Towards a Norwegian Spruce Bark Biorefinery

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Dedication

I would like to dedicate this body of work to my parents, I would not become who I am without your support and love. I love you, thank you!

Abstract

This project explores the development of a potential biorefinery based on Norwegian spruce bark using Soxhlet, microwave pyrolysis and subcritical water extraction processes in order to yield chemicals, materials and bioenergy. Spruce bark contains 18% ethanol extractives (Soxhlet extraction) including a significant amount of phenols and condensed tannins (39 mg GAE/g) commensurate with lignocellulosic matter. Component analysis of spruce bark revealed: cellulose (25%), hemicellulose (8%), lignin (25%), ethanol extractives (18%), ash (2%), moisture (9.2%), C (47.64%), H (5.91%), N (0.23%) and HHV (17.3 MJ/kg). Depending on the processing temperature, microwave pyrolysis of spruce bark yielded bio-oil (8%-14%) and bio-char (65%-75%) depending on pyrolysis temperature. The bio-oil mainly comprised phenolic compounds, such as: phenol, guaiacol, eugenol, and 2-methoxy-4-methylphenol, whilst the biochar gave a relatively high calorific value (26.6 MJ/kg at 240°C), compared with native spruce bark (22.4 MJ/kg), thus showing energy densification. Subcritical water extraction (SWE) yielded organic extractives (3%-8%), sugars (3%-6%) and residues (60%-70%). The organic extractive comprises phenols and furfurals, and a range of sugars, notably glucose and rhamnose, demonstrating the potential of SWE as a hydrolytic process for spruce bark. The sugars can be used for downstream fermentation processes, and the residues may be converted into nanocellulose or used as fuel (bioenergy). Thus, within the context of a potential biorefinery spruce bark affords high-value chemicals (phenols, tannins etc.), fermentable sugars, bio-char and cellulosic materials.

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Declaration

The research described in this thesis is original work, which I undertook at the University of York during 2016-2017. This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as References. Except where stated, all of the work contained within this thesis represents the original contribution of the author. Certain parts of the work described herein were undertaken in collaboration with other researchers, they are fully acknowledged below.

Acknowledgements

First and foremost, I would like to thank my supervisor Dr. Avtar Matharu and the Green Chemistry Centre of Excellence at the University of York for giving me the opportunity to study for an MRes in an area I'm interested in. I'm really appreciate for my supervisor's suggestions and guidance. Thank you to Dr. Vitaliy Budarin and Dr. Hannah Briers for your help and training in microwave process. Special thanks to my friends Hao Xia, Long Zhou, Yang Gao and Zhicheng Jiang, thank you for your help in both my research work and daily life. Great thanks to everyone in GCCE for your help in the lab, it has been a great honour to be a member of GCCE.

Abbreviations

AC	Activated Carbon
ATR-IR	Attenuated Total Reflection – Infra Red
BDC	Biorenewables Development Centre
CHN	Carbon Hydrogen Nitrogen
CR	Conversion Rate
CTs	Condensed Tannins
EA	Ethyl Acetate
FC	Folin–Ciocalteu
FW	Forestry Waste
GAE	Gallic Acid Equivalent
GC	Gas Chromatography
HHV	Higher Heating Value
HMF	5-Hydroxymethylfurfural
HPLC	High Performance Liquid Chromatography
LA	Levulinic Acid
MS	Mass Spectrometry
MW	Microwave
NMR	Nuclear Magnetic Resonance
OOP	Out of Plane

OPEC	Oil Producing Economic Countries
Py-GC/MS	Pyrolysis-Gas Chromatography /Mass Spectrometry
RE	Renewable Energy
REACH	Regulation, Evaluation and Authorisation of CHemicals
SBR	Spruce Bark Residue
$scCO_2$	Supercritical Carbon Dioxide
SDG	Sustainable Development Goal
SFE	Supercritical Fluid Extraction
STA	Simultaneous Thermal Analysis
Str	Stretch
SWE	Subcritical Water Extraction
TGA	Thermo Gravimetric Analysis
TPC	Total Phenolic Content
UK	United Kingdom
UN	United Nations
UoY	University of York
USA	United States of America

1. Introduction & Aims

1.1 Global overview

Since the beginning of 20th century, global population has increased fourfold to 7.4 billion and with an average increase over 1.2% yearly.[1] Material consumption also continues to increase. In 2010, 72 Gt materials were used in production worldwide annually, and material consumption is expected to reach 100Gt by 2030.[2] An increasing population within a finite domain, i.e., planet Earth, which will be resource-stressed in the future is unsustainable unless remediation strategies are set in place now. The vast majority of resources, whether they be chemicals, materials and/or energy are derived from petroleum feedstock (crude oil). The latter is finite and thus a restricted, unsustainable resource but also heavily polluting and climate change unfriendly. According to data from OPEC (Figure 1), in 2016 world proven crude oil reserves reached 1492 billion barrels but world oil demand was 95.115 million barrels/day.[3] With demand and reserves out of synchronization and the fact that economically accessible crude oil reserves will deplete in 43 years then the need for alternative sources of sustainable and renewable energy, materials and chemicals is immediate. The use of renewable feedstocks such as biomass can play an important role for a sustainable society.

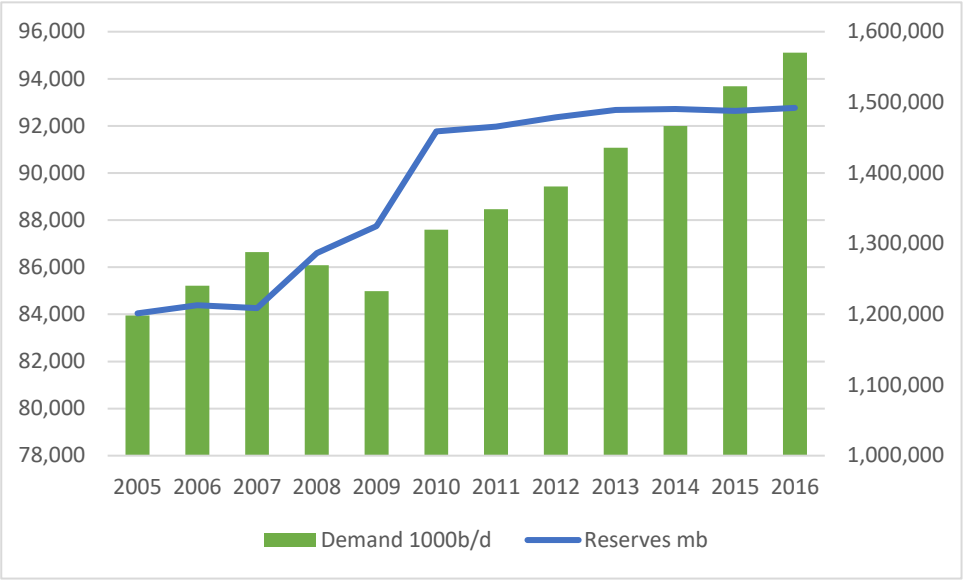


Figure 1 World proven oil reserves compared with world oil demand in past 12 years.

Biomass is a traditional source of solid bioenergy, *i.e.*, its open source burning as fuel (energy) is still practiced in many developing countries.[4] Bioenergy can arise many different sources as exemplified in Figure 2. In 2008, it was estimated that biomass provided about 10.2% (50.3 EJ/yr) of the annual global primary energy supply.[5]

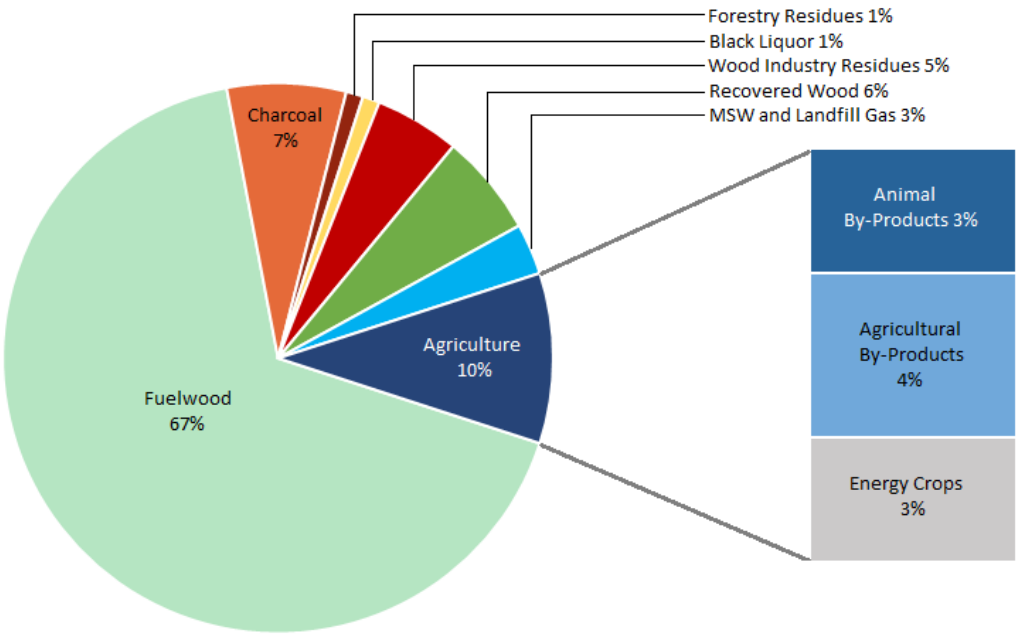


Figure 2 Shares of global primary biomass sources for energy.[4]

Global bioenergy use has steadily grown worldwide in absolute terms in the last 40 years, the use of solid biomass for electricity production is particularly important in pulp and paper plants and in sugar mills. The scope of this thesis is not bioenergy *per se* as it also encompasses liquid (bioethanol, biobutanol) and gaseous biofuels (biomethane, biohydrogen) so the reader is recommended the following references.[6-8] Biomass can provide more than just bioenergy as it also represents a source of sustainable chemicals and materials.

In 2015, the United Nations (UN) recognised the importance of sustainability in their report entitled: 'Transforming our World: the 2030 Agenda for Sustainable Development,' which aims to protect the planet, people, alleviate poverty and enhance peace. Seventeen Sustainable Development Goals (SDGs, Table 1) were actioned from this report with each goal further subdivided into several specific 'targets'.

SDG 12 is of particular relevance to this thesis. SDG 12 is responsible for sustainable consumption patterns and production and aims to reduce or minimize impact of waste, improve climate change and foster circular- rather than linear economies by 'doing more with less', *i.e.*, a more resource-efficient society.

A move towards utilization of forestry residues such as Norwegian spruce bark as a source of chemicals, materials and bioenergy (solid, liquid and gas) instead of crude oil fits the needs of SDG 12. Specifically: Target 2: By 2030, achieve the sustainable management and efficient use of natural resources, and; Target 5: By 2030, substantially reduce waste

generation through prevention, reduction, recycling and reuse. All SDGs are partly inter-related and thus SDGs 7, 13 and 15 are also relevant in the overall context of this research.

Table 1 Sustainable Development Goals.

Goal 1	End poverty in all its forms everywhere.
Goal 2	End hunger, achieve food security and improved nutrition and promote sustainable agriculture.
Goal 3	Ensure healthy lives and promote well-being for all at all ages.
Goal 4	Ensure inclusive and equitable quality education and promote lifelong learning opportunities for all.
Goal 5	Achieve gender equality and empower all women and girls.
Goal 6	Ensure availability and sustainable management of water and sanitation for all.
Goal 7	Ensure access to affordable, reliable, sustainable and modern energy for all.
Goal 8	Promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all.
Goal 9	Build resilient infrastructure, promote inclusive and sustainable industrialization and foster innovation.
Goal 10	Reduce inequality within and among countries.
Goal 11	Make cities and human settlements inclusive, safe, resilient and sustainable.
Goal 12	Ensure sustainable consumption and production patterns.
Goal 13	Take urgent action to combat climate change and its impacts.
Goal 14	Conserve and sustainably use the oceans, seas and marine resources for sustainable development.
Goal 15	Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss.
Goal 16	Promote peaceful and inclusive societies for sustainable development, provide access to justice for all and build effective, accountable and inclusive institutions at all levels.
Goal 17	Strengthen the means of implementation and revitalize the global partnership for sustainable development.

1.2 Green Chemistry and Biorefineries

Chemistry is always associated with danger, toxicity and pollution. In this case, a move towards green chemistry becomes very important.[9] Green Chemistry is defined as the ‘‘design of chemical products and processes to reduce or eliminate the use and generation of hazardous substances”.[10] An important aspect of Green Chemistry is at the design or concept stage. The design is a profession of human intention and one cannot design by accident.[11] Green Chemistry is governed by 12 principles as outlined below and as first defined by *Anastas and Warner*[10].

12 Principles of Green Chemistry:

1. Waste prevention is better than treatment or clean up.
2. Chemical synthesis should maximise the incorporation of all starting materials.
3. Chemical synthesis should ideally use and generate non-hazardous substances.
4. Chemical products should be designed so as to be non-toxic.
5. Catalysts are superior to reagents.
6. The use of auxiliaries should be minimized.
7. Energy demands in chemical syntheses should be minimized.
8. Raw materials should increasingly be renewable.
9. Derivitisations should be minimized.
10. Chemical products should break down into innocuous products.
11. Chemical processes require better control.
12. Substances should have minimum potential for accidents.

As we seek a future sustainable world through the practice of SDGs and the 12 principles of green chemistry, then the use of renewable feedstocks (Principle 8) is becomes more important for future chemical industries that were/are historically dependent on crude oil. Biomass comprises cellulose, hemicellulose and lignin (also known as lignocellulosic) as its three main structural components in addition to starches, oils and proteins. Their conversion in to smaller chemical moieties upon application of an appropriate technology or process (fermentation, pyrolysis, liquefaction) yields potentially high-value downstream products (Figure 3)[12-15], thus mimicking a conventional petroleum refinery but instead based on biomass, *i.e.*, a biorefinery.

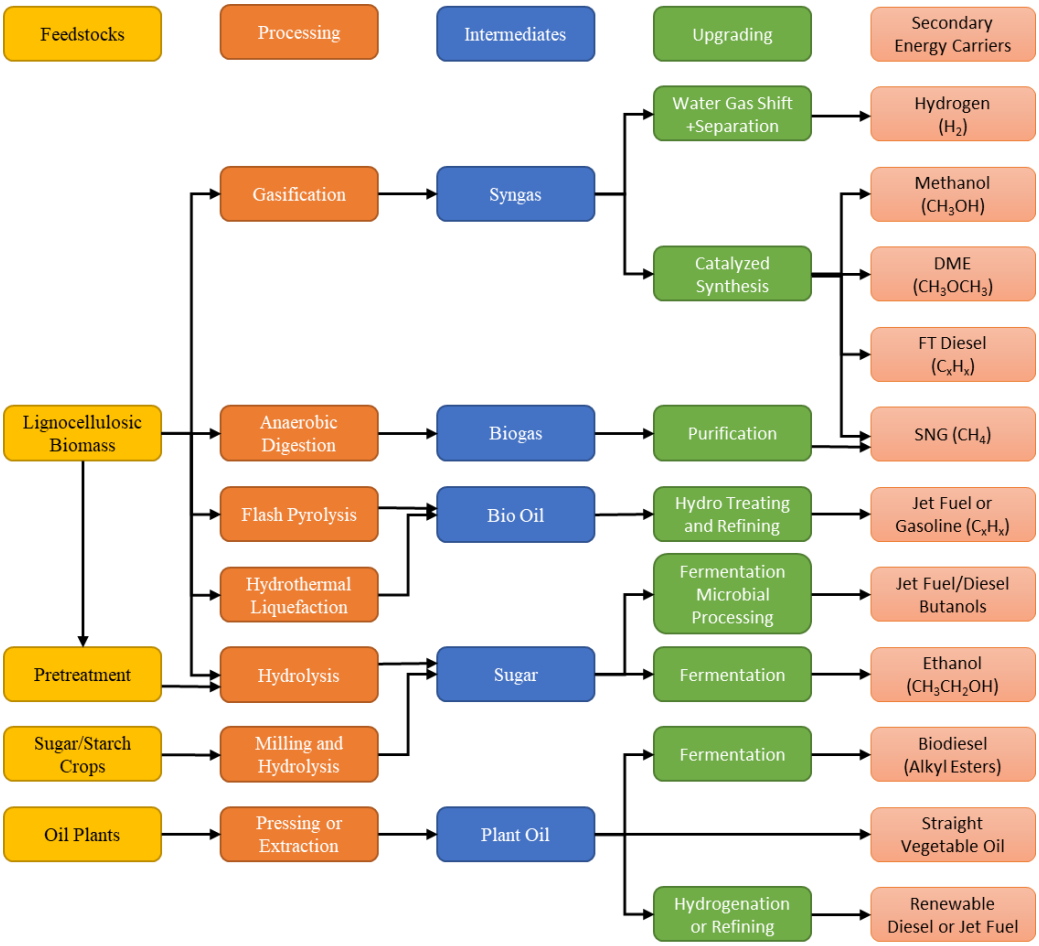


Figure 3 Overview of lignocellulosic biomass, sugar/starch crops and oil plants (feedstocks) and the biorefinery processes.

Early biorefineries focused on single technology and single feedstock such as vegetable oil or starch to biodiesel or bioethanol, respectively. However, these have a public backlash because the feedstock was in direct competition with food or feed, *i.e.*, food versus feed versus fuel debate.[16] In future biorefineries will be those based on feedstocks that are non-food competitive, can receive flexible feedstock, and generate zero waste whilst outputting (bio)energy, chemicals and materials all year round.

1.3 Aims

Forestry residues include the by-products of pulp and sawmills (bark, sawdust and shavings), tending and thinning residues (top, branches, leaves and needles) and residues during harvest, a major source of biomass for energy (burnt).[17, 18] A global forest map (Figure 4) acknowledges that Scandinavia, Siberia, Southeast Asia, Central Africa, Canada and Latin America have the highest tree cover density. In 2015, China accounted for approximately 21% tree coverage which was estimated to yield 511.63 Mt tree residues.[19] Norwegian

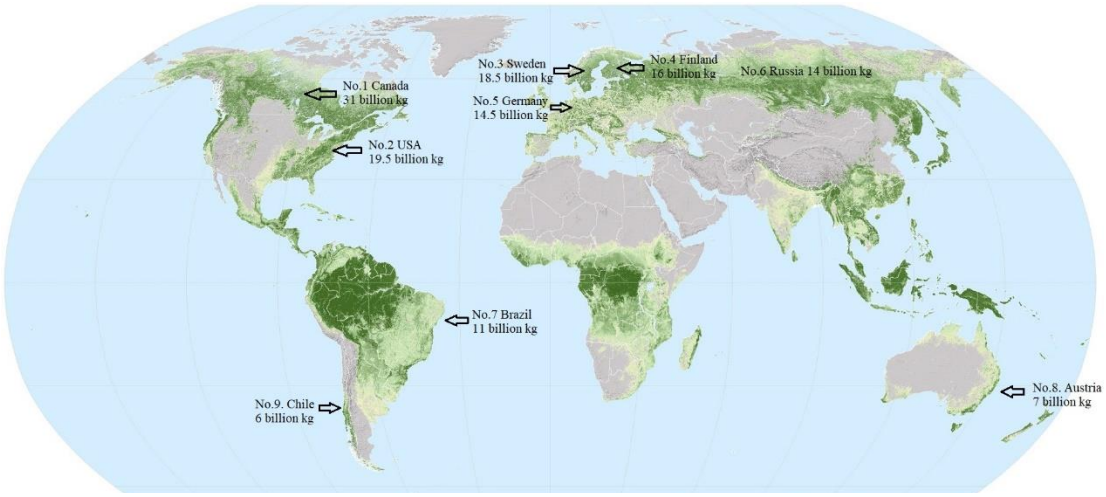


Figure 4 Global forest map & Top 9 wood export countries

spruce residues represent an important renewable feedstock within the context of biorefineries.

Thus, the over-arching aim is to explore the development of a potential biorefinery based on Norwegian spruce bark, a high volume waste product of pulp and paper producing industries, which yields chemicals, materials and bioenergy. A schematic of the process to achieve the desired outputs is shown in Figure 5.

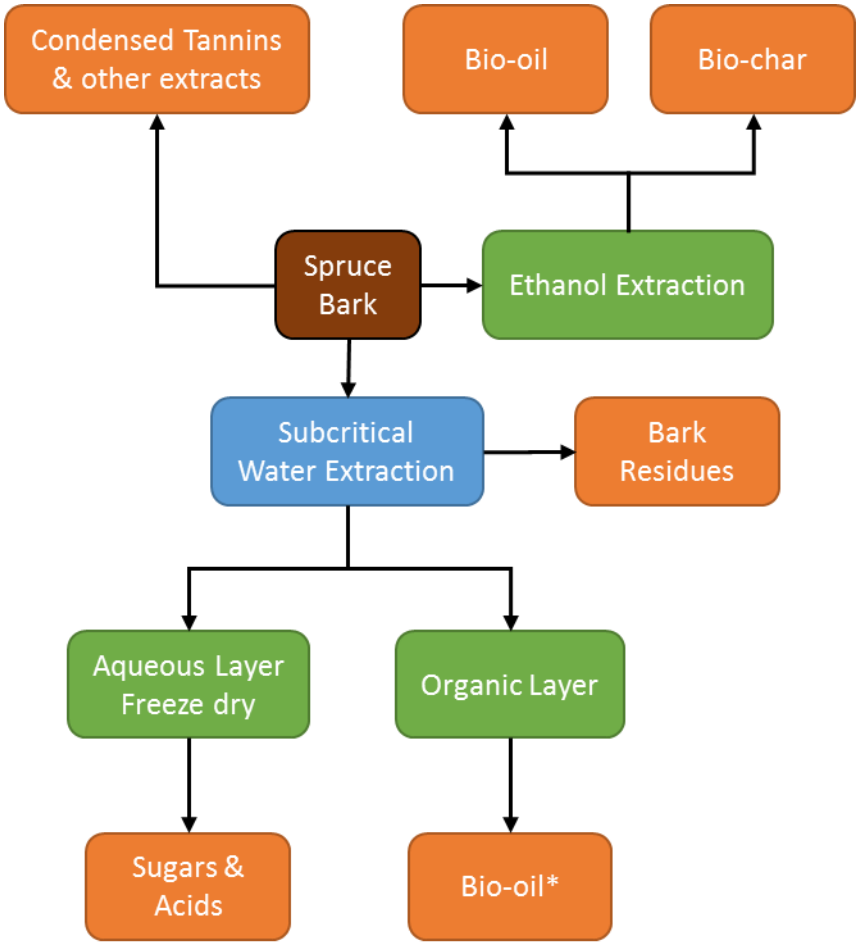


Figure 5 Flow sheet of this project.

Initially, extractives from spruce bark will be isolated using continuous hot solvent extraction, *i.e.*, Soxhlet, with ethanol to remove condensed tannins and resinous matter. Thereafter, spruce bark will be subjected to sub-critical water extraction and microwave

pyrolysis. The former is envisaged to partially hydrolyse spruce bark to afford a sugar-rich aqueous fraction, non-aqueous organic fraction (ethyl acetate soluble) and residual matter, whilst the latter will result in the formation of a bio-oil (semi-wet) and biochar. Pyrolysis also affords non-condensable gases, which will not be collected as part of this work. The energy content of chars formed and residues obtained will be determined so as to have an initial idea of the chemical and energy potential of spruce within the context of a biorefinery. Thus, the objectives of this research are to:

i. undertake component analysis of spruce bark - CHN, theoretical HHV, calorific value, and cellulose, hemicellulose and lignin content;
ii. determine extractives and total phenol content by solvent extraction of spruce bark with ethanol;
iii. undertake microwave pyrolysis of spruce bark to determine bio-oil yield and composition (GCMS), biochar yield and calorific value, and;
iv. undertake subcritical water extraction of spruce bark to determine organic extractives and sugar composition upon hydrolysis at different temperatures.

1.4 Norwegian Spruce & Bark

A spruce is a tree of the genus *Picea*, a genus of about 35 species of coniferous evergreen trees within the Pinaceae family, found in the northern temperate and boreal (taiga) regions of the earth. Spruces are large trees, about 20-60 metres tall when mature, and distinguished by their whorled branches and conical form. Norwegian spruce (*Picea abies*) is a species of

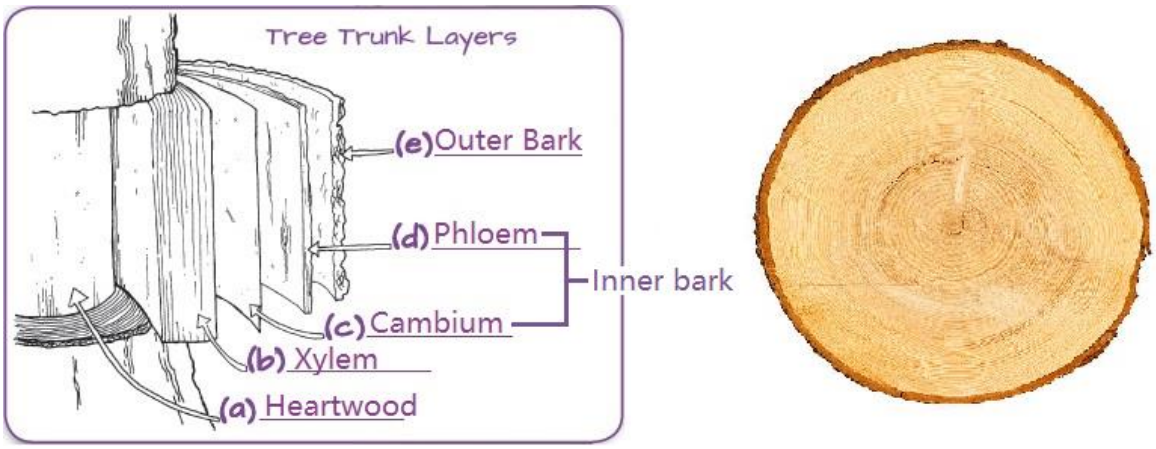


Figure 6 Tree Trunk Profile.

spruce native to Northern, Central and Eastern Europe. Spruce bark is usually considered as waste in the wood pulping and forestry industry, representing 10-20% of the tree trunk[20], but has the potential of realising valuable resource material for industries.[21] For example, the bark is a rich source of phenolic compounds such as condensed tannins.[22-26] The main components of spruce bark comprise cellulose, hemicellulose and lignin.

1.4.1 Cellulose

Cellulose is a linear homopolymer of glucose residues connected by β-(1-4)-glycosidic bonds (Figure 7) which thermally fragment above 300°C, and is the most common organic polymer on the Earth with total production of 10^{11}-10^{12} tons annually.[27-29] Cellulose is

biodegradable, renewable, decomposes rather than melts, and is insoluble in most solvents due to hydrogen bonding and crystallinity.[29, 30]

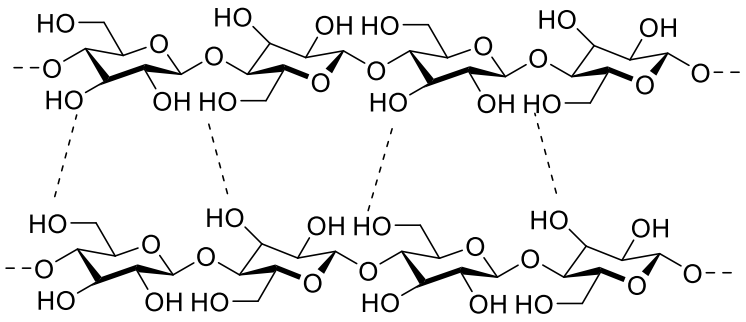


Figure 7 Structure of Cellulose.

First generation bioethanol is derived from starch-based plants, which are normally used as food and feed, due to the growth of population in the world, first generation bioethanol is not very sustainable. The second generation cellulose-based bioethanol production from agricultural residues or forestry waste via hydrolysis and fermentation is a more promising approach.[31]

1.4.2 Hemicellulose

Hemicelluloses are complex short chain, amorphous, branched polysaccharides, composed of pentoses (xylose, arabinose), hexoses (mannose, glucose, galactose), and sugar acids (Figure 8).[32] Chain lengths of hemicellulose polymers are much shorter than those of cellulose.[33] Hemicelluloses contribute to strengthening the cell wall by interaction with cellulose and lignin.[34]

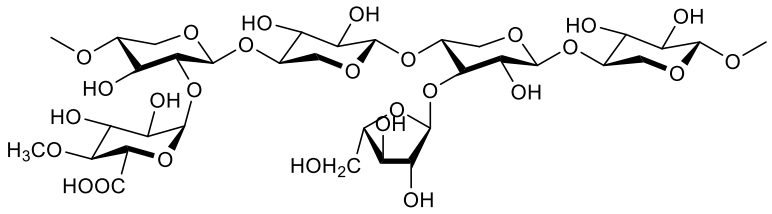


Figure 8 Structure of Hemicellulose

Generally, hemicelluloses in hard wood such as birch, walnut and willow contain mostly xylans, whereas hemicelluloses in soft wood such fir, pine and spruce contain mostly glucomannans.[32]

1.4.3 Lignins

Lignin is condensed phenolic-like aromatic polymer that binds and cross-links micro cellulose fibres and hemicellulosic cell wall components.[35-37,] Lignin accounts for 30% by weight in softwood, while this share falls to 20% - 25% in hardwood.[38,39] Lignin is mainly an amorphous tridimensional polymer of three primary units (Figure 9): sinapyl (3,5-dimethoxy-4-hydroxycinnamyl), coniferyl (3-methoxy-4-hydroxycinnamyl), and *p*-coumaryl (4-hydroxycinnamyl) alcohols, joined by ether and C-C linkages.[38] Lignin is an important source of aromatic compounds, namely phenols.

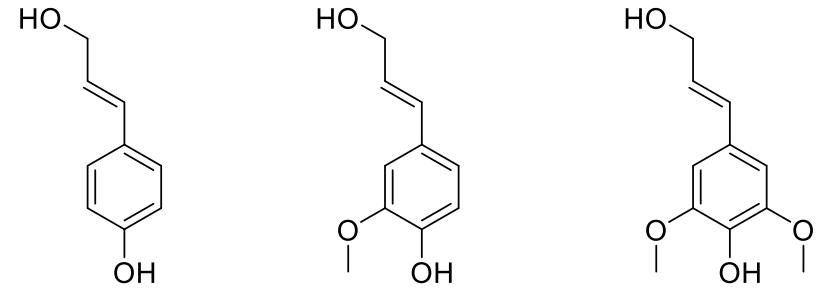


Figure 9 Lignin monolignols.

Significant quantities of lignin are produced as a by-product of pulp and paper processing which are often burnt to generate power,[36] as their conversion in to chemically important phenols at significant yield and purity is still problematic.

1.4.4 Polyphenols / Condensed Tannins

Condensed tannins (CTs) are one of the most abundant secondary metabolites in nature. These high molecular-weight polyphenols can be found mainly in the bark of conifers and legumes.[40] Condensed tannins are oligomers or polymers of flavan-3-ols (Figure 10). There are 2 different subgroups of CTs, they are procyanidins (PC) have two OH-groups, and prodelphinidins (PD) have three OH-groups in the B-ring.[41] Spruce bark tannins are identified mainly as procyanidins.[42]

Condensed tannins have some typical properties including protein complexation and inhibition, high antioxidant capacity and chemical reactivity which have been utilised in the pharmaceutical, nutraceutical, adhesives and leather tanning industries.[43] CTs extracted from the softwood bark have been proven to be suitable compounds in the formulation of environmentally friendly adhesives and resins.[44] Polyphenols including condensed tanning, flavonoids, and phenolic acids are very important antioxidant.[45] The antioxidant activity of phenolic compounds is mainly due to electron reduction potential of phenolic radical is lower than that of oxygen radicals, and phenoxyl radicals are generally less reactive than oxygen radicals. Therefore, phenolic compounds can sweep reactive oxygen intermediates to stop further oxidative reactions.[46, 47]

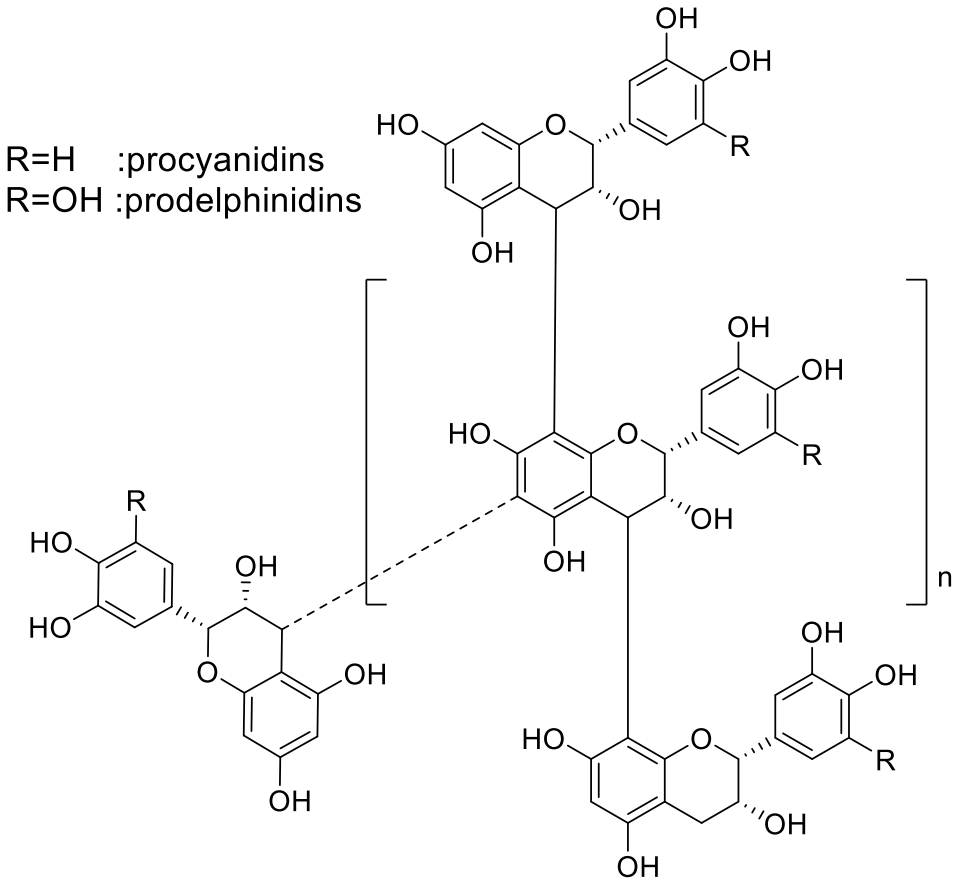


Figure 10 Structure of Condensed Tannin.

1.5 Extraction Processes

Extraction means the separation of a substance or a type of substances from a matrix. It is a traditional way to obtain soluble compounds in biomass. According to like dissolves like theory, solvents with different polarity will extract different extractives. Conventional solvent extraction is the simplest way to extract, but is not energy efficient, large amount of solvent will be used, the separation method – usually is distillation – also has a large energy demand. Extraction technologies have been developed to separate applicable compounds from biomass to obtain highly purified products.[48]

1.5.1 Soxhlet Extraction

The Soxhlet extractor was invented by *Franz von Soxhlet* in 1879.[49] A Soxhlet Extractor has three main sections: A percolator (flask and condenser) which circulates the solvent, a thimble (usually made of thick filter paper) which retains the solid to be laved, and a siphon mechanism, which periodically empties the thimble. The sample is placed in the thimble, the thimble-holder is filled with condensed solvent gradually during the operation, and the solvent comes from the flask. When the liquid reaches the overflow level, a siphon aspirates the solution back into the flask.[50] In Soxhlet extraction process, the solid phase (feedstock) and the liquid phase (extracts and solvent) are detached, no further separation process is needed.

1.5.2 Subcritical Water Extraction

Subcritical water extraction (SWE), also known as pressurized hot water or super-heated water extraction, is a technique that relies on polarity of water decreasing with increasing temperature. It is well known that the boiling point of water increases when the temperature increases, in this condition, physical and chemical properties of water as a solvent, such as its dielectric constant and solubility parameter, will change dramatically.[48] The relationship between the dielectric constant of water and temperature is shown in Figure 11.[51] At room temperature (25^{o} C), the dielectric constant of water is around 80 and decreases to about 33 at 200^{o} C.[52] This value is similar to some organic solvents such as ethanol or methanol. Therefore, this shows that SWE can be applied for extracting nonpolar compounds and water

can be used to replace organic solvents, partly. However, in the subcritical/superheated water conditions biomass is known to hydrolyse even in the absence of acids or alkalis.

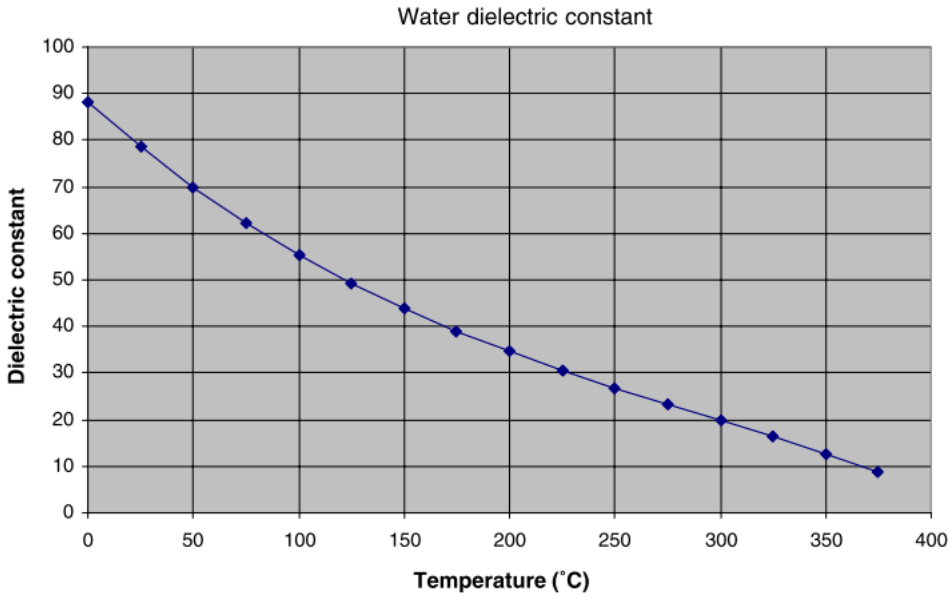


Figure 11 Graphical representation of dielectric constant of water vs. temperature.

1.6 Microwave Pyrolysis

Pyrolysis of biomass can be described as the direct thermal decomposition process of organic matrix in the absence of oxygen to obtain char, syngas and bio-oil.[53] Bio-oil is considered as an alternative to petroleum-based sources for a wide range of solvents, fuels and chemicals and other products.[54] Pyrolysis was performed on different temperatures, the solid part contains varying degrees of ashed inorganic materials, and any unconverted organic solid and carbonic residues produced from thermal decomposition of the organic compounds. The liquid fraction (bio-oil) is a complex mixture of organic compounds.

Microwaves are a form of electromagnetic radiation with wavelengths ranging from one meter to one millimeter; with frequencies between 300 MHz (100 cm) and 300 GHz (0.1 cm). Microwave heating has long been recognized as a rapid and energy-efficient mode of heating, with its most visible application being in domestic cooking. Microwave heating is caused by the direct absorption of microwave energy by components of the material as the microwave passes through the material.[55] The visualized difference between two heating methods is shown in Figure 12.[56] Compared with conventional heating method, microwave heating can be more efficient, due to its rapid, selective, volumetric, and uniform heating.[57, 58] Conventional pyrolysis of cellulose was carried out at temperatures >350 ^{o} C, while microwave pyrolysis of cellulose occurs at ~180^{o} C.

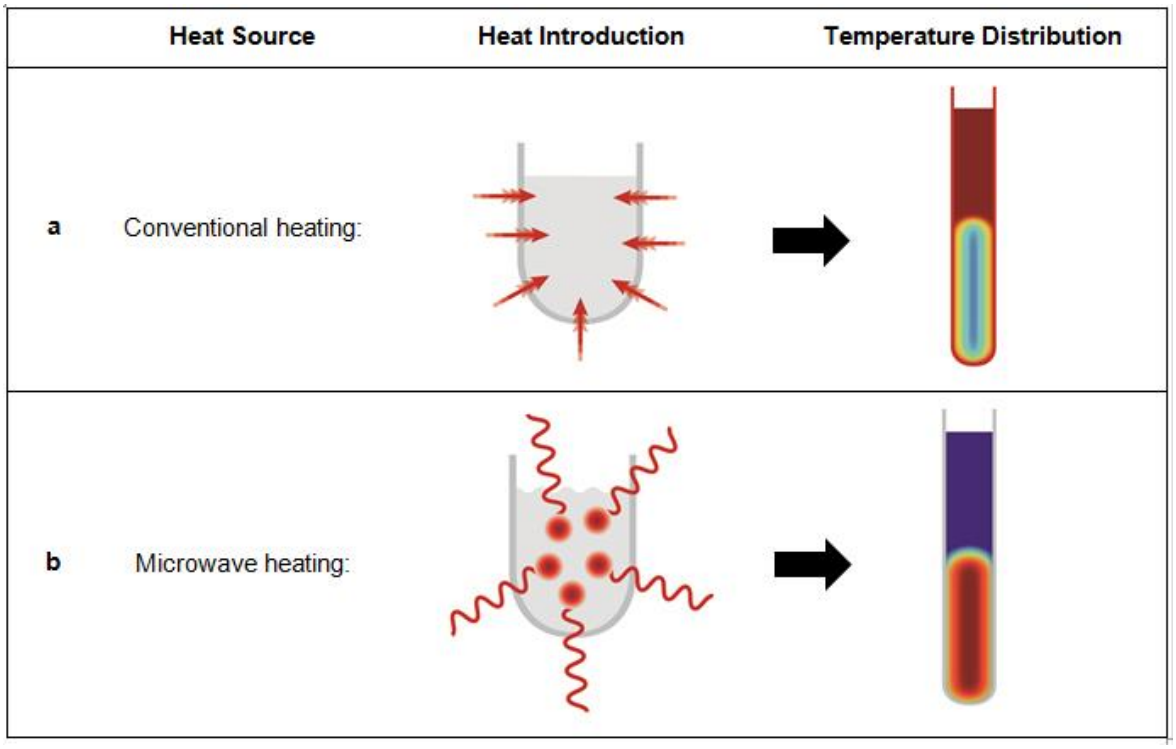


Figure 12 Microwave Heating Compare with Conventional Heating.

2. Experimental

2.1 Materials and Reagents

Norwegian Spruce was received from Umeå, Sweden. The biomass constituted the bark from numerous trees that had been recently felled for lumber. Samples of the biomass were then separated, through air drying until a constant weight was observed (circa three weeks) and a small portion refrigerated at 5 ℃. A small sample of the dry biomass and all of the refrigerated wet biomass were then milled as a whole. All milling was carried out using a Glen-Creston mill, with a 2 mm mesh.

Water content was tested in oven at 105°C, overnight. Ash content was tested in high temperature furnace at 800°C overnight.

All solvents and reagents used in work up procedures, *i.e.* ethanol, ethyl acetate, heptane, sulfuric acid, nitric acid, magnesium sulfate, potassium dichromate, Mohr's salt, acetic acid, calcium nitrate and sodium thiosulfate were obtained from Sigma Aldrich (now known as Merck) and used as supplied without further purification.

2.2 Instruments & Method

2.2.1 Conventional Solvent Extraction

Spruce bark (20 g) was heated under reflux in either ethanol (100 ml) contained in a round-bottom flask for 5 h. Thereafter, the mixture was cooled, filtered (gravity) and evaporated *in vacuo* to give either a viscous brown oil (ethanol extract, 8.8%).

2.2.2 Soxhlet Extraction

Spruce bark (4.2 g) was put in a Soxhlet thimble, ethanol (50 ml) was heated under reflux contained in a round-bottom flask for 2 h. Thereafter, the mixture was cooled and evaporated *in vacuo* to give a brown solid (18.2%).

2.2.3 Microwave Pyrolysis

Spruce bark (0.3 g or 1 g) was placed in either 10 ml or 35 ml microwave tubes and subjected to pyrolysis with a CEM Discover microwave reactor for 10 min in dynamic mode at different temperature as detailed in Table 2. Post pyrolysis, bio-oil was extracted in to ethanol and evaporated to give a viscous saddle brown oil. The bio-oil was analysed by IR and GCMS (see Results and discussion). The biochar (residue) was further washed with ethanol and acetone, dried and its calorific value determined via oxygen bomb calorimetry.

Table 2 Conditions of MW pyrolysis experiments.

Run	Vessel/ml	Bark /g	Temp. /°C	Power /W	Time /min
1	10	0.3	160	200	10
2	10	0.3	180	200	10
3	10	0.3	200	200	10
4	10	0.3	220	200	10
5	10	0.3	240	200	10
6	35	1	160	200	10
7	35	1	180	200	10
8	35	1	200	200	10
9	35	1	220	200	10
10	35	1	240	200	10

2.2.4 Subcritical Water Extraction

SWE was conducted in a batch reactor. Spruce bark (5 g) was heated under different temperatures (120, 140, 160, 180 and 200℃) in water (200 ml) for 2 h. The aqueous solution obtained after filtration was extracted with ethyl acetate (2 x 50 ml). The combined organic extract was evaporated in *vacuo* to give a deep yellow oil. The aqueous fraction was retained for sugar analysis via HPLC (see Results and Discussion section).

2.3 Cellulose, Hemi-cellulose and Lignin Content

The cellulose, hemi-cellulose and lignin content in spruce bark and pyrolysis residues was determined via titrimetry as reported in the literature and outlined below (Figure 13)[59]. The average deviation of the titration was less than ±1 wt. %.

2.3.1 Determination of Cellulose

The dry biomass (~0.03 g) was treated with a 1:8 nitric-acetic acid mixture (5 ml) for 25 min, in order to dissolve intercellular substances and separating the cellulose into single fibers. The resultant cellulose was oxidized by $K_2Cr_2O_7$ and H_2SO_4 (10 ml, 0.5 mol/L) to give carbon dioxide and water as shown in eq. 1.

$$C_6H_{10}O_5 + 4K_2Cr_2O_7 + 16H_2SO_4 = 6CO_2 + 4Cr_2(SO_4)_3 + 21H_2O \text{ ------Eq. 1}$$

The residual potassium dichromate was titrated with Mohr's salt [$(NH_4)_2Fe(SO_4)_2$] (Eq.2) solution (0.1mol/L). The same amount of $K_2Cr_2O_7$- H_2SO_4 which was not reacted with

cellulose was also titrated with $(NH_4)_2Fe(SO_4)_2$ solution. Then, the cellulose content was calculated based on the amounts of $(NH_4)_2Fe(SO_4)_2$ solution was used.

$$K_2Cr_2O_7 + 6FeSO_4 + 7H_2SO_4 = 3Fe_2(SO_4)_3 + Cr_2(SO_4)_3 + K_2SO_4 + 7H_2O \text{ ------Eq. 2}$$

2.3.2 Determination of Hemicellulose

Starch and other water-soluble carbohydrates, which would interference the titration of hemicellulose, were removed by boiling biomass (0.1 g) in 80% aqueous $Ca(NO_3)_2$ solution for 5 min. After washing with water, the residue was treated with 2 mol/L HCl in water bath for 45 min to hydrolyze the hemicellulose. After neutralizing with NaOH solution, total sugar content was determined by the copper-iodine method. Reducing sugars from the hydrolysis of hemicellulose reduced Cu(II) to Cu_2O. Through determination of the Cu_2O content, the hemicellulose content was determined. By adding sulfuric acid to the solution, the KIO_3 and KI (eq. 3) contained in the alkaline copper solution would react and release iodine under acidic conditions.

$$KIO_3 + 5KI + 3H_2SO_4 = 3I_2 + 3K_2SO_4 + 3H_2O \text{ ------Eq. 3}$$

Iodine reacted with Cu_2O (eq. 4) in presence of oxalic acid.

$$Cu_2O + I_2 + H_2C_2O_4 = CuC_2O_4 + CuI_2 + H_2O \text{ ------Eq. 4}$$

The residual iodine was titrated with a $Na_2S_2O_3$ solution (eq. 5).

$$2\ Na_2S_2O_3 + I_2 = Na_2S_4O_6 + 2NaI \text{ ------Eq. 5}$$

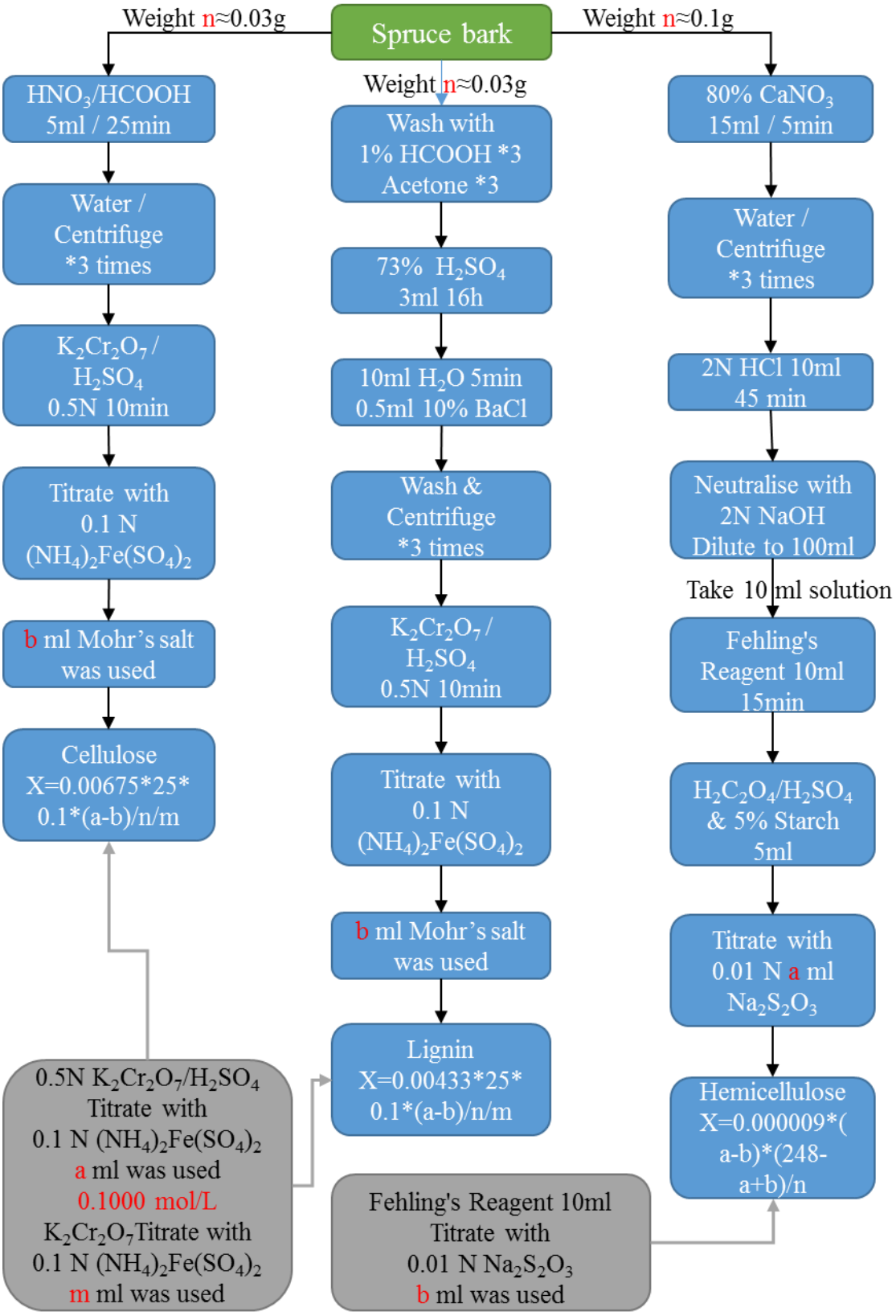


Figure 13 Flow Chart of Titration method.

2.3.3 Determination of Lignin

The dry biomass (~0.03 g) was placed in centrifuge tube. After the sugar, organic acid and other water soluble compounds were removed using 1% acetic acid, the chlorophyll, fat and other fat soluble compounds were removed using acetone. After the biomass was dried, sulfuric acid (73%, 3 ml, 16 h) was used to remove cellulose and hemicellulose. After washing with water, lignin was oxidized by $K_2Cr_2O_7$ with H_2SO_4 (eq. 6).

$$C_{11}H_{12}O_4 + 8\ K_2Cr_2O_7 + 32\ H_2SO_4 = 11CO_2 + 8K_2SO_4 + 8Cr_2(SO_4)_3 + 32H_2O \quad \text{----Eq. 6}$$

The remaining $K_2Cr_2O_7$ was titrated with a $(NH_4)_2Fe(SO_4)_2$ solution, which was the same as for the titration of cellulose (see Eq. 2).

2.3.4 Total Phenolic Content

Total phenolic content (TPC) is usually determined by Folin–Ciocalteu Method. The Folin-Ciocalteau procedure is a colorimetric method for analysis of phenolics/ antioxidant activity by reduction of a molybdo-tungstophosphate complex. It was originally developed for analysing proteins.[60] The metric produced by the test is a '*gallic acid equivalent*' (GAE), a relative measure of performance compared with gallic acid.

Standard gallic acid solution, the desired extract and sodium carbonate solution were prepared within 1 week before test. Gallic acid solution and sample solution were diluted to different concentration, reacted with Folin–Ciocalteu reagent and sodium carbonate in dark room for 2 h, and the UV-vis spectrum recorded. The UV absorption at 765 nm was measured in order to determine TPC.

2.4 Instrumentation

2.4.1 CHN Analysis

Samples was submitted to and tested by analytical services, Department of Chemistry, University of York. The sample was placed in a nickel sleeve and injected into a high temperature furnace (975°C) and burnt in high purity oxygen under static conditions.

The HHV (Higher Heating Value) of biomass and biochars were determined using the modified Dulong's formula[61] (eq. 7).

$$\mathrm{HHV(MJ/kg)} = \frac{33.5 \times \mathrm{wt.\%C}}{100} + \frac{142.3 \times \mathrm{wt.\%H}}{100} - \frac{15.4 \times \mathrm{wt.\%O}}{100} \qquad Eq.7$$

2.4.2 Oxygen Bomb Calorimetry

The calorific value of spruce bark and bio-char were determined using a Parr 6200 Calorimeter with a standard 1108 Oxygen Bomb. Dry biomass (~0.5 g) was placed in a plate inside the oxygen bomb, and fired by a heating wire. The temperature of water (2 L) was tested before and after the burning process to calculate the calorific value.

2.4.3 ATR-IR

Attenuated Total Reflection Infra-Red spectroscopy measurements were performed on a Perkin Elmer *Spectrum 400* instrument. Spectrum was taken from 4000 cm^{-1} to 600 cm^{-1} at 4 scans, with a spectral resolution of 2 cm^{-1}, with blank window for background.

2.4.4 GC-MS

Gas chromatography–mass spectrometry (GC-MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. MW pyrolysis bio-oil and organic layer product in SWE was analysed by GC-MS, was performed on *Clarus 500* Gas Chromatograph, and *Clarus 560 S* Mass Spectrometer, Perkin Elmer Company. Samples were analysed General-2 method, start from 50^{o} C, holding time 4 min, heating rate 8 ^{o}C/min, end at 290^{o} C with 10 min holding time.

2.4.5 Thermo Gravimetric Analysis

TGA is a method of thermal analysis in which changes in physical and chemical properties of materials are measured as a function of increasing temperature, or as a function of time. TGA was performed on STA-625 instrument. A small amount of sample (~10 mg) was accurately weighed into an aluminum TGA cup and heated under the nitrogen flow of 50ml/min to avoid oxidation of the sample. The programmed temperature was started at room temperature and then increased up to 625^{o}C at a rate of 10 ^{o}C /min. The mass of sample and heat flow are tested in the process, the chart can show us the changes of weight and heat.

2.4.6 Py-GCMS

Pyrolysis – gas chromatography – mass spectrometry (Py-GCMS) is a method of chemical analysis in which the sample is heated to decomposition producing pyrolysis products that are separated by gas chromatography and detected using mass spectrometry.

Py-GC/MS results were obtained from BDC, University of York. The modular system comprised a CDS Analytical 5250-T Trapping Pyrolysis Autosampler (UK) as the pyrolysis unit, Agilent Technologies 7890B GC System (USA) as gas chromatography unit, and Agilent Technologies 5977A MSD (USA) as mass spectrum unit. The sample was loaded into the pyrolysis unit and pyrolyzed at 600 °C for 10 s. The volatile materials released were carried into the GC/MS unit by nitrogen for analysis. The following GC/MS parameters were applied: GC inlet temperature at 350 °C, initial temperature at 40 °C for 2 min, ramp rate at 10 K/min until 300 °C, holding at 300 °C for 30 min, and split ratio with 50:1. Volatile compounds were identified by comparing the mass spectra with NIST Lab database. A standard sample mixture of four compounds, cresol/vanillin/2-methoxyphenol (guaiacol)/Eisoeugenol, was also subjected to pyrolysis and GC/MS in order to verify the mass spectral identities.

3. Results and Discussion

Herein, the results and discussion is divided in to 4 parts:

i. Component analysis of spruce bark;

ii. Solvent extraction of spruce bark with ethanol

iii. Microwave pyrolysis of spruce bark, and;

iv. Subcritical water extraction of spruce bark.

3.1 Component analysis of Spruce Bark

3.1.1 Elemental analysis and theoretical Higher Heating Value (HHV)

The elemental analysis of spruce bark obtained from Umea, Sweden, comprised: C: 47.64%; H: 5.91%; N: 0.26%; Rest: 46.19%. Assuming the 'rest' is mainly oxygen then spruce bark is lignocellulosic rich (see section 3.1.2 for cellulose, hemicellulose and lignin content) and with minimal nitrogen-containing compounds. The latter may arise from proteins or proteinaceous matter within the bark.

From knowledge of the elemental composition, the theoretical HHV can be determined using a modified version of the Dulong equation[61] (see eq. 4, Experimental Section). Assuming that the oxygen content is 49.16% ('rest'), the theoretical HHV for spruce bark is 17.3 MJ/kg. This value will be compared later in section 3.3.2 in relation to the actual calorific value of biochars produced as a consequence of microwave pyrolysis.

3.1.2 Cellulose, Hemicellulose and Lignin content.

Spruce bark is an example of lignocellulosic biomass and thus the cellulose, hemicellulose and lignin content was determined according to the protocol outlined in Figure 13.

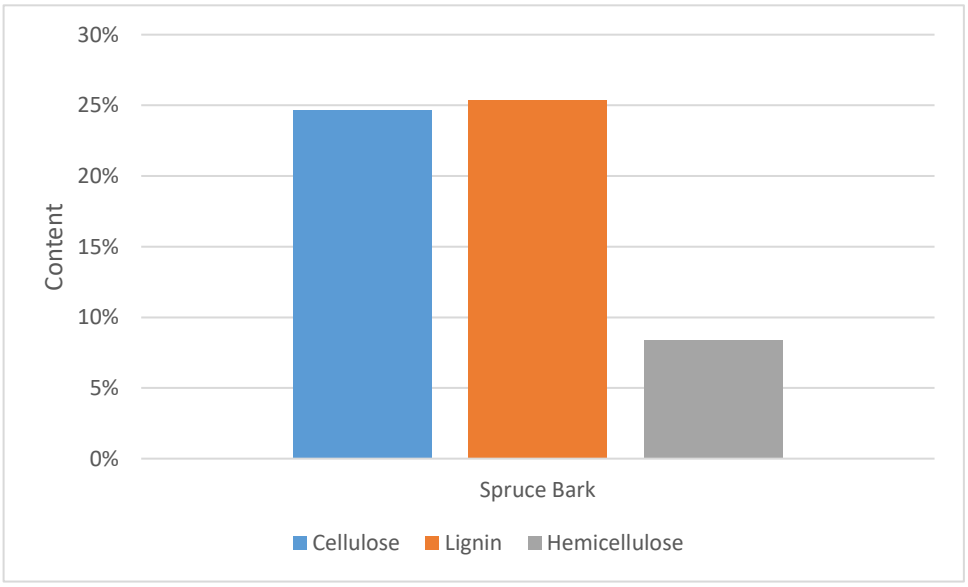


Figure 14 Cellulose, Hemicellulose and Lignin content of spruce bark.

As seen in Figure 14, spruce bark contains 25% cellulose, 25% lignin and 8% hemicellulose. Compared with literature[62] (cellulose, 26 %; hemicellulose, 9 %; lignin, 11%; extractives, 32%), our spruce bark has higher lignin content and lower extractive content, this may because of the age of raw material, older trees may contain more lignin and less volatiles/extractives.

3.1.3 Thermo gravimetric analysis of spruce bark

The thermogram for mass loss (decomposition) versus temperature of spruce bark in an atmosphere of nitrogen from 30 - 625^{o}C at a heating rate of 10 ℃ min^{-1} is shown in Figure 15. Initial decomposition of 9.2% from room temperature to approximately 150^{o}C is

usually associated with moisture loss (water) and volatiles. The second main mass loss from approximately 150 – 300ºC, centred at 248ºC, is associated with decomposition of hemicellulose (approximately 20%). Thereafter, decomposition in the range 300-400ºC, centred at 342°C corresponds to cellulose decomposition (approximately 30%). Decomposition of organic matter from 400-625ºC is attributed to lignin (approximately 15% but could be more as the decomposition profile has not plateaued to completion at 625ºC). At 625ºC, the residual matter was estimated to be 25% and assumed still to contain lignin and some inorganic matter. This was confirmed by heating native spruce bark in a muffle furnace at 800ºC for 4 h which gave an ash yield of 2%.

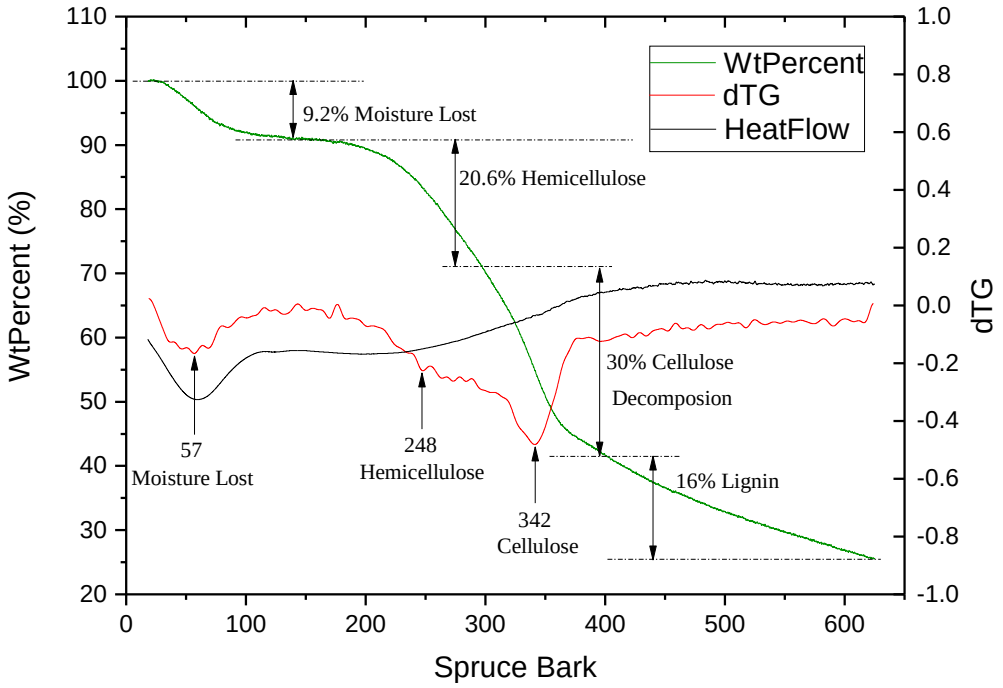


Figure 15 STA 625 data of Spruce Bark.

3.1.4 ATR-IR of spruce bark

The ATR-IR spectrum of feedstock spruce bark is shown in Figure 16. The broad absorbance band centred at 3311 cm^{-1} corresponds to O-H str vibration mode characteristic of moisture and lignocellulosic matter. Characteristic absorbance bands at 2922 and 2850 cm^{-1} are indicative of C-H str vibrational modes predominantly of aliphatic (saturated) regions. C-H str vibrational modes for unsaturated and aromatic structures usually occur slightly above 3000 cm^{-1} but are not seen as they are probably masked by the strong, broad O-H str vibration. However, possible C-H oop vibrational mode characteristic of aromatic compounds, e.g., lignin, is noted at 835 cm^{-1}. [63] Interestingly, weak absorbance bands are observed at 1735 cm^{-1} characteristic of the carbonyl stretching mode which may be tentatively assigned to acetyl moieties in hemicellulose.

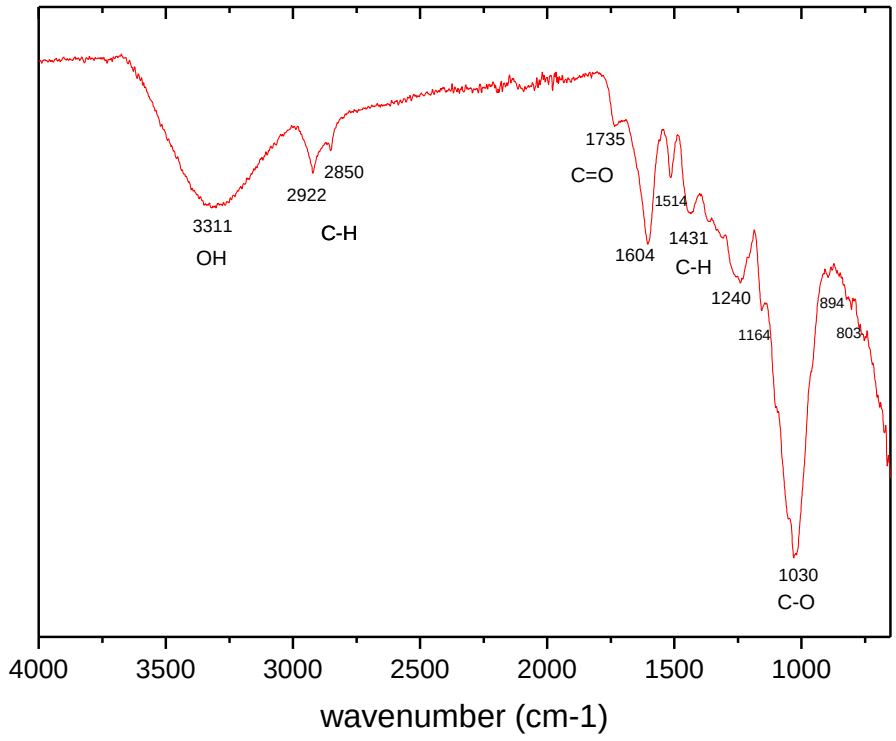


Figure 16 FT-IR Spectrum of spruce bark.

3.1.5 Pyrolysis-GCMS (py-GCMS)

Pyrolysis-GCMS is a powerful technique that provides preliminary information with respect to potential compounds that can removed unadulterated as well those that arise as a result of thermal decomposition. Spruce bark was subjected to py-GCMS and the full list of compounds detected with possible matches is listed in the Appendix 1. Herein, Table 3 only shows those compounds with a NIST library probability match of >80%. Interestingly, decomposition products of carbohydrates are evidenced, for example, acetic acid and furfural. A variety of phenolic compounds are detected arising from decomposition of lignin, condensed tannins and polyphenols. Oleic acid and long chain alcohols, e.g. 1-heptatriacotanol, sitosterols, are detected which would be present unadulterated, *i.e.*, not decomposition products of lignocellulose.

Table 3 Py-GCMS data.

Time (mins)	Name	Formula	Probability
2.196	2-Butanone, 3-methyl-	$C_5H_{10}O$	80.12
2.71	Acetic acid	$C_2H_4O_2$	87.16
3.842	(1-Allylcyclopropyl)methanol	$C_7H_{12}O$	82.19
4.024	1,3,5-Cycloheptatriene	C_7H_8	87.51
4.31	Pentanal, 2,4-dimethyl-	$C_7H_{14}O$	88.08
5.179	Furfural	$C_5H_4O_2$	85.37
5.567	2-Hexanone, 6-hydroxy-	$C_6H_{12}O_2$	80.28
6.379	Cyclopropanecarboxylic acid, cyclohexylmethyl ester	$C_{11}H_{18}O_2$	80.63
6.893	1,2-Cyclopentanedione	$C_5H_6O_2$	88.41
7.773	Phenol	C_6H_6O	88.82
8.688	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	$C_6H_8O_2$	89.63
8.814	1-Amino-2,6-dimethylpiperidine	$C_7H_{16}N_2$	80.15
9.534	Ethanone, 1-(1-cyclohexen-1-yl)-	$C_8H_{12}O$	82.82
10.059	Maltol	$C_6H_6O_3$	84.5
10.905	4-Hydroxy-non-2-ynoic acid, ethyl ester	$C_{11}H_{18}O_3$	83.73
11.134	Creosol	$C_8H_{10}O_2$	93.78

11.374	Resorcinol	$C_6H_6O_2$	80.33
12.357	Phenol, 4-ethyl-2-methoxy-	$C_9H_{12}O_2$	92.55
12.665	1,2-Benzenediol, 3-methyl-	$C_7H_8O_2$	86.49
12.871	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	88.13
13.431	2,5-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	82.23
13.888	Cyclohexene, 1,5,5-trimethyl-6-acetylmethyl-	$C_{12}H_{20}O$	80.05
14.654	Phenol, 2-methoxy-5-(1-propenyl)-, (E)-	$C_{10}H_{12}O_2$	91.8
16.86	Melezitose	$C_{18}H_{32}O_{16}$	81.91
18.072	Acetamide, *N*-methyl-*N*-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	$C_{11}H_{18}N_2O_2$	83.34
20.277	Estra-1,3,5(10)-trien-17.beta.-ol	$C_{18}H_{24}O$	87.3
20.929	1-Heptatriacotanol	$C_{37}H_{76}O$	85.55
21.832	1-Heptatriacotanol	$C_{37}H_{76}O$	83.96
21.946	Oleic Acid	$C_{18}H_{34}O_2$	89.88
22.129	1-Heptatriacotanol	$C_{37}H_{76}O$	83.61
24.712	Pimaric acid	$C_{20}H_{30}O_2$	84.1
25.341	Pregn-4-ene-3,20-dione, 16,17-epoxy-, (16.alpha.)-	$C_{21}H_{28}O_3$	84.5
25.627	Hydrocortisone Acetate	$C_{23}H_{32}O_6$	84.84
29.536	beta-Sitosterol acetate	$C_{31}H_{52}O_2$	81.6
31.753	gamma-Sitosterol	$C_{29}H_{50}O$	84.62

3.2 Solvent Extraction of Spruce Bark

3.2.1 Extractive Yield

The extractive yields with hot ethanol (reflux and Soxhlet) are reported in Table 4. Ethanol Soxhlet extraction gave a slightly extractive yield (18.2%) compared with ethanol reflux (15.5%). Ethanol, a polar solvent, will extract polar organic molecules, e.g., phenols, condensed tannins[25], sugars but also ethanol as it is a water-miscible solvent, hence, the seemingly high yield. The analysis of the Soxhlet extract is reported later in section 3.2.2.

Table 4 Yield of solvent extraction % dry mass.

Method	Yield (%)
EtOH (reflux)	15.5
EtOH (Soxhlet)	18.2

3.2.2 Extract Analysis

3.2.2.1 ATR-IR

Although the Soxhlet extract is a multi-component mixture its ATR-IR was recorded (Figure 17) in order to undertake a first, broad-brush, look see in order to ascertain functional groups present. As expected, a broad absorbance band centred at 3305 cm^{-1} corresponding to the O-H str was observed characteristic sugars, small molecule phenols

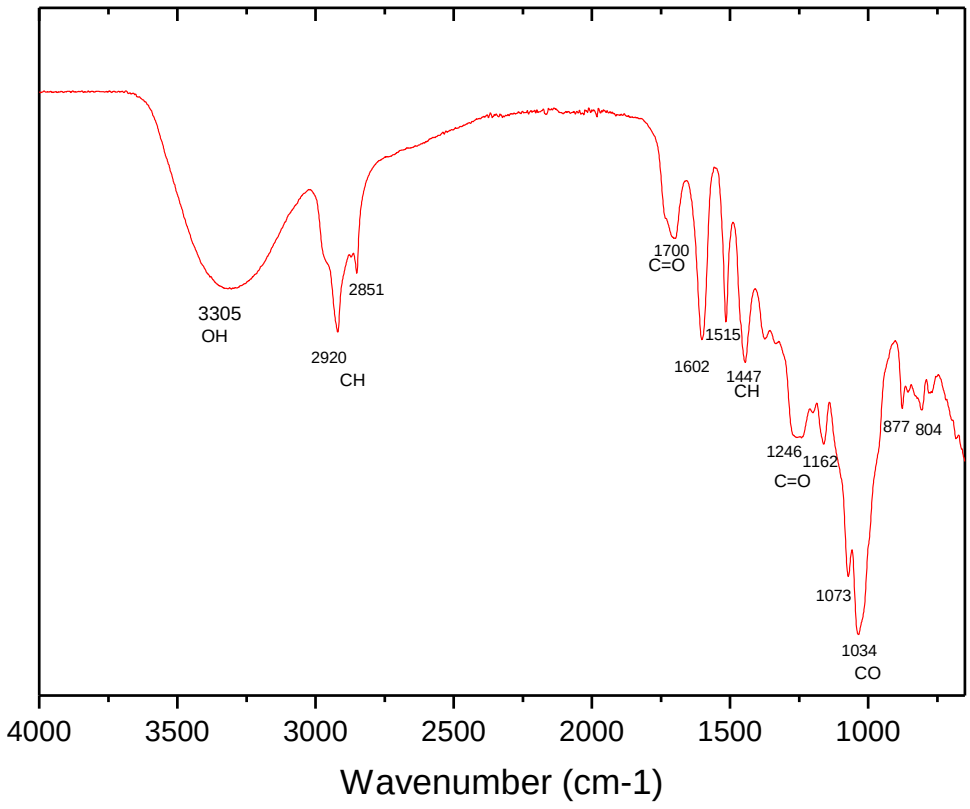


Figure 17 IR spectrum of Soxhlet extracts

and residual water. Sugars are notoriously hygroscopic thus complete removal of water using rotary evaporation *in vacuo* is difficult. Also, care must be taken to avoid using extreme reduced pressure and/or high temperatures may lead to loss of volatiles and/or decomposition products. The absorbance bands at 2920 and 2851 cm^{-1} are characteristic of the C-H str. A weak carbonyl stretching absorbance is noted at 1700 cm^{-1} and strong C-O and C-O-C vibrational modes are detected at 1246, 1162, 1073 and 1034 cm^{-1}. GC-MS was performed to better ascertain the composition of the Soxhlet extract as discussed in section 3.2.2.2.

3.2.2.2 GCMS

As the Soxhlet extract may contain non-volatile sugars to avoid column damage, a small portion of the Soxhlet extract was treated with ethyl acetate in order to isolate organic compounds. The resulting ethyl acetate extract was then subjected to GC-MS. Figure 18 shows the annotated chromatograph. The annotations are based on spectral matches with respect to the NIST library instrument software. A significant number of peaks are associated with small molecule phenolic compounds, in particular 2-methoxy-5-methylphenol, often-characteristic precursors of lignin, polyphenols and condensed tannins which are not observed due to their high boiling point. Thus, the total phenolic content was determined using the Folin–Ciocalteu methodology giving 39 mg GAE/g spruce extract. Interestingly, this value is considerably higher than that of Ghitescu *et al*.[22] who reported 13.32 mg GAE/g spruce extract when spruce bark was extracted with 70% ethanol aqueous solution at 60^{o} C for 45 min.

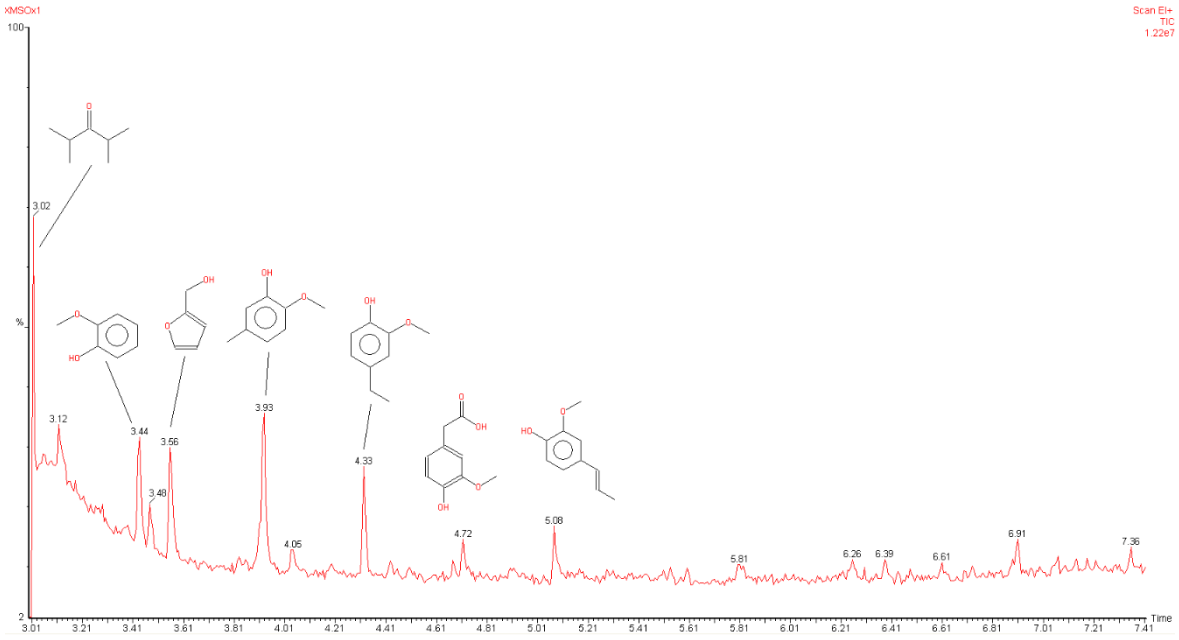


Figure 18 GC-MS chromatography of Soxhlet extracts.

3.3 Microwave Pyrolysis of Spruce Bark

The microwave pyrolysis of spruce bark was expected to afford bio-oil and biochar of differing yield and composition based on pyrolysis conditions employed (see Experimental Section 2.2.3 and Table 2).

3.3.1 Pyrolysis Bio-oil

3.3.1.1 Bio-oil Yield

Yields of bio-oil from microwave pyrolysis at different temperatures are shown in Figure 19. The bio-oil yield decreases as the pyrolysis temperature increases from 160 to 240°C at 20°C intervals. The decrease appears to be gradual up to 200°C, plateaus between 200 and 220°C (in fact rises slightly), but then falls again at 240°C. During 200 and 220°C

microwave-induced decomposition of cellulose is observed in addition to hemicellulose decomposition, which occurs at lower temperatures. The bio-oil yield drops at 240°C due to losses arising from *insitu* decomposition of the oil either in to biochar or non-condensable gases. The latter were not collected thus lost to the atmosphere.

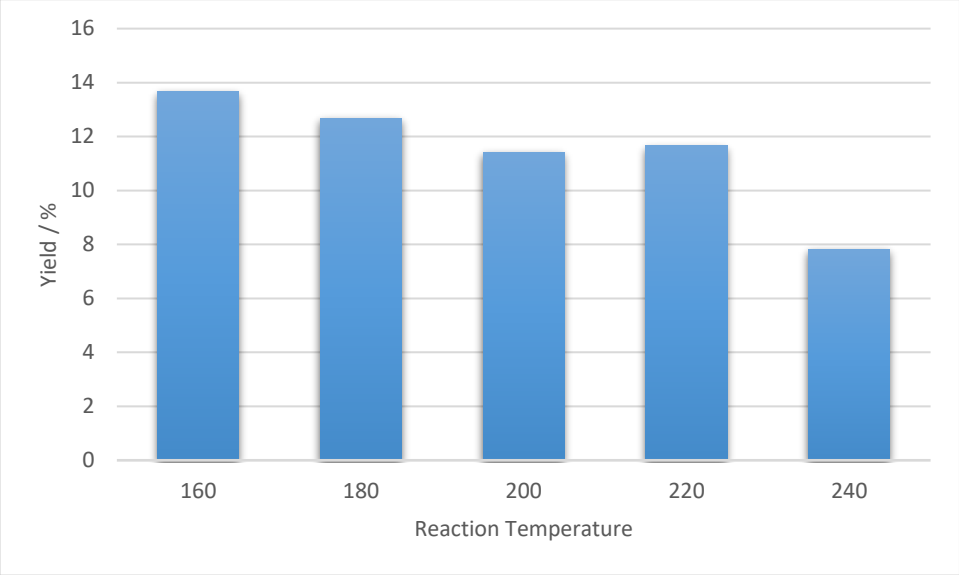


Figure 19 Yields of bio-oil from MW pyrolysis.

3.3.1.2 GCMS of pyrolysis bio-oil.

The GC-MS chromatogram for microwave pyrolysis bio-oil (180 °C) is shown in Figure 20, and selected compounds based on >80% probability match with the NIST library are listed Table 5. Again, the compounds listed need to be considered with care as they are library matches. Nevertheless, good evidence of phenolic compounds associated lignin and tannins is observed. For example, the mass spectrum of 2-methoxyphenol is shown in Figure 21 a, the peak at 124 m/z is assigned to molecular ion, the peak at 109 m/z is assigned to the ion $C_6H_5O_2^+$ when methyl group was removed. The mass spectrum 2-methoxy-4-methylphenol is shown in Figure 21 b, the peak at 138 m/z is assigned to molecular ion, the peak at 123 m/z is assigned to the ion without methyl group.

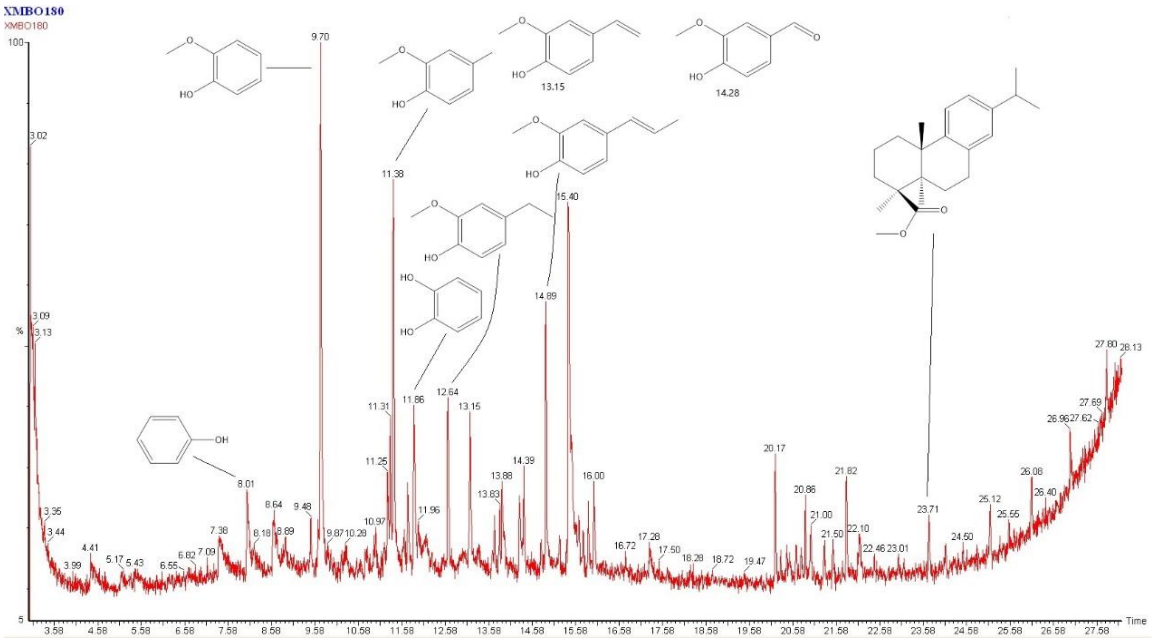


Figure 20 GC-MS chromatogram of MW pyrolysis bio-oil.

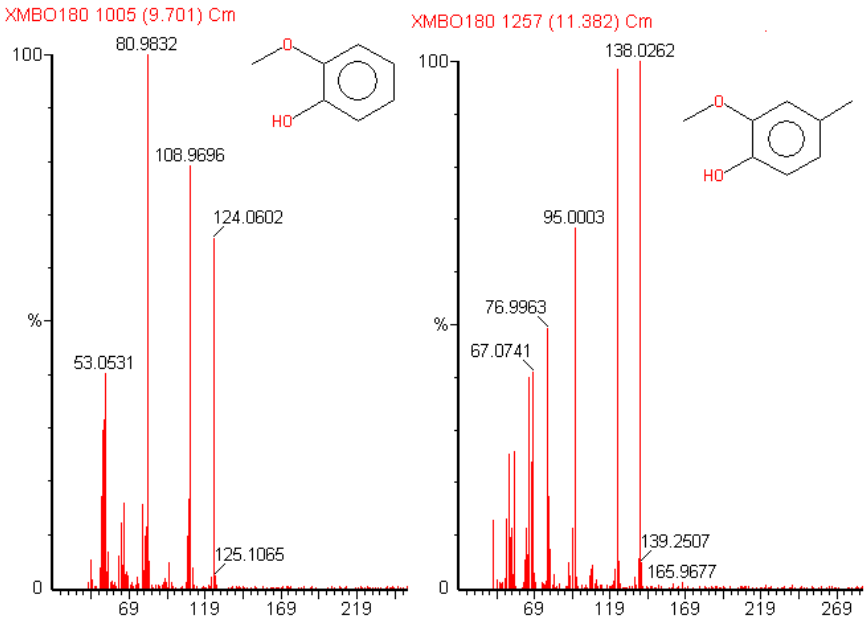
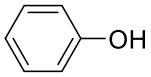
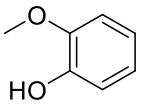
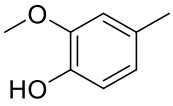
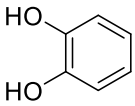
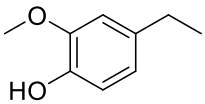
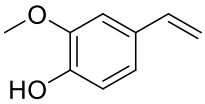
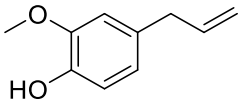
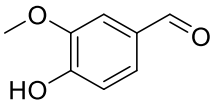
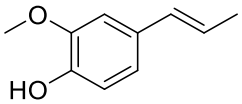
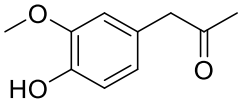


Figure 21 Mass Spectrum of a) 2-methoxyphenol and b) 2-methoxy-4-methylphenol.

Table 5 Chemicals and Structures recognized from GC-MS of pyrolysis bio-oil.

Time/min	**Compound**	**Structure**
8.01	Phenol	
9.70	2-methoxy-Phenol, Guaiacol	
11.38	2-Methoxy-4-methyl-phenol	
11.86	1,2-Benzenediol	
12.64	4-Ethyl-2-methoxy-phenol	
13.15	2-Methoxy-4-vinyl-phenol,	
13.72	Eugenol	
14.28	Vanillin	
14.89	2-Methoxy-4-(1-propenyl)-phenol	
15.87	1-(4-hydroxy-3-methoxyphenyl)-2-propanone	

In order to remove the interference of condensed tannins, the spruce bark after ethanol extracted was used as the feedstock of microwave pyrolysis experiment.

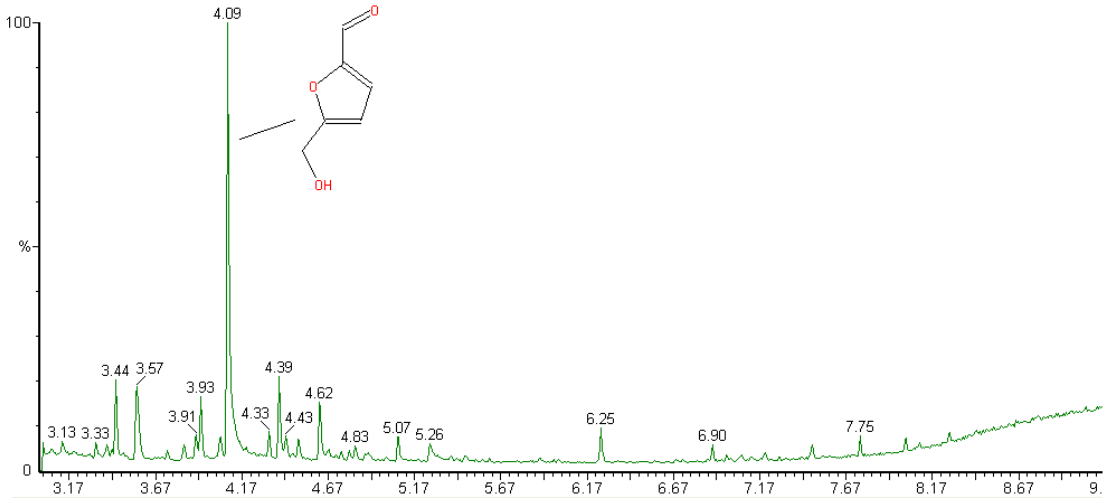


Figure 22 GC-MS chromatogram of extracted bark.

The GC-MS chromatogram of pyrolysis after ethanol extraction is shown in Figure 22, from the chromatogram, we can see the largest peak corresponds to HMF the most common product from the degradation of polysaccharide.

The mass spectrum of HMF is shown in Figure 23, from the spectrum we can indicate that the molecular weight is 126 (molecular ion), the peak at 109 m/z /is assigned to the remove of –OH group, the 97 m/z peak is assigned to the mass loss of aldehyde group (29 m/z). The signals of $C_3H_5^+$, $C_4H_5^+$ and $C_4H_5O^+$ are corresponded to peaks at 41, 53 and 69 m/z. From the peaks in Figure 19, the signal of HMF is not shown. Before ethanol extraction, spruce bark contains approximately 1/5 phenolic compounds including condensed tannins, excluding lignins, we can surmise that this part of polyphenols absorbed microwave

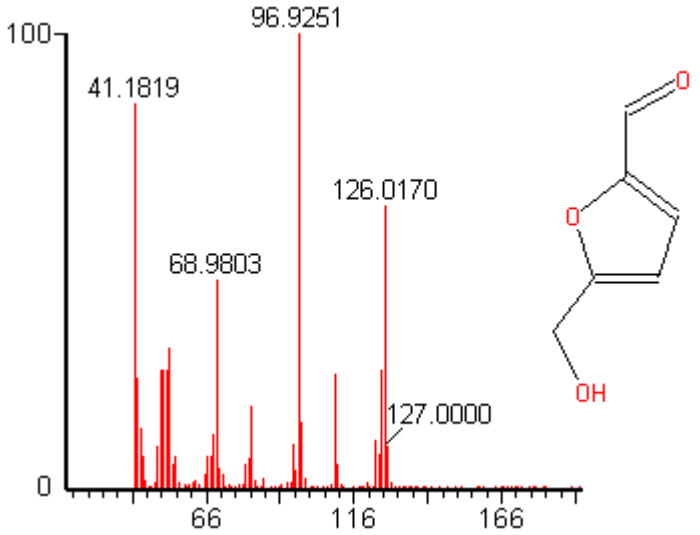


Figure 23 Mass Spectrum of HMF.

energy and decomposed at first. Nevertheless, when the phenols were removed by ethanol extraction, cellulose and hemicellulose are able to absorb microwave energy, therefore, HMF became main component in pyrolysis bio-oil.

3.3.2 Pyrolysis *Bio-char*[*]

3.3.2.1 Pyrolysis *Bio-char* yield

The pyrolysis *bio-char* yield spruce bark at different temperatures is shown in Figure 24. A similar trend to bio-oil yield as discussed earlier was observed, *i.e.*, the bio-char yield decreases with increasing pyrolysis temperature. The decrease in biochar yield is commensurate with increasing formation of non-condensable pyrolysis gases.

Biochar is a form of bioenergy and the process of pyrolysis often leads to energy densification. Thus, the calorific values, as determined by oxygen bomb calorimetry, of

[*] The term *biochar* is italicised because at low temperatures incomplete pyrolysis of the feedstock is noted.

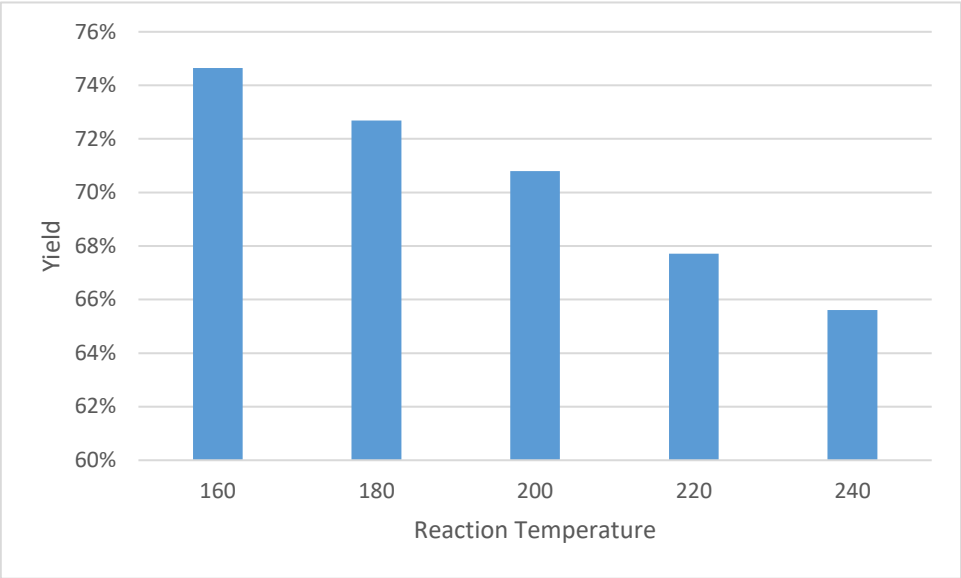


Figure 24 Yield of Bio-char from MW pyrolysis.

native spruce bark, its pyrolysis chars, and chars from pine wood, wheat straw and chestnut shell, are listed in Table 6.

Table 6 Calorific value (MJ/kg) of spruce bark, bio-char & data from literature.

Sample	**Calorific Value (MJ/kg)**
Spruce Bark	22.4
Bio-char 160	24.9
Bio-char 180	25.3
Bio-char 200	25.7
Bio-char 220	26.1
Bio-char 240	26.6
Pine wood biochar 1[64]	27.1
Pine wood biochar 2[65]	27.8
Wheat straw biochar[65]	22.0
Chestnut shell biochar[66]	25.9

Compared with spruce bark itself, all resulting biochars (160, 180, 200, 220 and 240) show energy densification. The energy densification increases with increasing pyrolysis temperature, e.g., the calorific value of bio-char 240 (26.6 MJ/kg) is 4.2 MJ/kg larger than for spruce bark itself (22.4 MJ/kg). Compared with certain literature materials, pinewood biochar has a higher calorific value than for all the biochars produced from spruce bark

whilst all the biochars produced (including spruce bark itself) have a higher calorific value than wheat straw. Pinewood and spruce bark have a similar lignocellulosic composition; especially lignin content of approx. 25%, whilst wheat straw has a lignin content of about 15%, which accounts for the latter's low embedded energy. Biochar 180, 220 and 220 have comparable calorific values to chestnut shell biochar. However, it must be stressed that the comparisons made with literature data do not take in to account different processing conditions for char formation.

As stated earlier the term *biochar* is defined loosely because at low pyrolysis temperatures incomplete pyrolysis occurs. In order to confirm incomplete pyrolysis, the cellulose, hemicellulose and lignin content of biochar-160 was determined and reported in Table 7.

Table 7 Determination of 3 component before and after pyrolysis at 160°C.

Sample	**Cellulose/%**	**Lignin/%**	**Hemicellulose/%**
Spruce Bark	24.7	25.3	8.4
Bio-char/160°C	22.8	38.5	4.8

Compositional analysis of biochar-160 in comparison with native spruce bark (Table 7) shows a significant amount of cellulose was still retained whilst approximately 40% of hemicellulose remains. Interesting, the lignin content increases from 25.3% (spruce bark to 38.5% (biochar-160). As cellulose and hemicellulose decompose to small molecules, e.g. furans, these tend to polymerise forming intractable, condensed compounds known as humins. The formation of humins from 5-hydroxymethyl furfural (HMF) is shown in Figure 25. Humins as intractable by-products will give an over estimate for lignin content using the titration method.

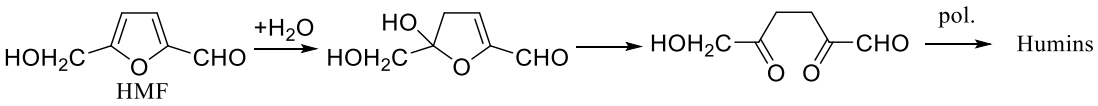


Figure 25 HMF conversion pathway to humins.[70]

3.4 Subcritical Water Extraction

SWE of spruce bark was performed using conditions as listed in Experimental, section 2.2.4. Post-SWE treatment, the cooled water was extracted with ethyl acetate to determine yield of organic extractives which are depicted as a bar-graph in Figure 26. The composition of the organic extractive was determined by GC-MS as outlined in section 3.4.2 whilst the sugar content of the 'organic'-free water was analysed by HPLC (see Section 3.4.3).

3.4.1 Organic Extractive Yield

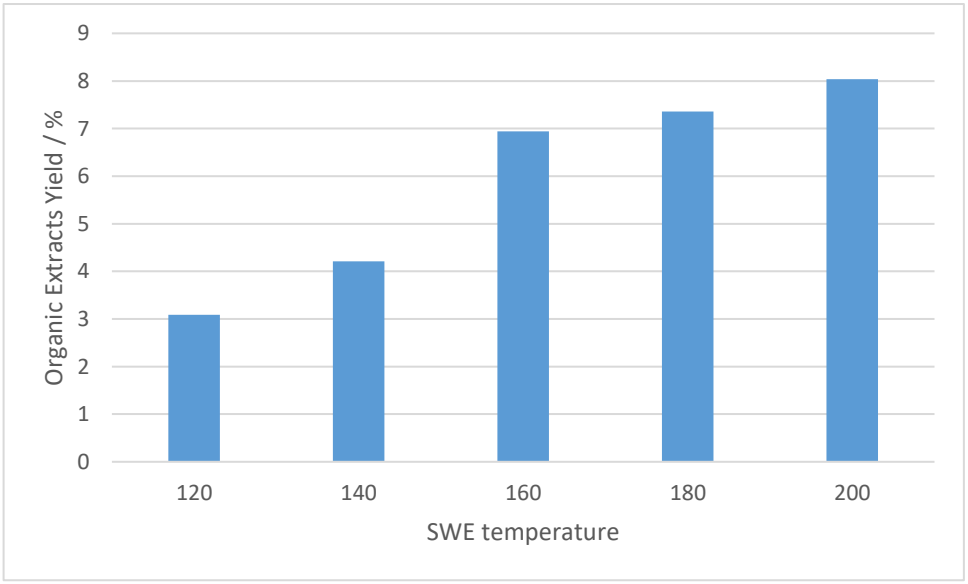


Figure 26 Yield of Ethyl Acetate Extracts in SWE.

The yield of ethyl acetate soluble organic extractives increases as the temperature increases (Figure 26). Maximum yield (8%) was achieved at 200^{o}C compared with a relatively low yield (~3%) at 120^{o}C. There appears to be significant step change in extractive yield from 140 to 160^{o}C, which may be associated with significant hemicellulose decomposition/leaching.

3.4.2 GC-MS analysis of Extractives

The GCMS chromatograms of the extractives as a result of SWE at 140^{o}C and 200^{o}C are shown in Figure 27.

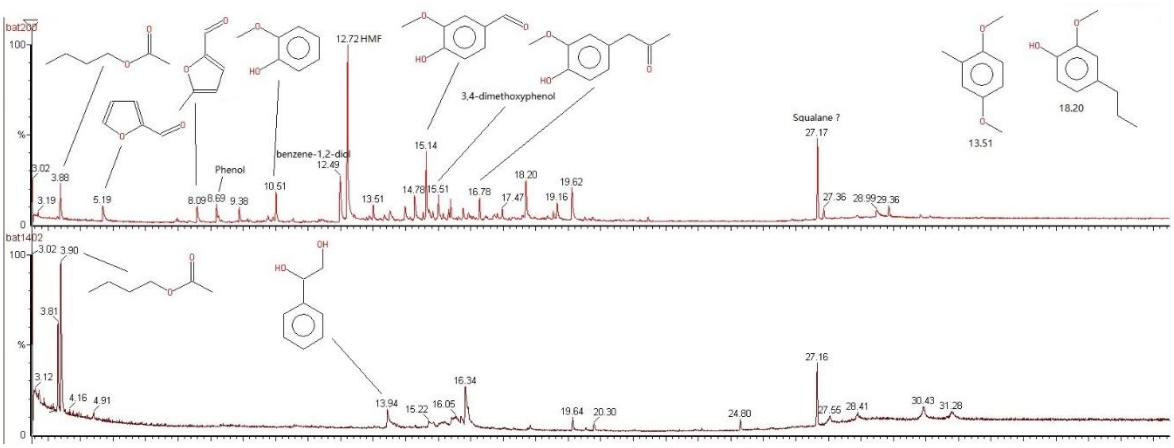


Figure 27 Comparison of GCMS of EA extracts of SWE at 200^{o} C and 140^{o} C.

At a simplistic, visual level, the chromatogram for SWE 200^{o}C shows far more components than the corresponding chromatogram for SWE 140^{o}C. Based on probability matches with respect to the NIST library software the annotations in Figure 27 depict possible compounds. At SWE 120^{o}C, it appears the hot water has limited effect on the structure of spruce bark whilst at SWE 200^{o}C significant compounds are observed commensurate with lignocellulosic hydrolysis.

3.4.3 Proton NMR of organic extractives.

As an aside, the 1 H NMR of the ethyl acetate extract of SWE 200°C was recorded (Figure 28). Although the spectrum is of a mixture of compounds, signals are observed in the aromatic region thus partially correlating with aromatic compounds seen in the GCMS of SWE 200°C. Of particular note, is the evidence of resonances for HMF as highlighted in Figure 28. The NMR's of other samples in contained in the Appendix.

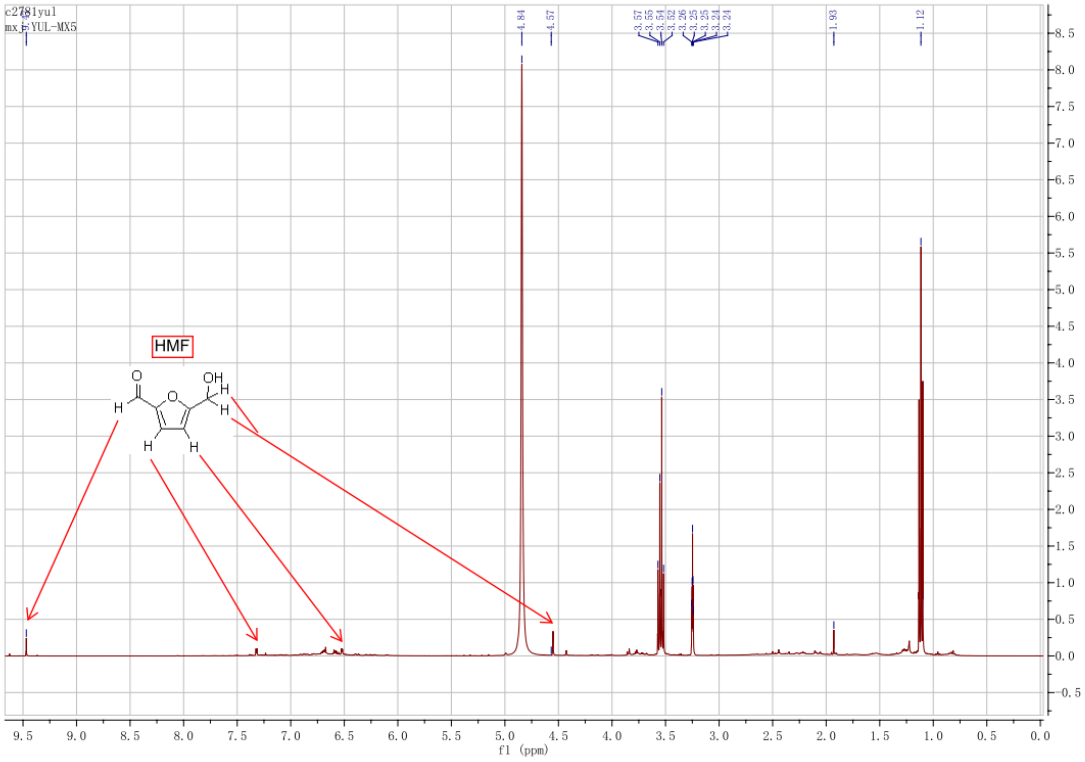


Figure 28 Proton NMR of EA extracts of SWE 200° C.

3.4.4 Analysis of Aqueous Layer

Table 8 HPLC data of SWE aqueous layer product. Unit: mg.

Amount/ mg	SWE 120	SWE 140	SWE 160	SWE 180	SWE 200
Cellubiose	1.70	5.42	0.00	0.00	0.00
Glucose	33.82	53.14	58.15	27.30	5.62
xylose & fructose	10.35	18.27	26.66	13.80	3.25

Rhamnose	75.25	174.10	137.98	0.72	0.00
Levoglucosan	2.84	4.05	8.84	17.52	21.93
Lactic Acid	1.37	0.71	2.79	4.81	9.26
Formic Acid	10.15	15.47	17.10	21.82	24.90
Acetic Acid	2.24	9.13	19.01	23.57	34.86
Levulinic Acid	0.00	7.62	13.93	27.97	37.19
HMF	0.00	0.24	2.24	6.76	6.06
Levoglucosanone	5.39	10.90	16.55	25.24	33.22
Furfural	1.27	2.98	10.90	15.65	12.39

Table 8 shows the amount of sugars, acids and furfurals can be obtained from 5 g spruce bark via batch reactor SWE at different temperatures. The data is shown more intuitively in Figure 29.

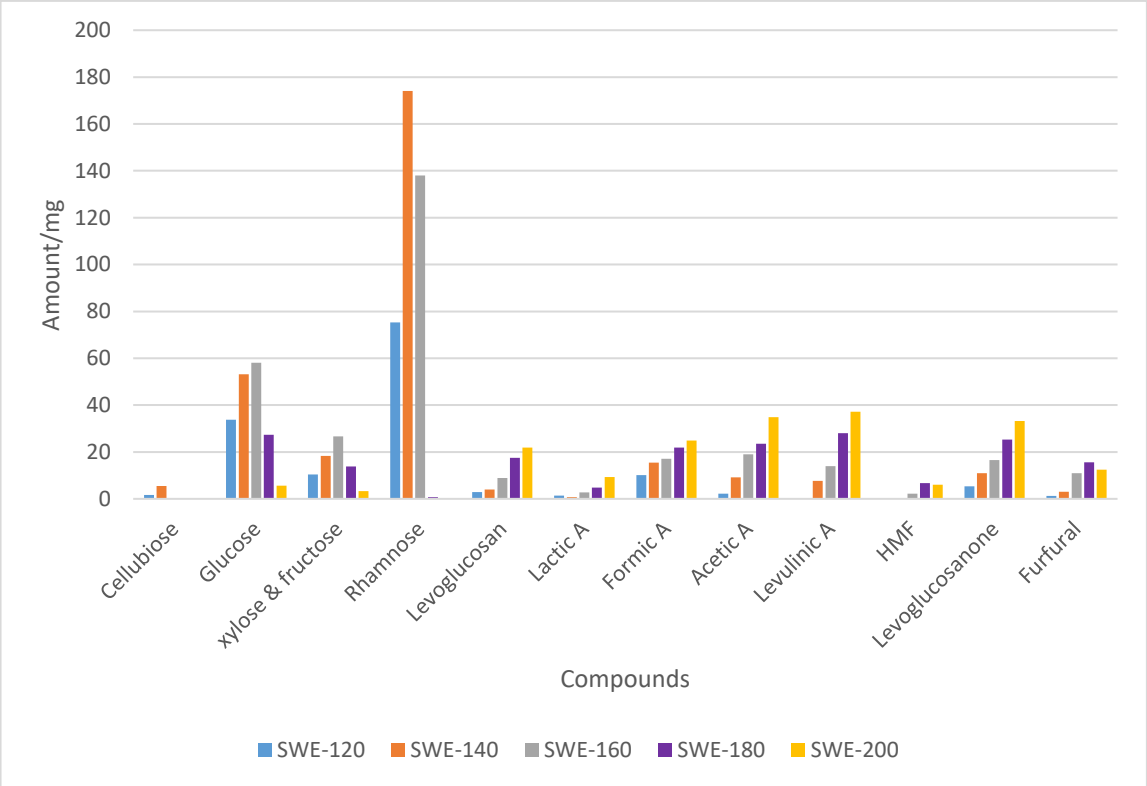


Figure 29 Bar Chart of HLPC data of SWE aqueous layer product.

From Figure 29 we can see that cellubiose can hardly be detected, only small amount in SWE-120 and 140 samples is seen. The content of glucose, xylose and fructose increase

when reaction temperature increases from 120℃ to 160ºC, then starts to fall at 180℃. An interesting result shows that the amount of rhamnose between thetemperature range 140-180℃ is higher than any other product, peaking at 140℃, but no rhamnose was detected in sample SWE120 and 200. The amount of levoglucosan and levoglucosanone increases when reaction temperature increases. In the meantime, the amount of all acids shows the same trends as well. Thus, SWE is playing an important role in hydrolysis of spruce bark and the composition and distribution of components thereof.

3.4.5 Analysis of Spruce Bark Residues after SWE

To look at the effect of SWE on native spruce bark, the cellulose, hemicellulose and lignin content of the post-residues (SBR) was determined (Table 9).

Table 9 Titration data of spruce bark residue (SBR) after SWE.

Samples	Cellulose/%	Hemicellulose/%	Lignin/%	SBR Yield/%
SBR-SWE-120	27.37	11.22	22.63	69.20
SBR-SWE-140	28.26	6.91	28.64	66.06
SBR-SWE-160	27.21	3.35	30.70	61.79
SBR-SWE-180	24.99	2.27	37.34	60.82
SBR-SWE-200	17.96	0.45	42.74	59.31

From the data, we can acknowledge that both cellulose and hemicellulose reduce when temperature increases, but lignin is opposite. Combined with yields of these bark residues and the content of raw material, the conversion rate (CR, see Table 10) of cellulose, hemicellulose and lignin in SWE at different temperatures was calculated.

Table 10 Conversion rate (CR) of 3 component in SWE.

	Conversion Rate / %		
Samples	**Cellulose**	**Hemicellulose**	**Lignin**
SBR-SWE-120	19.35	2.61	35.12
SBR-SWE-140	26.11	46.76	27.15
SBR-SWE-160	37.50	77.31	31.38
SBR-SWE-180	40.07	83.96	12.87
SBR-SWE-200	59.49	97.04	6.23

According to the data shown in Table 10, conversion of cellulose and hemicellulose increase with reaction temperature, about 60% of cellulose and almost all hemicellulose was decomposed at 200°C SWE. But for lignin conversion, the CR reduces at higher temperature.

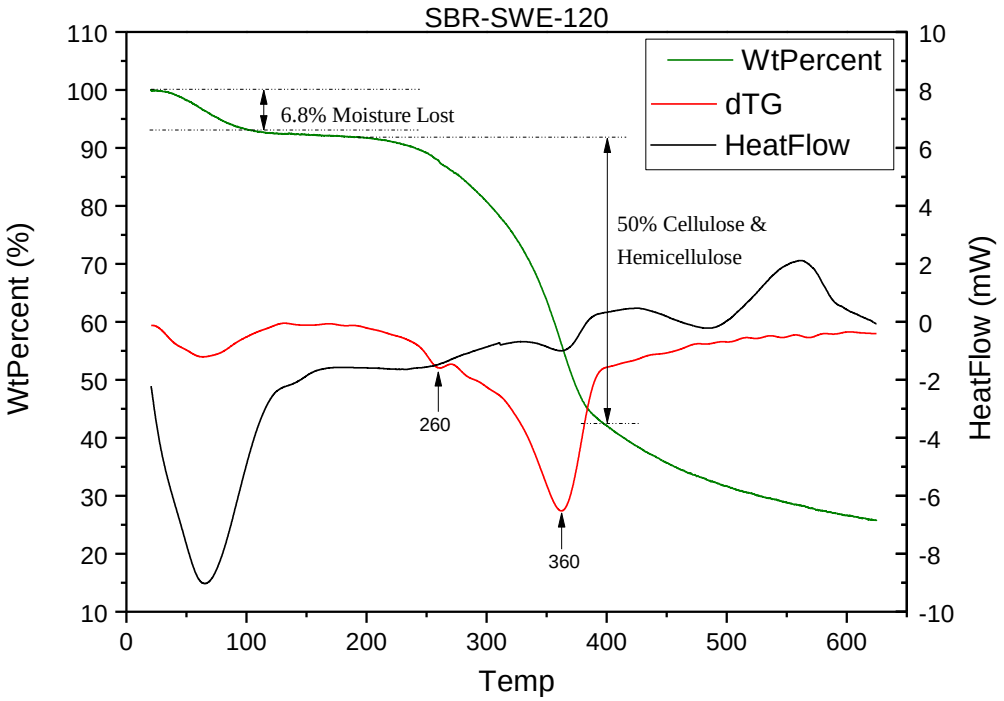


Figure 30 STA-625 data of SBR-SWE-120.

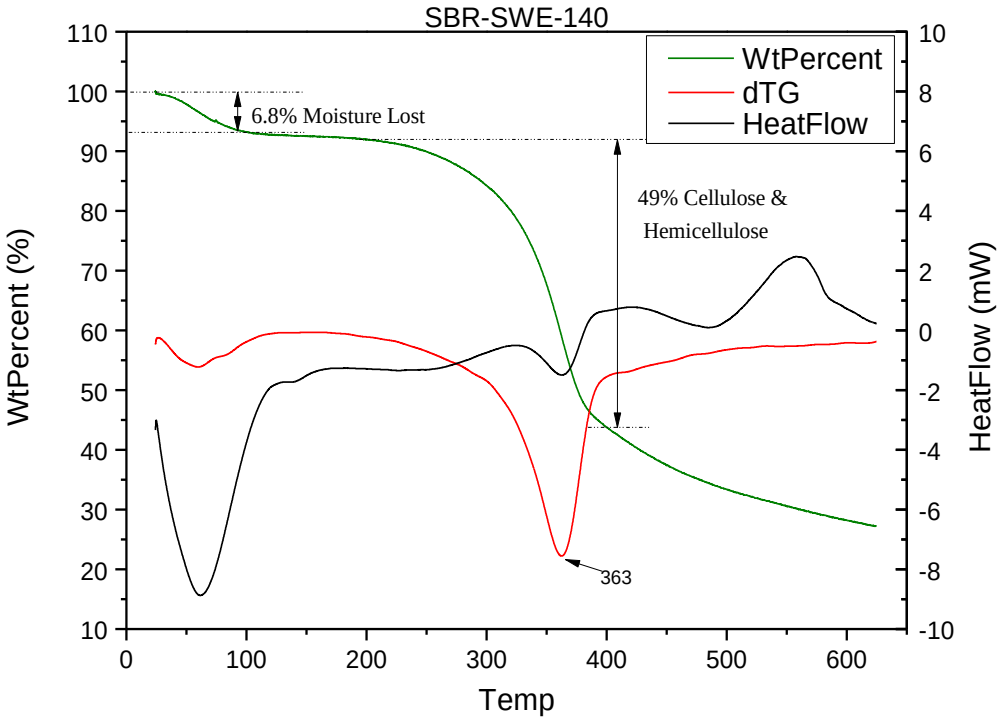


Figure 31 STA-625 data of SBR-SWE-140.

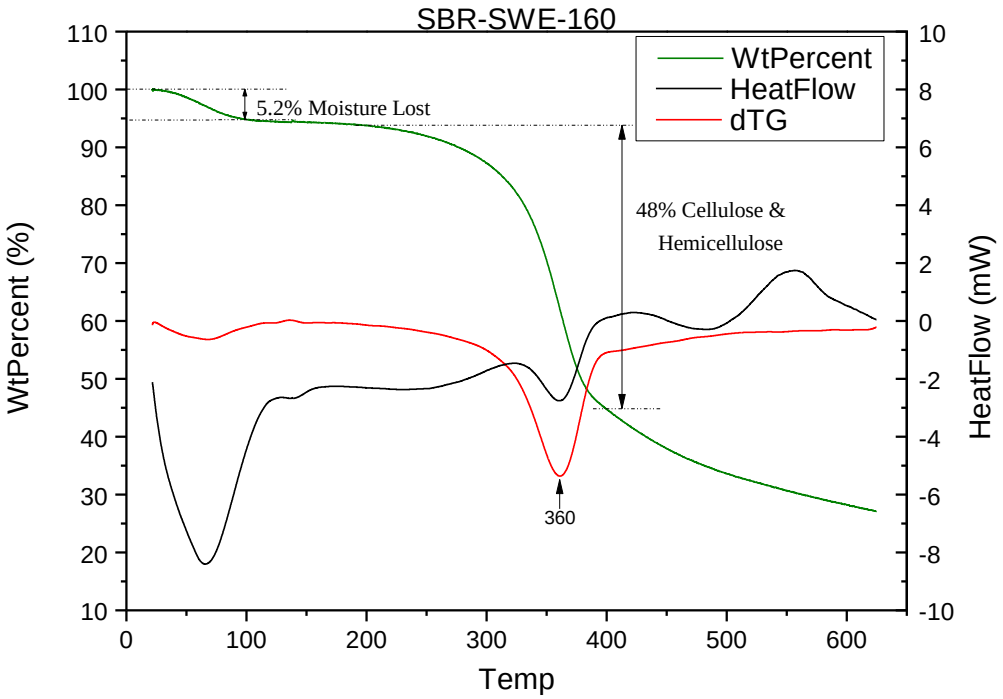


Figure 32 STA-625 data of SBR-SWE-160.

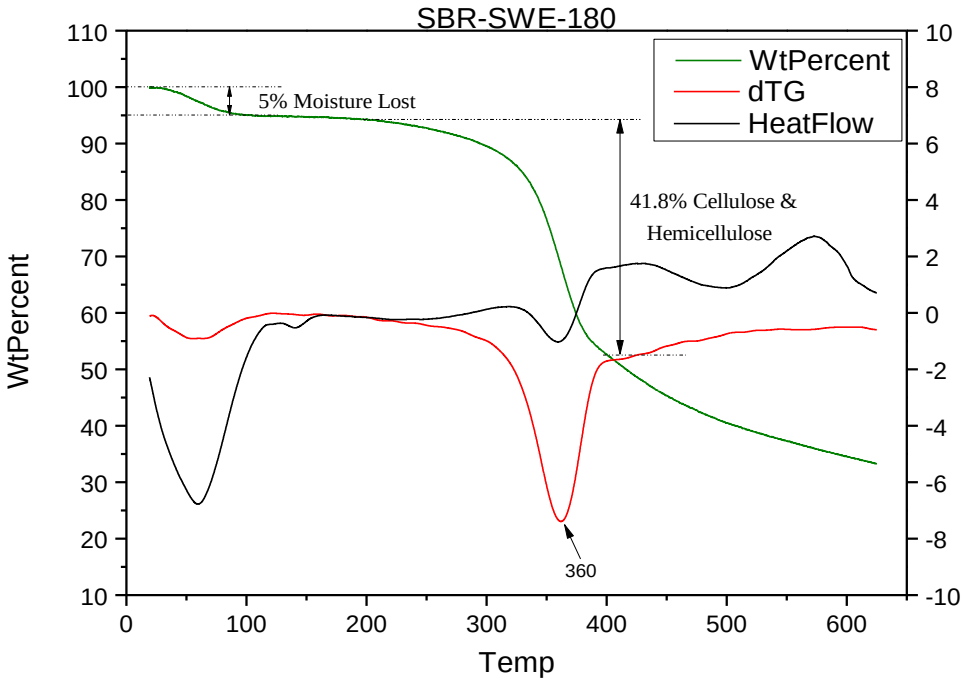


Figure 33 STA-625 data of SBR-SWE-180.

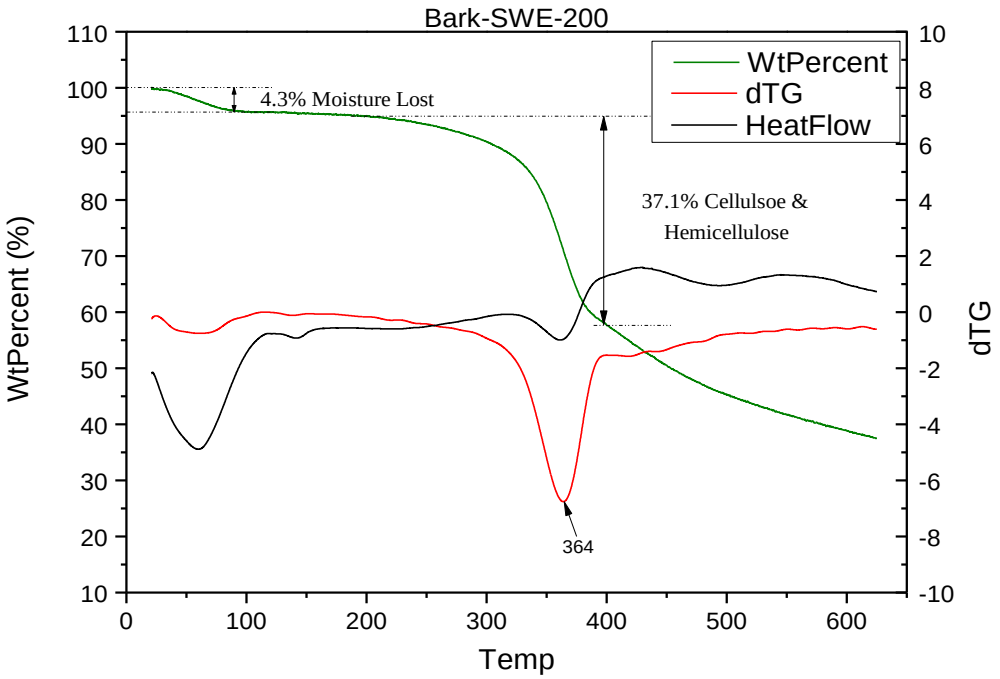


Figure 34 STA-625 data of SBR-SWE-200.

The TG analyses of the SBRs are shown in Figures 30 -34. The cellulose decomposition temperature remains relatively constant at approximately. Most notably, the combined percentage of hemicellulose and cellulose decreases with increasing temperature, *i.e.*, SBR 120, 50%; SBR 140^{o}C, 49%; SBR 160, 48%; SBR 180, 42%, and; SBR 200, 37%. The main change across this temperature range is initially dissolution of hemicellulose below 180^{o}C, in fact almost complete removal, and thereafter above 180^{o}C, onset of significant cellulose dissolution.

4. Conclusions and Future Work

Spruce bark is a large value waste in industry, often burnt for energy directly every year. However, spruce bark is source of chemicals and materials beyond just bioenergy.

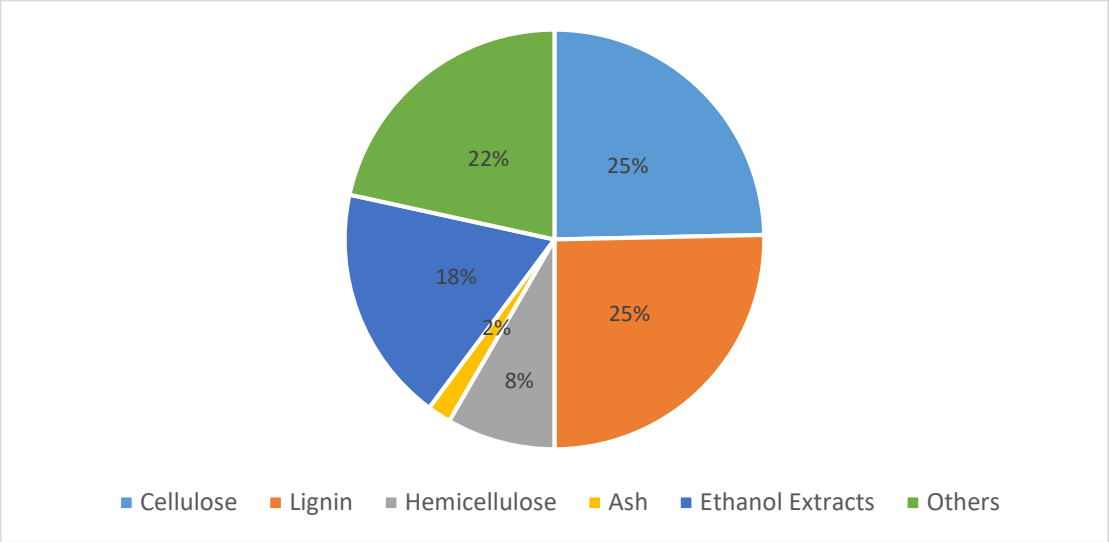


Figure 35 Composition of Spruce Bark.

In summary this research shows that spruce bark contains 25% cellulose, 8% hemicellulose, 25% lignin, 2% ash and 18% ethanol extractives (Figure 35). Spruce bark is rich in condensed tannins which is 39 mg GAE, the tannins are used to protect spruce tree from beetles, and also can be used as antioxidant and environmentally friendly adhesives and resins. Condensed tannins are obtained easily by simple ethanol extraction and the ethanol can be reused after distillation.

Spruce bark is a potential source of sugars via SWE hydrolysis and bio-oil and bio-char via microwave pyrolysis. Depending on processing conditions, phenolic compounds, fermentable sugars and energy-densified biochars are obtained.

To conclude, this research is a starting point towards a potential biorefinery from spruce bark as outlined in Figure 36. At present biorefineries are still in their infancy and thus economically uncompetitive with respect to petroleum refineries. However, the latter has benefitted with over a 100 years research, development, technology and markets to such an extent that they are very efficient processes but dirty and polluting. Research in to development of biorefineries should continue for the betterment of society and for a sustainable future.

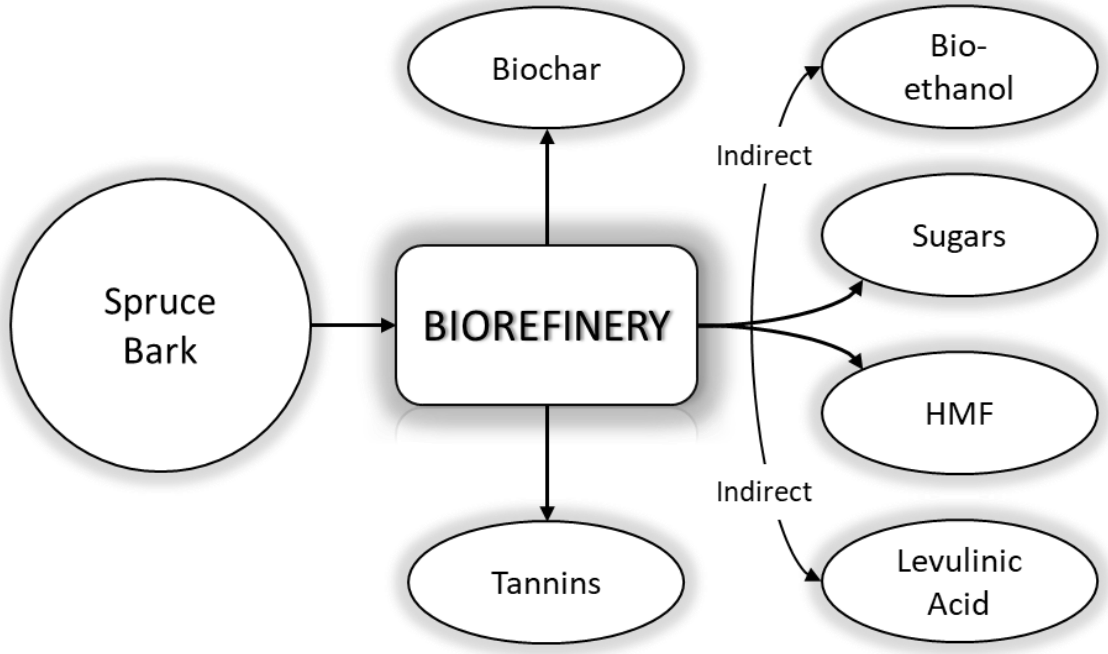


Figure 36 Towards a potential spruce bark biorefinery.

Future work:

Future work should explore the use of activated carbon with spruce bark to see microwave pyrolysis could be enhanced giving either higher bio-oil or bio-char yield but at lower temperatures. Activated carbon is a very efficient adsorber on microwave radiation

Several experiments of microwave pyrolysis in presence of activated carbon have been performed but insufficient, consistent data was produced to warrant reporting in this thesis. Nevertheless, some acceleration in oil yield was observed at low processing temperatures and future work should explore controllability of pyrolysis to give constant product yield and composition.

5. References

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Appendix

Appendix 1 Py-GCMS data of Spruce Bark

No.	RT	Name	Formula	Mass (DB)	Area %	Probability
2	2.196	2-Butanone, 3-methyl-	$C_5H_{10}O$	86.1	2.01 %	80.12
3	2.276	4-Cyclopentene-1,3-diol, trans-	$C_5H_8O_2$	100.1	2.17 %	76.07
4	2.71	Acetic acid	$C_2H_4O_2$	60	6.77 %	87.16
5	2.847	2-Propanone, 1-hydroxy-	$C_3H_6O_2$	74	2.50 %	75.45
6	2.996	5-Hexen-2-one	$C_6H_{10}O$	98.1	0.49 %	78.06
7	3.213	Furan, 2,5-dimethyl-	C_6H_8O	96.1	1.00 %	70.54
8	3.647	4-Cyclopentene-1,3-diol, trans-	$C_5H_8O_2$	100.1	0.65 %	75.08
9	3.842	(1-Allylcyclopropyl)methanol	$C_7H_{12}O$	112.1	0.56 %	82.19
10	4.024	1,3,5-Cycloheptatriene	C_7H_8	92.1	0.49 %	87.51
11	4.104	Acetic acid, (acetyloxy)-	$C_4H_6O_4$	118	1.32 %	70.65
12	4.31	Pentanal, 2,4-dimethyl-	$C_7H_{14}O$	114.1	0.62 %	88.08
13	4.402	Carbonic acid, allyl hexyl ester	C10H18O3	186.1	0.47 %	75.77
14	4.516	Propanoic acid, 2-oxo-, methyl ester	C4H6O3	102	1.27 %	75.75
15	4.596	Trimethylsilyl cyanide	C4H9NSi	99	0.99 %	78.37
16	5.179	Furfural	C5H4O2	96	4.08 %	85.37
17	5.567	2-Hexanone, 6-hydroxy-	C6H12O2	116.1	1.14 %	80.28

18	5.762	2-Hexanone, 6-hydroxy-	C6H12O2	116.1	1.30%	70.91
19	6.379	Cyclopropanecarboxylic acid, cyclohexylmethyl ester	C11H18O2	182.1	0.49%	80.63
20	6.665	2H-Pyran, 3,4-dihydro-	C5H8O	84.1	0.97%	75.6
21	6.893	1,2-Cyclopentanedione	C5H6O2	98	2.53%	88.41
22	7.088	3-Nonynoic acid	C9H14O2	154.1	0.41%	75.19
23	7.396	Spiro[2.4]heptan-4-one	C7H10O	110.1	1.72%	76.97
24	7.682	2,4-Diaminopyrimidine	C4H6N4	110.1	0.53%	74.61
25	7.773	Phenol	C6H6O	94	1.05%	88.82
26	8.116	Oxazolidine, 2,2-diethyl-3-methyl-	C8H17NO	143.1	1.53%	74.99
27	8.482	Aziridine, 2-(1,1-dimethylethyl)-1,3-dimethyl-	C8H17N	127.1	1.14%	77.15
28	8.688	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	C6H8O2	112.1	1.81%	89.63
29	8.814	1-Amino-2,6-dimethylpiperidine	C7H16N2	128.1	0.49%	80.15
30	9.351	Phenol, 3-methyl-	C7H8O	108.1	1.76%	77.36
31	9.534	Ethanone, 1-(1-cyclohexen-1-yl)-	C8H12O	124.1	4.86%	82.82
32	10.059	Maltol	C6H6O3	126	0.70%	84.5
33	10.642	2-Dodecenoic acid	C12H22O2	198.2	0.59%	74.22
34	10.905	4-Hydroxy-non-2-ynoic acid, ethyl ester	C11H18O3	198.1	0.43%	83.73
35	11.134	Creosol	C8H10O2	138.1	2.61%	93.78
36	11.374	Resorcinol	C6H6O2	110	2.31%	80.33
37	11.705	trans-2-Decenoic acid	C10H18O2	170.1	0.53%	79.43
38	12.357	Phenol, 4-ethyl-2-methoxy-	C9H12O2	152.1	0.74%	92.55

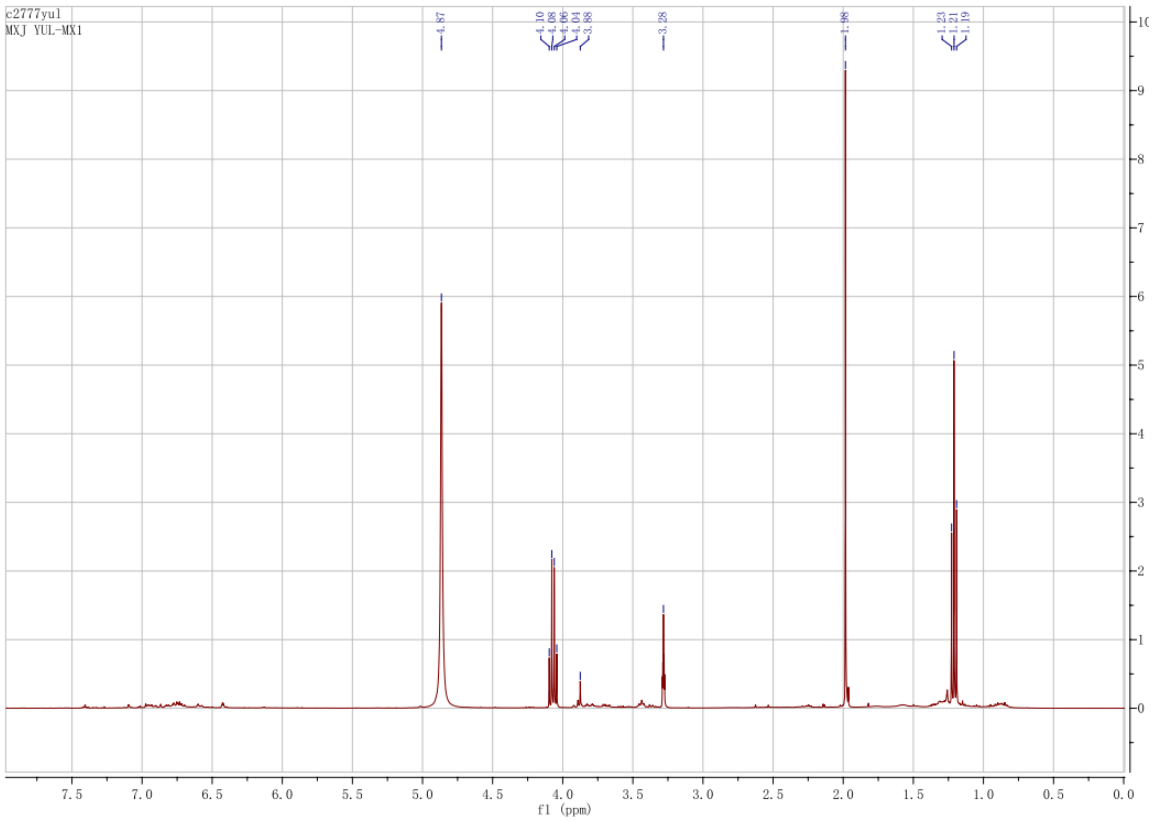
39	12.665	1,2-Benzenediol, 3-methyl-	C7H8O2	124.1	2.73%	86.49
40	12.871	2-Methoxy-4-vinylphenol	C9H10O2	150.1	2.13%	88.13
41	13.431	2,5-Octadecadiynoic acid, methyl ester	C19H30O2	290.2	0.58%	82.23
42	13.888	Cyclohexene, 1,5,5-trimethyl-6-acetylmethyl-	C12H20O	180.2	0.54%	80.05
43	14.437	2-Ethylcyclohexylamine, N-(2-chloropropylidene)-, N-oxide	C11H20ClNO	217.1	0.44%	77.77
44	14.654	Phenol, 2-methoxy-5-(1-propenyl)-, (E)-	C10H12O2	164.1	1.23%	91.8
45	16.22	Melezitose	C18H32O16	504.2	0.57%	80.36
46	16.357	Melezitose	C18H32O16	504.2	0.69%	78.35
47	16.677	Melezitose	C18H32O16	504.2	0.52%	80
48	16.86	Melezitose	C18H32O16	504.2	1.83%	81.91
49	17.146	d-Mannose	C6H12O6	180.1	3.75%	75.87
50	17.374	Melezitose	C18H32O16	504.2	3.17%	79.92
51	18.072	Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	C11H18N2O2	210.1	0.41%	83.34
52	20.277	Estra-1,3,5(10)-trien-17.beta.-ol	C18H24O	256.2	1.17%	87.3
53	20.929	1-Heptatriacotanol	C37H76O	536.6	1.06%	85.55
54	21.832	1-Heptatriacotanol	C37H76O	536.6	1.20%	83.96
55	21.946	Oleic Acid	C18H34O2	282.3	2.00%	89.88
56	22.129	1-Heptatriacotanol	C37H76O	536.6	0.90%	83.61
57	23.741	1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxy-1,4-bis(hydroxymethyl)-1,7,9-trimethyl-, [1S-(1.alpha.,1a.alpha.,2.alpha.,5.beta.,5a.beta.,6.beta.,8a.alpha.,9.alpha.,10a.alpha.)]-	C20H28O6	364.2	0.41%	87.58

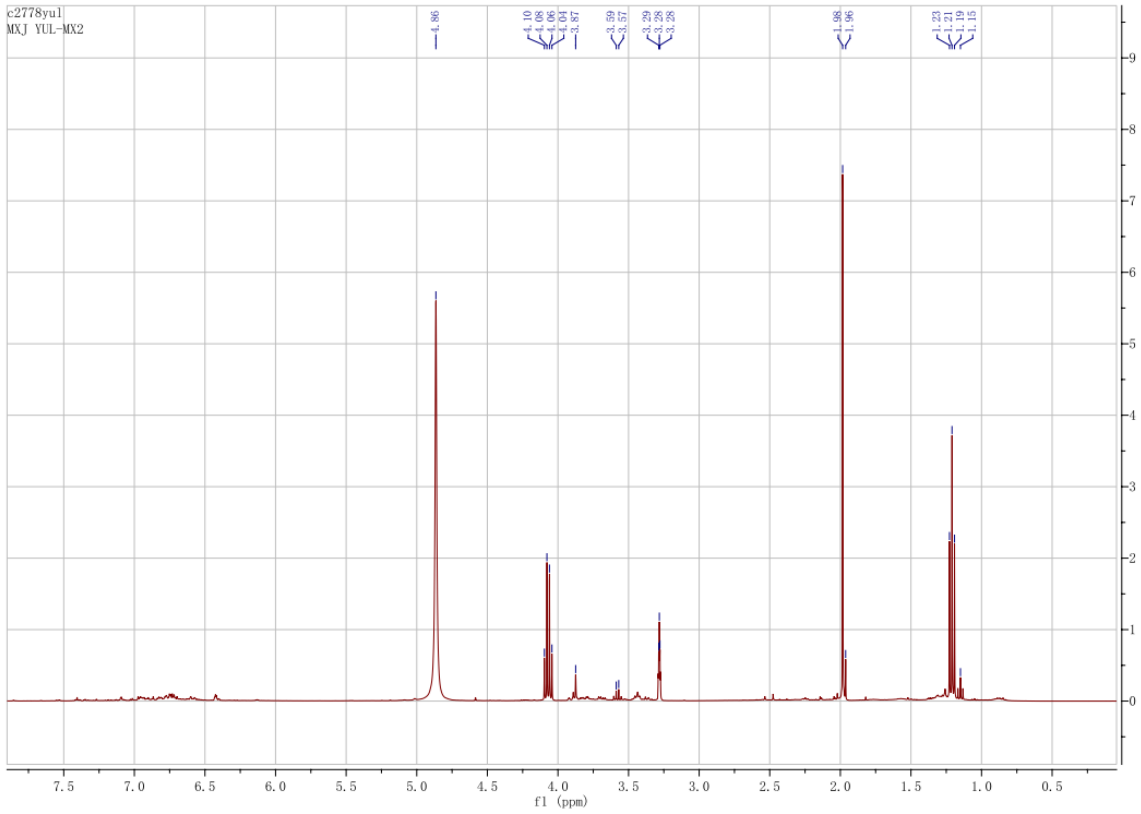
58	23.809	Butanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-11-oxo-1H-2,8a-methanocyclopenta[a]cyclopropa[e]cyclodecen-6-yl ester, [1aR-(1a.alpha.,2.alpha.,5.beta.,5a.beta.,6.beta.,8a.alpha.,9.alpha.,10a.alpha.)]-	C24H34O6	418.2	0.80 %	85.7
59	24.049	1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxy-1,4-bis(hydroxymethyl)-1,7,9-trimethyl-, [1S-(1.alpha.,1a.alpha.,2.alpha.,5.beta.,5a.beta.,6.beta.,8a.alpha.,9.alpha.,10a.alpha.)]-	C20H28O6	364.2	0.43 %	89.99
60	24.358	1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxy-1,4-bis(hydroxymethyl)-1,7,9-trimethyl-, [1S-(1.alpha.,1a.alpha.,2.alpha.,5.beta.,5a.beta.,6.beta.,8a.alpha.,9.alpha.,10a.alpha.)]-	C20H28O6	364.2	1.11 %	90.99
61	24.495	1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxy-1,4-bis(hydroxymethyl)-1,7,9-trimethyl-, [1S-(1.alpha.,1a.alpha.,2.alpha.,5.beta.,5a.beta.,6.beta.,8a.alpha.,9.alpha.,10a.alpha.)]-	C20H28O6	364.2	0.52 %	88.15
62	24.598	1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxy-1,4-bis(hydroxymethyl)-1,7,9-trimethyl-, [1S-(1.alpha.,1a.alpha.,2.alpha.,5.beta.,5a.beta.,6.beta.,8a.alpha.,9.alpha.,10a.alpha.)]-	C20H28O6	364.2	1.09 %	86.12
63	24.712	Pimaric acid	C20H30O2	302.2	1.73 %	84.1
64	24.849	1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxy-1,4-bis(hydroxymethyl)-1,7,9-trimethyl-, [1S-(1.alpha.,1a.alpha.,2.alpha.,5.beta.,5a.beta.,6.beta.,8a.alpha.,9.alpha.,10a.alpha.)]-	C20H28O6	364.2	1.28 %	85.68
65	25.124	1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxy-1,4-bis(hydroxymethyl)-1,7,9-trimethyl-, [1S-(1.alpha.,1a.alpha.,2.alpha.,5.beta.,5a.beta.,6.beta.,8a.alpha.,9.alpha.,10a.alpha.)]-	C20H28O6	364.2	1.00 %	85.37
66	25.341	Pregn-4-ene-3,20-dione, 16,17-epoxy-, (16.alpha.)-	C21H28O3	328.2	1.92 %	84.5
67	25.627	Hydrocortisone Acetate	C23H32O6	404.2	0.77 %	84.84

68	25.729	Butanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5a-hydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-6,11-dioxo-1H-2,8a-methanocyclopenta[a]cyclopropa[e]cyclodecen-5-yl ester, [1aR-(1a.alpha.,2.alpha.,5.beta.,5a.beta.,8a.alpha.,9.alpha.,10a.alpha.)]-	C24H32O6	416.2	0.76%	86.59
69	25.947	Butanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5a-hydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-6,11-dioxo-1H-2,8a-methanocyclopenta[a]cyclopropa[e]cyclodecen-5-yl ester, [1aR-(1a.alpha.,2.alpha.,5.beta.,5a.beta.,8a.alpha.,9.alpha.,10a.alpha.)]-	C24H32O6	416.2	0.50%	86.63
70	26.232	Gibberellic acid	C19H22O6	346.1	1.21%	75.7
71	26.381	Butanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5a-hydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-6,11-dioxo-1H-2,8a-methanocyclopenta[a]cyclopropa[e]cyclodecen-5-yl ester, [1aR-(1a.alpha.,2.alpha.,5.beta.,5a.beta.,8a.alpha.,9.alpha.,10a.alpha.)]-	C24H32O6	416.2	0.87%	86.31
72	28.107	1-Phenanthrenecarboxylic acid, tetradecahydro-7-(2-methoxy-2-oxoethylidene)-1,4a,8-trimethyl-9-oxo-, methyl ester, [1S-(1.alpha.,4a.alpha.,4b.beta.,8.beta.,8a.alpha.,10a.beta.)]-	C22H32O5	376.2	0.77%	77.03
73	29.227	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl-, 5,9,9a-triacetate, [1aR-(1a.alpha.,1b.beta.,4a.beta.,5.beta.,7a.alpha.,7b.alpha.,8.alpha.,9.beta.,9a.alpha.)]-	C26H36O8	476.2	0.45%	81.2
74	29.341	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl-, 5,9,9a-triacetate, [1aR-(1a.alpha.,1b.beta.,4a.beta.,5.beta.,7a.alpha.,7b.alpha.,8.alpha.,9.beta.,9a.alpha.)]-	C26H36O8	476.2	0.51%	78.75
75	29.536	.beta.-Sitosterol acetate	C31H52O2	456.4	1.61%	81.6
76	31.753	.gamma.-Sitosterol	C29H50O	414.4	1.36%	84.62
77	32.953	Stigmasta-3,5-dien-7-one	C29H46O	410.4	0.51%	67.39
78	33.513	5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 9,9a-bis(acetyloxy)-1,1a,1b,2,4a,7a,7b,8,9,9a-decahydro-2,4a,7b-trihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-, [1aR-	C24H32O9	464.2	0.43%	71.55

		(1a.alpha.,1b.beta.,2.beta.,4a.beta.,7a.alpha.,7b.alpha.,8.alpha.,9.beta.,9a.alpha.)]-				

Appendix 2 Proton NMR spectrum of organic extractives in SWE-120,140,160 and 180 samples (From top to bottom).

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4.08
4.06
4.04
3.87
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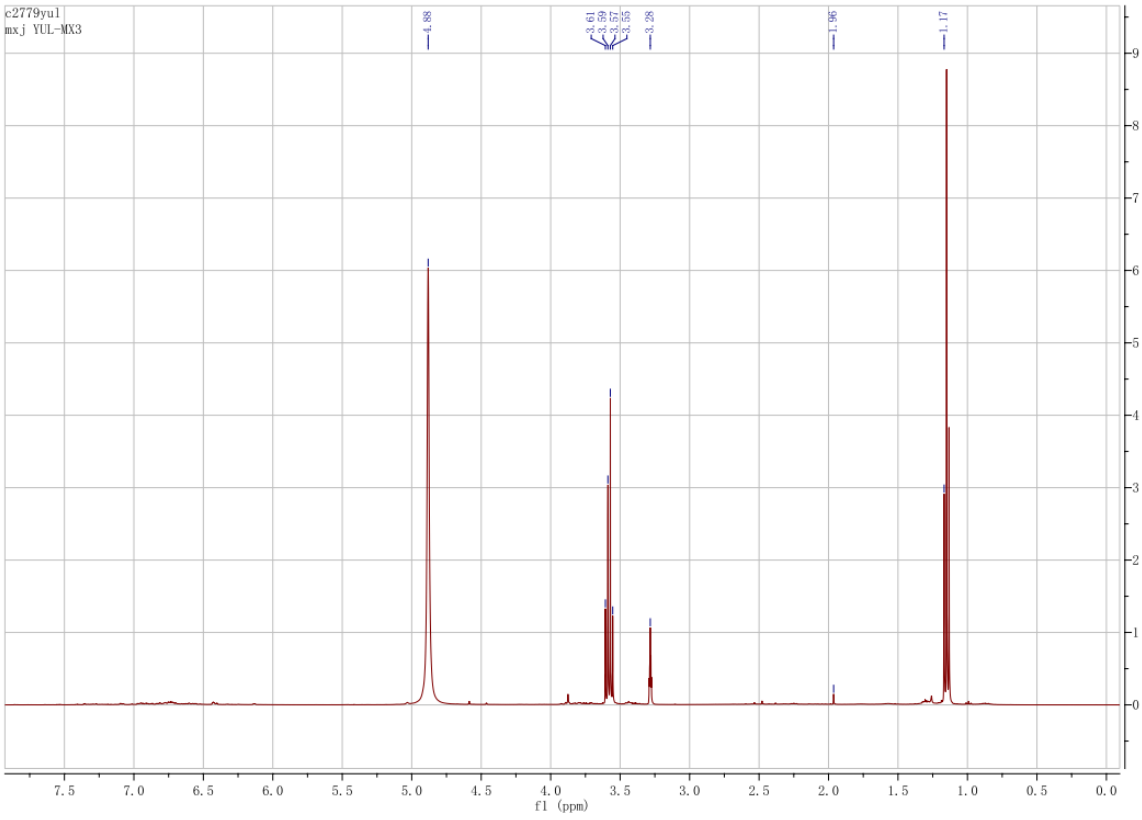
3.28
3.26
1.98
1.96
1.23
1.21
1.19
1.15
f1 (ppm)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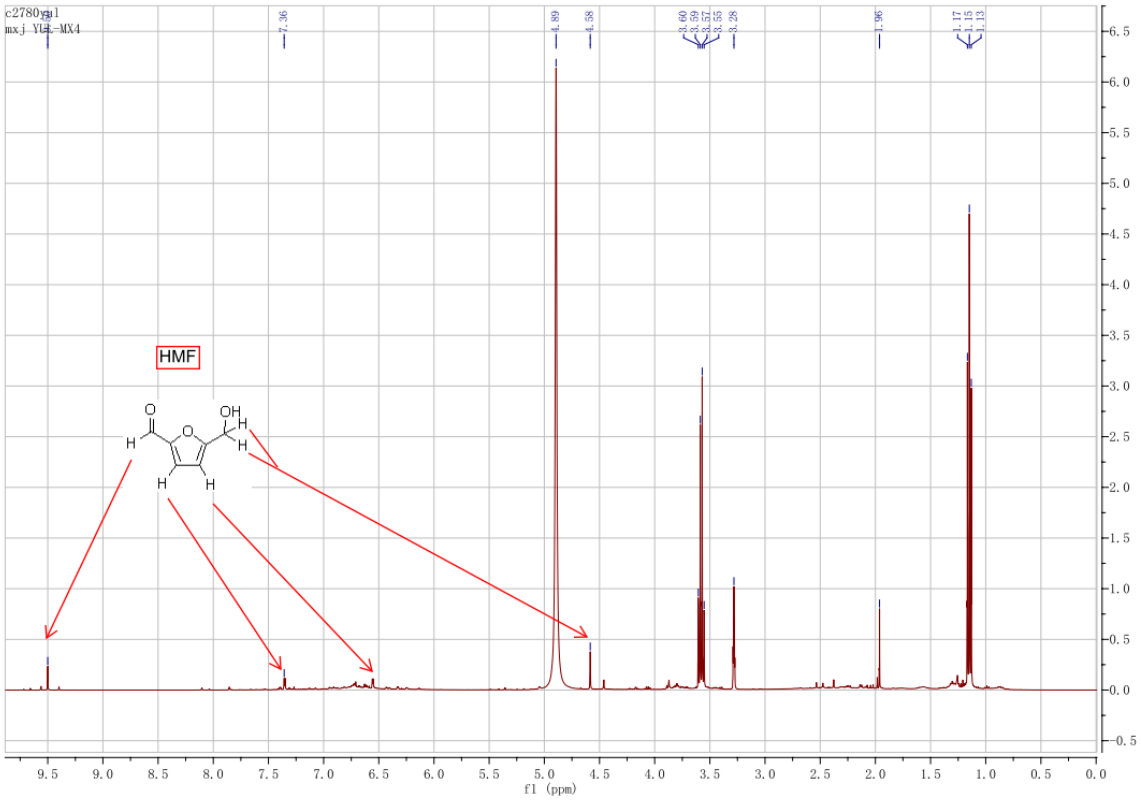
1.17
f1 (ppm)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3.57
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1.96
1.17
1.15
1.13
f1 (ppm)