzenoids. The chemical composition of SC-CO_2 fractions are presented in Table 4 and total 99 compounds were identified and hexadecanoic acid was found to be the major compound (23–52%) in these fractions. The SC-CO_2 fractions contained benzenoids (9.1–26.2%), furanoids (0.8–5.6%), pyranoids (0.1–2.1%) and more than 50% aliphatic compounds. The biomass contained cellulose (43 wt%), hemicellulose (33 wt%), and lignin (21.5 wt%), which mainly contributes to the formation of these chemical components in the extracted bio-oil. Cellulose and hemicelluloses degrade under cycloreversion, and degradation product obtained could be aliphatic compounds

Table 2
^1H NMR of bio-oil supercritical CO_2 fractions (percentage of total hydrogen).

Chemical shift (ppm)	Type of hydrogen	Bio-oil[a]	10 MPa	25 MPa	30 MPa
6.5–9.0	Aromatic	1.0 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	–
5.0–6.5	Phenolic (OH) or olefinic proton	3.6 ± 0.3	6.5 ± 0.2	7.4 ± 0.3	8.2 ± 0.3
3.4–4.5	Ring join methylene (Ar–CH_2–Ar)	2.4 ± 0.3	3.8 ± 0.3	4.4 ± 0.2	3.9 ± 0.2
2.1–3.3	CH_3, CH_2 and CH to an aromatic ring	9.3 ± 0.5	18.6 ± 0.5	19.2 ± 0.4	18.9 ± 0.5
1.6–2.0	CH_2 and CH β to an aromatic ring	2.4 ± 0.2	6.3 ± 0.3	6.2 ± 0.2	6.2 ± 0.3
1.0–1.6	β-CH_3, CH_2 and CH γ or further from an aromatic ring	24.8 ± 0.7	54.1 ± 0.6	52.7 ± 0.7	53.6 ± 0.6
0.5–1.0	CH_3 γ or further from an aromatic ring	3.5 ± 0.3	9.1 ± 0.3	8.9 ± 0.5	8.8 ± 0.4

[a] Bio-oil contained about 50% of water peak at 5.1 ppm, which is not taken into consideration.

Table 3
Yield and chemical classes of bio-oil fractions obtained by SC-CO_2 and column chromatography.

Bio-oil fractions (SC-CO_2 and CC[a])	Yield (%)	Chemical classes (%)									
		1	2	3	4	5	6	7	8	9	10
10 MPa	6.8 ± 0.4	5.8	5.6	2.1	3.1	23.1	1.7	23.0	2.2	8.6	–
25 MPa	18.2 ± 0.8	2.8	2.4	1.2	1.6	28.3	1.7	26.0	0.3	16.8	0.4
30 MPa	20.7 ± 0.8	0.3	0.8	–	0.3	8.8	0.4	52.0	1.0	18.8	0.6
Pentane	1.0 ± 0.2	1.2	1.2	0.2	0.5	9.0	0.3	49.1	5.5	3.9	1.1
Carbon tetrachloride	1.8 ± 0.2	1.9	0.9	0.4	1.5	6.5	0.4	40.1	8.6	4.2	5.1
Benzene	11.0 ± 0.3	9.8	2.3	2.0	3.5	18.5	1.1	19.8	0.5	12.0	2.8
Diethyl ether	11.2 ± 0.4	6.1	1.7	1.9	1.0	13.5	1.3	12.9	7.8	24.0	2.5
Chloroform	4.0 ± 0.2	4.0	4.6	2.1	1.2	11.8	0.7	10.8	2.4	28.6	4.7
Methanol	8.7 ± 0.8	3.2	1.2	0.8	1.0	9.2	0.3	16.0	4.0	15.8	19.0

1, low molecular mass fatty acids/esters; 2, furanoids; 3, pyranoids; 4, benzenoid hydrocarbons; 5, oxygenated benzenoids; 6, low molecular mass alcohols/aldehydes/ketones; 7, hexadecanoic acid; 8, hydrocarbons; 9, high molecular mass alcohols; 10, high molecular mass waxy components.
[a] CC: column chromatography.

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Table 4
Chemical composition of supercritical CO_2 fractions of bio-oil.

Compound	10 MPa	25 MPa	30 MPa	RRI cal	RRI lit	MS data
Propanoic acid	0.4 ± 0.1	0.1	0.1	–	668	–
3,4-Dimethyl-2-pentene	0.6 ± 0.1	–	–	–	679	–
Ethyl cyclopentane	0.1	–	–	–	–	–
2,3-Dimethyl hexane	0.1	–	–	–	–	–
Methyl-1-heptene	0.1	–	–	–	–	–
Toluene	0.5 ± 0.1	0.2 ± 0.1	–	–	749	–
3-Butenioc acid	1.2 ± 0.3	0.5 ± 0.2	–	–	–	–
Crotonic acid	0.4 ± 0.1	0.1	–	–	–	–
1-Octene	0.3 ± 0.1	0.2 ± 0.1	–	–		–
Butanoic acid	0.2 ± 0.1	0.2 ± 0.1	0.1	821	827	–
Isovaleric acid	0.2 ± 0.1	0.1	–	834	–	–
Furfural	0.4 ± 0.1	0.3 ± 0.1	–	838	831	96 (95%), 95 (100%), 67 (10%)
2-Methyl butenoic acid	0.1	–	–	842	–	–
2-Methyl butanoic acid	0.1	0.1	–	846	839	–
Furanmethanol	1.8 ± 0.3	0.1	0.1	854	851	98 (80%), 81 (20%), 69 (40%), 41 (100%)
Ethyl benzene	0.8 ± 0.3	0.5 ± 0.2	0.1	868	855	–
p-Xylene	0.5±.2	0.2 ± 0.1	0.1	876	864	–
m-Xylene	0.4 ± 0.1	0.2 ± 0.1	0.1	880	872	–
Ethyl furan	1.8 ± 0.3	0.3 ± 0.1	–	884	–	96 (50%), 81 (100%), 67 (50%), 53 (95%)
2-Pentenoic acid	2.6 ± 0.2	1.3 ± 0.2	–	898	–	–
o-Xylene	0.1	0.1	–	893	889	–
Dihydro-4-2(3H)-furanone	0.2 ± 0.1	0.4 ± 0.1	0.1	918	913	–
Dihydro-pyran-2-one	2.1 ± 0.2	1.2 ± 0.1	–	943	945	–
Dihydro-4-methyl-2(3H)-furanone	3.2 ± 0.2	0.6 ± 0.2	0.2 ± 0.1	960	–	100 (10%), 71 (5%), 56 (80%), 41 (100%)
3-Methyl furaldehyde	t	–	0.1	966	–	110 (95%), 109 (100%), 81 (5%), 53 (70%)
5-Methyl-furaldehyde	0.2 ± 0.1	0.2 ± 0.1	–	969	968	–
Hexanoic acid	1.2 ± 0.2	0.4 ± 0.1	–	978	982	–
Phenol	0.6 ± 0.2	0.2 ± 0.1	0.1	982	986	–
1,3,5-Trimethyl benzene	0.7 ± 0.1	–	–	987	992	–
Methyl-3-methyl-oxo-butanoate	0.1	0.1	–	990	–	–
2,5-Dihydro-3,5-dimethyl-2-furanone	0.1	0.5 ± 0.1	0.2 ± 0.1	1004	–	112 (50%), 97 (20%), 69 (100%), 41 (90%)
Methyl-3-methyl-oxo-pentanoate	0.5 ± 0.1	0.3 ± 0.1	0.1	1026	–	–
o-Cresol	4.2 ± 0.4	1.9 ± 0.3	0.5 ± 0.2	1050	–	–
m-Cresol	1.7 ± 0.3	1.5 ± 0.2	1.2 ± 0.2	1060	–	–
p-Cresol	1.2 ± 0.2	1.0 ± 0.2	0.5 ± 0.1	1069	–	–
2-Methylene cyclohexanol	0.5 ± 0.1	0.7 ± 0.2	0.1	1079	–	112 (30%), 83 (80%), 67 (50%), 55 (100%)
1-Vinyl-cyclohexanol	0.6 ± 0.1	0.3 ± 0.1	0.1	1094	–	126 (15%), 111 (15%), 83 (95%), 55 (100%)
o-Guaicol	0.2 ± 0.1	0.2 ± 0.1	–	1097	1090	–
5-(Hydroxymethyl)-2-furaldehyde	0.1	0.1	–	1100	–	126 (25%), 97 (100%), 69 (50%), 41 (80%)
2-Hydroxy-3,5-dimethylcyclopent-2-en-1-one	0.1	0.1	–	1104	–	126 (100%), 97 (25%), 83 (60%), 55 (60%)
Guaicol[a]	1.2 ± 0.2	0.3 ± 0.1	–	1107	–	–
2,5-Dimethyl benzoquinone	0.2 ± 0.1	0.7 ± 0.2	0.8 ± 0.2	1121	–	136 (100%), 108 (50%), 79 (50%), 68 (60%)
1,2,3,6,7,7a-Hexahydro-5(H)-inden-5-one	0.3 ± 0.1	0.3 ± 0.1	0.1	1028	–	136 (40%), 108 (95%), 79 (40%), 39 (100%)
1-Ethoxy-4-methyl benzene	2.2 ± 0.3	1.8 ± 0.3	0.4 ± 0.2	1035	–	136 (70%), 108 (100%), 107 (80%), 79 (50%)
Methyl undecane	1.1 ± 0.3	0.3 ± 0.1	0.7 ± 0.2	1139	–	–
Benzoic acid	0.7 ± 0.2	1.0 ± 0.3	0.1	1170	–	122 (95%), 105 (100%), 77 (80%), 51 (50%)
4-Ethyl phenol	0.1	0.1	–	1174	1169	–
Dimethyl phenol[a]	0.1	0.2 ± 0.1	t	1177	–	122 (70%), 107 (100%), 91 (15%), 77 (40%)
Dimethoxy benzene[a]	0.7 ± 0.2	2.0 ± 0.3	0.4 ± 0.1	1199	–	138 (100%), 123 (40%), 95 (50%), 55 (20%)
2,3,5-Trimethyl-benzoquinone[a]	0.8 ± 0.1	1.1 ± 0.2	0.2 ± 0.1	1217	–	–
Ethyl cresol[a]	0.8 ± 0.2	1.8 ± 0.3	0.2 ± 0.1	1246	–	136 (30%), 121 (100%), 91 (20%), 77 (25%)
Isopropyl phenol[a]	0.1	1.2 ± 0.2	0.3 ± 0.1	1253	–	136 (40%), 121 (100%), 91 (20%), 77 (20%)
o-Phthaldehyde	1.0±.2	0.2 ± 0.1	0.2 ± 0.1	1256	–	134 (100%), 105 (25%), 77 (30%), 51 (30%)
Propyl phenol[a]	0.2 ± 0.1	t	t	1263	–	134 (100%), 133 (95%), 107 (50%), 77 (50%)
3-Propyl phenol	0.1	0.4 ± 0.1	0.1	1267	–	136 (30%), 121 (15%), 107 (100%), 77 (25%)
Trimethyl phenol[a]	0.1	–	–	1277	–	136 (60%), 121 (100%), 91 (25%), 77 (15%)
4-Ethyl-2-methoxy-phenol	t	–	–	1283	–	152 (75%), 137 (100%), 122 (10%), 91 (10%)
Dihydroxy acetophenone[a]	t	–	–	1290	–	152 (75%), 137 (100%), 109 (20%), 81 (25%)
Indanone	3.9 ± 0.3	1.8 ± 0.2	1.5 ± 0.2	1293	–	132 (100%), 104 (95%), 78 (60%), 63 (10%)
Methyl furanoate	t	–	–	1299	–	126 (15%), 110 (5%), 95 (100%), 39 (25%)
Methyl furoic acid	0.1	0.2 ± 0.1	0.1	1311	–	126 (100%), 109 (30%), 81 (25%), 69 (10%)
2,5-Dimethyl acetophenone	0.1	0.4 ± 0.1	t	1334	–	148 (60%), 133 (100%), 105 (50%), 79 (25%)
2,3-Dihydro-1-H-inden-5-ol	0.2 ± 0.1	0.3±.1	0.1	1356	–	134 (50%), 133 (100%), 105 (25%), 77 (15%)
Syringol	0.2 ± 0.1	0.2 ± 0.1	0.1	1360	–	154 (100%), 139 (50%), 111 (25%), 93 (30%)
Eugenol	0.5 ± 0.1	0.8 ± 0.2	0.2 ± 0.1	1367	1356	–
2-Methoxy-4-propyl phenol	0.1	0.1	–	1378	–	166 (20%), 137 (100%), 122 (15%), 94 (10%)
2,5-Dimethoxy ethylbenzene	0.1	0.1	0.1	1392	–	166 (100%), 151 (90%), 137 (70%), 121 (20%)
Vanillin	0.2 ± 0.1	0.3 ± 0.1	–	1411	1391	–
Z-Isoeugenol	0.2 ± 0.1	0.5 ± 0.1	–	1419	1402	–
Trimethoxy benzene[a]	t	0.1	–	1455	–	168 (100%), 153 (90%), 125 (50%), 79 (15%)
E-Isoeugenol	t	t	–	1463	1447	–
Propyl guaicol[a]	0.2 ± 0.1	0.6 ± 0.1	0.1	1470	–	166 (100%), 137 (100%), 122 (25%), 94 (20%)
Methyl vanillin[a]	0.3 ± 0.1	1.3 ± 0.2	0.1	1493	–	166 (100%), 165 (80%), 151 (10%), 95 (30%)
Acetovanillin[a]	0.5 ± 0.1	1.1 ± 0.2	0.1	1499	1490	–

(*continued on next page*)

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Table 4 (*continued*)

Compound	10 MPa	25 MPa	30 MPa	RRI cal	RRI lit	MS data
1,2-Dimethoxy propyl benzene	0.1	0.2 ± 0.1	0.1	1520	–	180 (10%), 151 (100%), 107 (10%), 91 (20%)
Vanillyl acetate	t	0.1	–	1535	1524	–
(4-Hydroxy-3-methoxyphenyl)-propanone	–	0.1	–	1543	1537	–
Homovanillyl alcohol[a]	0.6 ± 0.1	1.1 ± 0.2	0.5 ± 0.1	1551	–	–
Vanillic acid	t	0.1	–	1576	1566	–
(4-Hydroxy-3-methoxy phenyl)-propanone	–	0.1	–	1588	–	180 (50%), 137 (100%), 94 (15%), 77 (15%)
3-Ethoxy-4-methoxy benzaldehyde	0.1	0.3 ± 0.1	0.1	1592	–	180 (20%), 151 (100%), 123 (15%), 65 (10%)
Allyl syringol	0.1	0.3 ± 0.1	0.1	1608	1608	–
2-Butyl-decahydronaphthalene	t	t	–	1639	–	194 (25%), 137 (100%), 95 (20%), 81 (15%)
2-Acetyl-3-methoxy-benzoic acid	t	0.1	t	1648	–	194 (15%), 179 (100%), 135 (10%), 43 (25%)
4-Ethoxymethyl-2-methoxy phenol	–	0.2 ± 0.1	–	1670	–	210 (30%), 137 (100%), 122 (15%), 94 (10%)
5,7-Tetradecadien-1-ol	–	–	0.1	1674	–	–
Methyl vanillate	–	0.1	–	1691	1687	–
4-Hydroxy-5-methoxy-phenylacetyl formic acid	0.1	0.1	–	1704	–	–
2,4,6-Trimethoxy styrene	–	0.1	t	1745	–	–
2,4,5-Trimethoxy benzaldehyde	t	0.1	t	1750	–	–
2-Acetyl-3-methoxy-benzoic acid	–	0.6 ± 0.1	–	1759	–	–
2,3-Dimethoxy-benzenebutyric acid	1.1 ± 0.2	2.0 ± 0.2	0.7 ± 0.1	1782	–	–
1,3-Diphenyl-buta-1,2-diene	–	0.3 ± 0.1	–	1790	–	–
Hexadecanol[a]	5.1 ± 0.2	10.5 ± 0.4	11.4 ± 0.3	1880	1879	–
Hexadecanoic acid	23.0 ± 0.5	26.0 ± 0.7	52.0 ± 1.0	1960	1953	–
3,13-Octadecedienol[a]	2.1 ± 0.2	3.3 ± 0.2	3.1 ± 0.3	2043	–	–
Octadecanol[a]	1.1	2.4 ± 0.2	2.7 ± 0.1	2065	2070	–
Eicosanol[a]	0.3 ± 0.2	0.6 ± 0.1	1.5 ± 0.1	–	2265	–
Octyl adiapate	–	0.4 ± 0.1	0.6 ± 0.1	–	–	–

RRI cal: relative retention indices calculated, RRI lit: relative retention indices literature reported.
[a] Correct isomer not identified.

(Meire and Faix, 1999). Similarly transglycosylation of cellulose and hemicellulose leads to cyclic and bicyclic degradation products such as pyranoids and furanoids (Evans and Milne, 1987). One of the typical lignin degradation mechanisms occur via dehydration. That's the reason why several pyrolysis products of lignin have unsaturated side chains such as styrene and phenyl propenoid derivatives. It was also observed that the fractions obtained at 10–25 MPa were enriched with furanoids, pyranoids and benzenoids; whereas the high pressure fraction i.e. at 30 MPa contained improved percentage of waxy components such as fatty acids/alcohols and high boiling compounds. The fractions collected from bio-oil at low pressures were enriched with valuable compounds and can be treated as a source of valuable chemicals. Therefore, SC-CO_2 extraction was found to be a green process for extraction of valuable compounds from crude bio-oil without organic solvent residue. The commercial applications of bio-oil exist in the field of production of food aromas (Meire and Faix, 1999) indicating that the bio-oil is a source of valuable chemicals for food flavours.

For comparison purposes conventional liquid–liquid extraction of organic compounds from crude bio-oil was carried out. In the first step the liquid–liquid separation was carried out by using $CHCl_3$:Et_2O (1:1) and there after $CHCl_3$–Et_2O extracted bio-oil was fractionated by column chromatography. The benzene fraction obtained from column chromatography was superior in terms of yield and chemical composition. It was enriched with valuable compounds like furanoids (2.3%), pyranoids (2.0%) and benzenoids (22.0%). More value-added compounds were found in the benzene fraction followed by those in Et_2O fraction. But overall SC-CO_2 fractions were superior in quality compared to the column chromatographic fractions. The main drawback of the conventional process is that it requires huge amounts of organic solvent and is not recommended from environmental regulations point of view and also it has limited scope in food application.

4. Conclusion

The biomass under study contained high percentage of carbon (46.7 wt%) and low percentage of N (0.05 wt%) and S (0.01 wt%) which all together makes it the potential candidate for bio-oil production. The bio-oil derived from the wheat–hemlock biomass contains high percentage of water (45.0 wt%) with low calorific value of 18.6 MJ/kg. The SC-CO_2 effectively separates the water from the crude bio-oil and the fractionated bio-oil was found to have high caloric value (40.2–43.2 kJ/kg) indicating that its calorific value could be compared with those of diesel oil. ^{1}H NMR study indicates that the aromaticity of the oil was low, whereas the low pressure fractions (10–25 MPa) contained improved percentage of furanoids (2.4–5.6%), pyranoids (1.2–2.1%) and benzenoids (26.2–29.9%). But later fraction (30 MPa) contained improved percentage of fatty acids and alcohols (71.5%). The yield of valuable compounds from bio-oil was found to be less in conventional column chromatographic method compared to the SC-CO_2 process. From the chemical composition of SC-CO_2 fractions it can be conclude that hexadecanoic acid was found to be the major compound present in these fractions. However, hexadecanoic acid percentage in the fraction was found to be increased from 23% to 52% with increasing the extraction pressure from 10 to 30 MPa. There were 52 oxygenated benzenoids detected in SC-CO_2 fractions along with furanoids and pyranoids, which were valuable chemicals. The low pressure fractions (10–25 MPa) enriched with these valuable chemicals. The advantage of SC-CO_2 is that it produces solvent free chemicals and selectively separates the valuable compounds at different pressure which can be used for fine chemicals and food flavour. The SC-CO_2 was found to be an advanced separation process to enrich the valuable chemicals with low water content (~2.5%). The SC-CO_2 extraction process comes under green technology and can be implemented for value addition of bio-oil. This is the first report of its kind on selective separation of bio-oil using SC-CO_2.

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