A Barley Straw based Bio-refinery

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Abstract

The individual components of a biorefinery concept based on a ligno-cellulose biomass such as straw have been studied but a complete technical, economic and environmental analysis of such an integrated process is lacking. A detailed analysis on barley wax was performed as a part of the experiment. In addition to further research in wax, the aim of this project is to study the difference, also improvement made, when utilising wax extracted and un-extracted straw on thermal pyrolysis products. The composition of bio-oil char and biogas as pyrolysis products were also studied and the reproducibility of bio-oil using different microwave machines were investigated. The effect on product yield and composition when changing pyrolysis reaction temperature was also tested. The green chemistry metrics was applied to the whole process to measure the environmental impact of the experiment.

Wax was extracted from various kinds of barley straws by different methods and its chemical compositions were analysed using GC-MS and GC. The result indicated that components in the extracted wax can be grouped into several categories: fatty acids was the most dominating group in wax (32.5%), followed by fatty alcohols (14.6%), 14,16-hentriacontanedione (11.3%) and β-diketones (8.6%). When comparing to previous supercritical carbon dioxide (SC-CO_2) extraction results of other straws, barley wax has the highest yield. The yield from different variety barley straw also varies; Carat barley has a slightly higher yield than Saffron barley.

Previous studies show that wheat straw wax comprises a similar blend of compounds found in ladybird footprints (trace of material found on the path of the insect), which is the aphid's natural enemy and could cause the aphid avoidance.[1] In order to test whether other straw wax contains the similar semiochemicals and causes the same effect, previous column chromatography extracted wheat straw, barley straw, and apple peel wax was characterised by GC-MS and GC; and the research was particularly focused in branched and long chain alkane regions. The analysis showed wax extracts all contain similar long-chain and branched-chain components, which may also induce aphid avoidance.

Pyrolysis was applied on both raw and de-waxed straw to produce bio-oil, char and bio-gas. The difference in bio-oil produced using raw and de-waxed straw was mainly caused by wax. Through analysis, bio-oil was identified mainly containing aromatic compounds, where the high content compound in it was (acetyloxy)-acetic acid (7.0%), 3-methyl-1,2-cyclopentanedione(5.2%) and 2,6-dimethoxyphenol(4.6%). Bio-oil's reproducibility was tested on an additional CEM microwave machine. When increasing the pyrolysis temperature, bio-oil yield was slightly increased while char yield

was dramatically decreased. However the temperature impact on char was disappeared when reaction reached to 140°C and above.

Char and bio-gas were analysed by FTIR. From the spectra, the decrease intensity of char compared to straw was obvious and caused by decomposing of the chemical groups (e.g. phenolic groups, C-O group) during pyrolysis. The high concentrated peak in bio-gas spectrum was caused by the CO_2; other peaks were due to the exultance of CO, CO_2, CH_4, C_2H_4 and C_2H_6.

The environmental impact of both wax extraction and pyrolysis were investigated by performing calculation of their E-factors. The results showed that although the processes were generally environmental benign, there were great potential for improvements, such as employ solvent recycling and broaden products applications.

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Declaration

I declare that the thesis hereby submitted for the MSc by Research degree at the University of York is my own work and has not been previously submitted by me at another University for any degree.

Dan Han

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1 Introduction

Straw is an agricultural by-product; it is the dry stalks of cereal plants after the grain and chaff have been removed. Straw makes up about half of the yield of cereal crops such as barley, oats, rice, rye and wheat. However in contrast to mass production each year, there has been only a little commercial use. With the help of modern technology, straw can be identified as a source of potential high-value chemicals, and a sustainable energy resource.

Straw surface is covered with a layer of plant wax which could be used in wide applications such as cosmetics and surface coating. Other sources of natural wax are from plants, animals, minerals and fossil fuel. The problems associated with such sources have been lack of availability due to regional limitation, unfriendly processes due to usage of hazardous chemicals, and unsustainable due to harvesting from non-renewable sources.

In addition to traditional application of straw wax, literatures indicated certain chemicals in wheat straw wax could be used as semiochemical to cause aphid avoidance. When the wax was applied as a spray formulation to wheat plants, the time spent by parasitoids on the treated plant was significantly reduced compared to control plants. This indicates another potential of being useful for agriculture pest control.

Nowadays, the excessive use of fossil fuels speeds up the depletion of such natural resource dramatically, attempts to move to sustainable consumption has been a major focus. Deeper down the surface of straw, the major constituents of straw consist of cellulose, hemicelluloses and lignin, which are potentially valuable fuels.

Barley straw is focused in this project among the straw varieties. As the largest barley producer, European Union produces a large amount of straw every year and most of this straw is treated as waste. Therefore, in order to add value to wasted barely straw, suitable extraction and decomposition techniques with the proper process conditions are required, and a detailed analysis on products obtained through processes need to be performed.

Various methods were employed in prior works for straw wax extraction and biomass decomposition. SC-CO_2 and microwave technology are selected for the isolation of wax and pyrolysis products from barely straw due to their environmental friendly nature. The feasibility of integration of the two techniques is considered undoubted, however a thorough research is necessary in order to broaden the understanding of wax SC-CO_2 extraction and microwave pyrolysis and allow further commercial applications.

The essential aim of this project is to deliver a straw-utilising model system which captures and quantifies added-value products, identifies the downstream processes impact of biomass extraction and how by-products can be recycled into the bio-refinery.

In this project, barley (Saffron) straw was used as the primary resource for SC-CO_2 extraction and pyrolysis. A complete technical analysis of combined SC-CO_2 extraction and bio-refinery process was performed and products are analysed by GC-MS, GC or FTIR. Wax yield was compared to different variety barley straw and other straws. Impact on products from microwave pyrolysis by using raw straw and de-waxed straw as starting materials was investigated. Effects on pyrolysis yield and product compositions when changing reaction temperature were also studied.

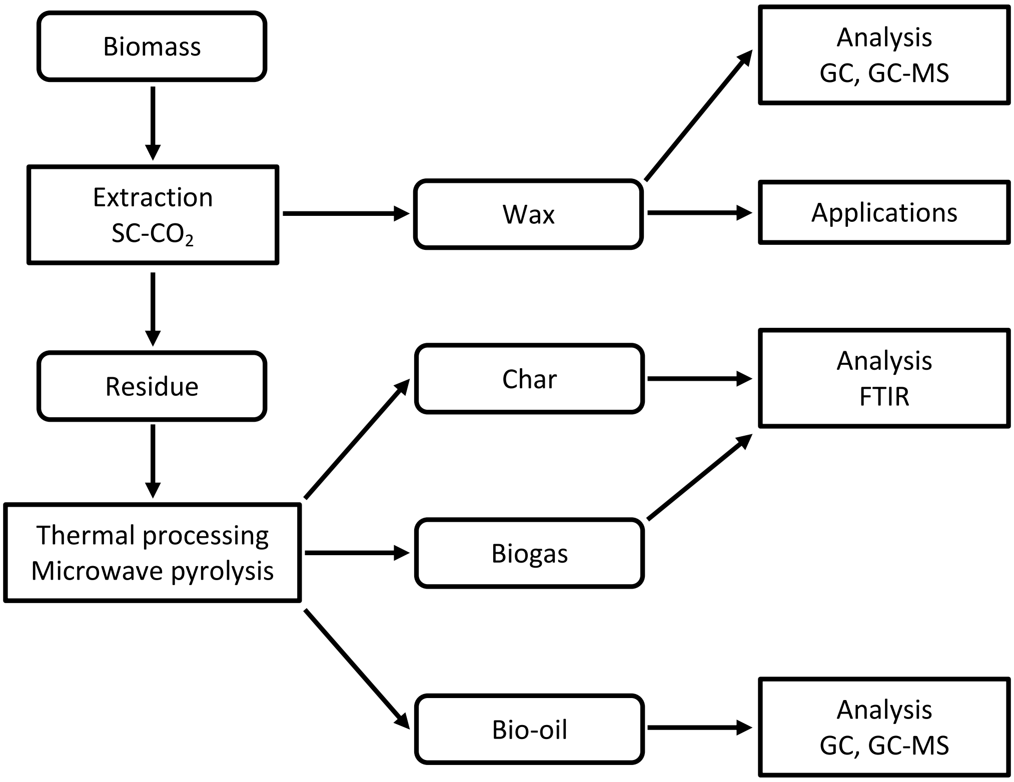


Figure 1 Integrated biorefinery process steps

1.1 Barley straw

1.1.1 Production and distribution

Barley is grown in many regions of the world for cultural as well as economic reasons, it is one of the most highly adapted of the cereal grains, with production in climates ranging from sub-Arctic to subtropical.[2] Barley is popularly grown as a summer crop in temperate areas, and is sown as a winter crop for tropical areas. Barley is the fourth most quantity produced cereal grain in the world that has an almost stable worldwide output. In 2009/10, the total production was 149 million tonnes[3], while the average production from 2000-2008 had been 136 million tonnes. The map below shows the distribution of production by country in 2005.

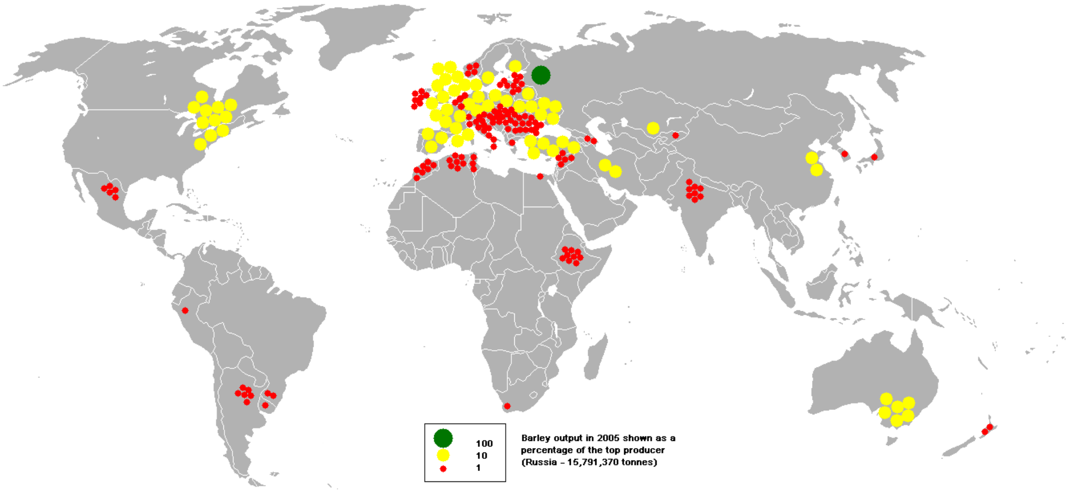


Figure 2 Barley output in 2005[4]

From the above graph, that European Union-27 is the largest barley producer. The recent data shows EU-27 has a barley production of 56 million tonnes, out of the worldwide production of 135 million tonnes in year 2010/2011, according to the data in the global trade website Alibaba[5]. As one of the main producer, UK's barley production counts for almost 10% within EU and 4% worldwide based on Food and Agriculture Organization of the United Nations.[4] The following graph and figure represent the UK domestic barley distribution and the production from 2000 to 2008.

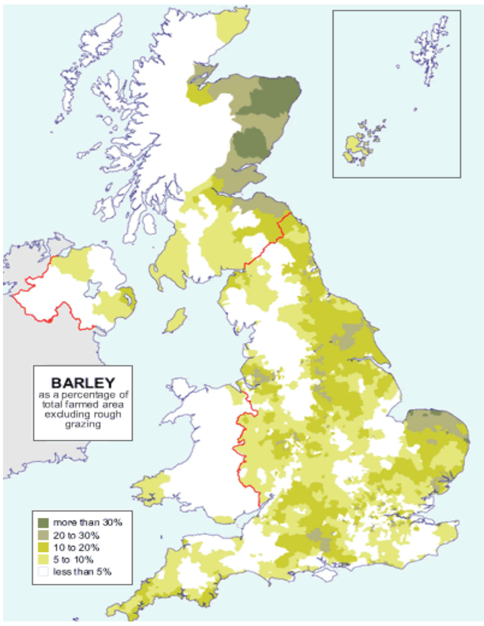


Figure 3 UK barley growing areas[6]

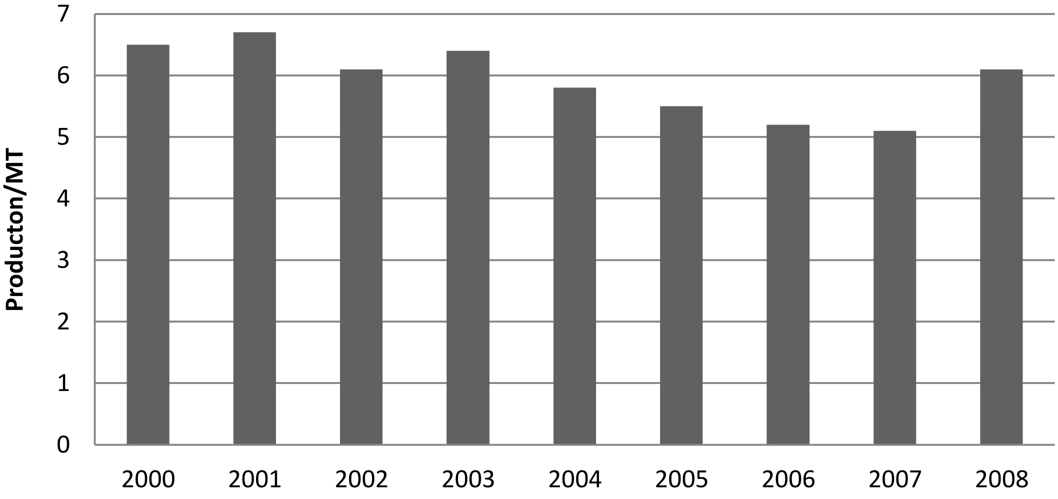


Figure 4 UK barley production from 2000--2008[4]

1.1.2 Variety of barley plant

The anatomy of barley shows numerous similarities to other grasses. Fertility differences in spikelet introduce two-rowed six-rowed barley classification. For each type there are three florets at every node, and in two-rowed barley only the central floret is fertile, whereas in six-rowed all florets are fertile.[7] Although this causes each head of the six-rowed barley to produce more seeds, the two-rowed varieties tend to have more tillers per plant and therefore yield similarly.

The majority barley used in this experiment is the 2-row variety Saffron barley. It has the following advantages:[8]

- It is widely grown (25% of the total certified seed production in 2007).
- Capable of giving yield (81%) similar to older 6-row varieties, such as Sequel.
- High untreated yield.
- Stiff straw.
- Good resistance to net blotch but susceptible to mildew.

1.1.3 *Taxonomy*

In Table 1, the classification of wheat in the binomial system is given, and plants are grouped using observable morphological characteristics. Cultivated barley is one of 31 *Hordeum* species, belonging to the tribe *Triticeae*, family *Poaceae*.[9]

[9]

Table 1 Taxonomy of barley

Taxonomy	
Kingdom	*Plantae* (Plants)
Subkingdom	*Tracheobionta* (Vascular plants)
Superdivision	*Spermatophyta* (Seed plants)
Phylum	*Magnoliophyta* (Flowering plants)
Class	*Liliopsida* (Monocotyledons)
Subclass	*Commelinidae*
Order	*Cyperales*
Family	*Poaceae* (Grass family)
Genus	*Hordeum* (Barley)

1.2 Utilization of straw

As an agriculture by-product, straw offers multiple usages since ancient, now modern technology offers more ways of straw utilization.

Traditionally, straw was burnt by farmers for plant disease control, increasing next year's yield, reducing the agrochemical usage et cetera. However straw is now generally chopped up and ploughed back into the land since the prohibition of burning in many countries (e.g. banned in England and Wales in April 1993).

In addition to being ploughed back to soil. Straw is majorly used as animal fodder, and also used as fuel, livestock bedding and fodder, thatching and basket-making. Furthermore, barley straw can be used f*or* algae control. During decomposition, barley straw has the capability to decrease the population of various algae types in several situations,[10] which the principle is explained in the next section. However due to the great amount of barley straw produced annually, only approximately 3% of biomass is being utilised.[11]

Viewed as a resource rather than a waste material, straw could become a chemical feedstock for industry and could be converted into a variety of high-value wax products and a number of energy and chemical products. Most straw, including barley, is naturally coated by a layer of crystalline-like wax; this wax can be extracted and has many uses in beauty industry and daily life. In addition, the energy potential of biomass could be well realised using pyrolysis conversion. Being an unconventional technology, pyrolysis converts biomass to clean fuel and valuable substance. Nowadays, with the excessive use of fossil fuels and the concerns over environmental protection, the research in renewable resources start to become a more and more important focus and attracted increasing world interest. Biomass including agricultural residues is one of the main renewable and carbon-neutral energy resources; the fuels derived from it often burn cleaner than fossil fuels. It has been estimated that biomass is capable of providing about 25% of global energy requirements.[12] The EU is also attempting to reduce CO_2 emissions, its members are required to increase the role of renewable resources for total energy production to 12 % by 2010 and ultimately to 20 % by 2020, with 33 % of electricity production needed to come from renewable resources.[13] If UK is to achieve the EU's objective of a 20% contribution from renewable energy by 2020, it is clear that biomass utilisation will have to increase substantially.

In this project, wax and bio-oil extracted from straw is the main target of interest. To understand the complexity of pyrolysis and apply such vital process on biomass,[14] it is very important to study the fundamentals and mechanisms of barley residues.

1.3 Key properties and chemicals of barley straw

1.3.1 Surface properties prevent lake blooms

Barley straw is used as a biodegradable substance for the inhibition of algae and cyanobacteria growth in aquatic reservoirs as mentioned in last section. The surface properties of barley straw, in this case, play a critical role. When straw is used for the algae growth restrictions, suitable surface properties allow microorganisms and fungi to adhere and decompose the straw. Accordingly, release the substances that are responsible for decreasing lake blooms. Since leaves and stems are the main components of barley straw, the multi- layer structure of these plant parts must be considered in order to understand these phenomena.[10]

The leaf and the stem retain a multi-layered structure; the top layer is a cuticle, which lies over the epidermal walls.[10] The cuticle is composed of an insoluble cuticular membrane impregnated by and covered with soluble wax. As shown below, cuticular wax covers only the outer part of a stem and leaves. The cuticle, including the wax layer, was believed to be formed in situ by modification of the cellulostic constituents of the epidermal cell walls.

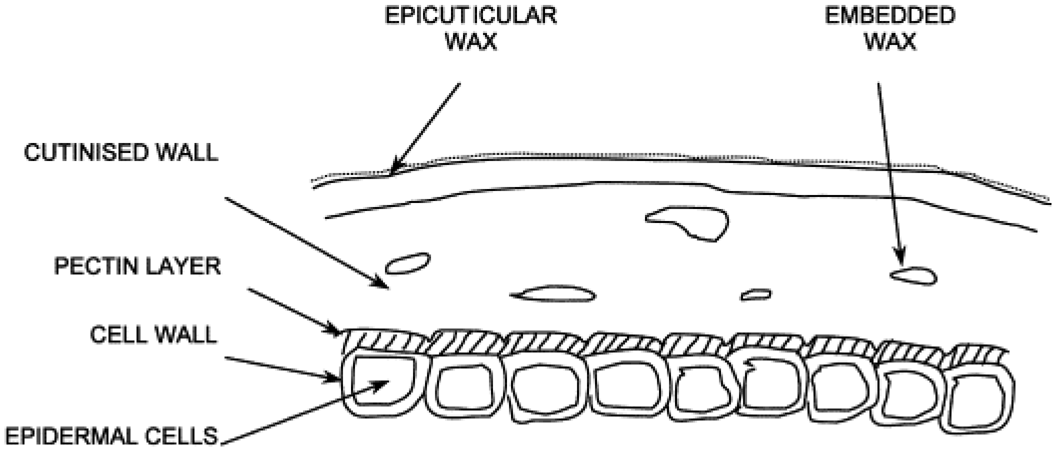


Figure 5 Cross-section of barley leaf.[10]

1.3.2 Function of cuticle waxes

The cuticle waxes are formidable barriers through which permeates diffuse with extremely low mobility. This property enables the plant to control efficiently the exchange of water, solutes, and even gases and vapours.[15] Microbial activities including adhesion, germination, and penetration were also controlled by the epicuticular waxes.[16] Another significant role of the epicuticular waxes is the enhancement of reflectance which results in the decrease of absorption of visible and infrared radiation and thus the leaf temperature and transpirational loss is reduced.[17]

Plant waxes are a very complex mixture. Previous report had also shown the content of lipid extracts affects the composition content of lignin, carbohydrate, ash and protein content in the biomass.[18] Generally waxes, also known as plant surface lipids, can be subdivided into categories of long-chain alkanes, branched chain alkanes, alkenes, esters of fatty acids and primary alcohols (monoesters, diesters, polyesters, estolides, and glycerides), free fatty acids and alcohols, aldehydes, ketones (β-diketones, substituted β -diketones), terpenoids, and phenolic substances.[19] A previous report also indicated that the barley early leaves produce mainly alcohols, but for the grown leaves and straw, the covering wax is rich in beta-diketones. The alcohols are transformed into ketones in the wax layer due to the oxidation process.[10]

1.4 Semiochemistry

As a source of high-value chemicals application in different area (e.g. coating, cosmetic, agrochemical), barley straw wax has a great potential to become a source of valuable chemicals.[20] One of the applications of wax is as semiochemicals to cause aphid avoidance. A semiochemical is a generic term used for a chemical substance or mixture that carries a message. Semiochemistry is the isolation, chemical characterisation, synthesis and bioassay of such chemicals.[21] Female aphid avoided leaves visited by its main enemy ladybird, *Coccinella septempunctate* adults and larvae during the previous 24 hours. Ethanol extracts of C.septempunctata adults and larvae also induced avoidance responses by *A.ervi*. According to previous literature, after hexane extraction, two of the hydrocarbons *n*-tricosane ($C_{23}H_{48}$) and *n*-pentacosane ($C_{25}H_{52}$) were found responsible for *A.ervi*'s avoidance.[1] As semiochemicals are naturally occurring, environmentally friendly, and species-specific compounds that do not result in the development of insecticide resistance, it offers the potential opportunity to work as an alternative to synthetic pesticides.[22] As aphids are the major arable crop pest throughout western and Northern Europe, the deployment of semiochemicals can therefore potentially reduce direct feeding damage and virus transmission in crops.[23] Many studies of their synthesis as the alternative choice for pest management programs are going on. Synthetically manufacture of highly pure semiochemicals such as some insect pheromones is already possible.

No studies, however, have been carried out on other straw or fruit waxes. There is a great opportunity facilitated by the use of renewable resources, as the production of insect semiochemicals are not just expensive but also difficult and expensive to synthesize from oil-based chemicals.[24]

1.5 Wax removal

However, the traditional extraction method of using volatile organic solvents has many disadvantages including low selectivity and harmful to environment. Therefore an alternative extraction method is needed. In this section, the environmentally friendly supercritical carbon dioxide technique and the widely used Soxhlet method were employed for wax removal. Hexane was used as solvent for its good wax selectively and low environmental impact.

1.5.1 SC-CO$_2$ extraction

A supercritical fluid is a substance above its critical temperature and critical pressure. Under such conditions, the distinction between gases and liquids does not apply and the substance can only be described as a fluid.

Above 304.2K (31.2^{o}C) and 72.8atm carbon dioxide behaves as a supercritical fluid and shows properties of both a liquid and a gas. It fills the container like a gas, and dissolves substances like a liquid. The phase diagram of carbon dioxide is shown below in Figure 6.

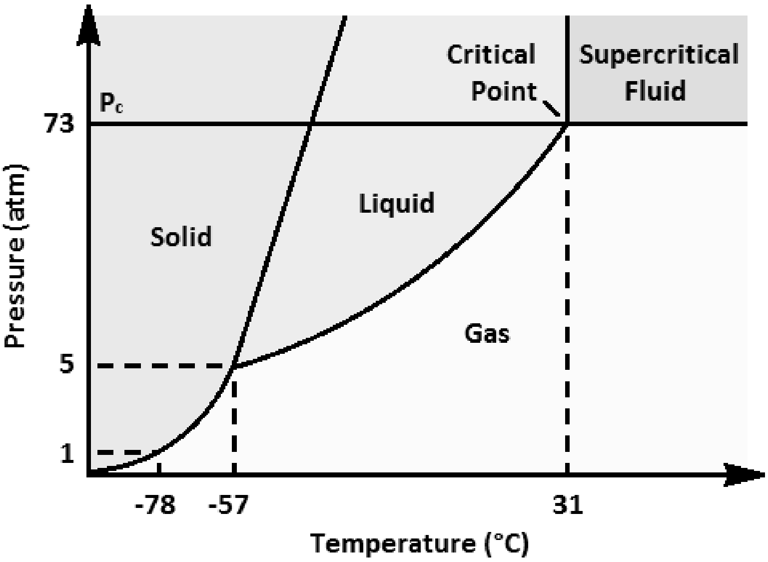


Figure 6 The Phase diagram of carbon dioxide

Supercritical carbon dioxide (SC-CO$_2$) is an excellent non-polar solvent for many organic compounds.[24] It has been likened to a solvent resembling hexane, though with some hydrogen-bonding acceptor capability and some dipole selectivity. Alkenes, alkanes, aromatics, ketones and alcohols (up to a relative molecular mass of around 400) dissolve in SC-CO$_2$. Very polar molecules such as sugars or amino acids and most inorganic salts are insoluble. The lipid-laden supercritical SC-CO$_2$ from the extractor is passed through a heated metering valve where the SC-CO$_2$ is depressurized and the extracted wax collected in a stainless vessel while CO$_2$ is vented out.[25]

Table 2 The advantages and disadvantages of using SC-CO$_2$

Advantages	Disadvantages
Non-toxic	Needs high pressures
Non-flammable	Greenhouse gas
Relatively unreactive	
Inexpensive	

Less solvent residues in products
Lower environmental impact
Penetrating power of a gas, solvent power of a liquid
Smaller solvent disposal costs

Literature data indicated the importance of removing wax from biomass prior further analysis for obtaining accurate results.[26] Previous work that have been done in this department demonstrated wheat straw could be used as a source of high value wax products through SC-CO_2[24] and the best yield is achieved with pressure of 350 bars. Considering the similar property shared between wheat straw and barley straw, SC-CO_2 technology and the reaction pressure were adapted in this research for barley wax extracting.

1.5.2 Soxhlet extraction

Conventionally, the most widely used method for extraction of plant natural products is Soxhlet extraction.[27] The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is used and recycled. However, the method greatly relies on the solubility characteristics of the particular species involved. Therefore, Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent.

Three classified categories solvents can be applied for Soxhlet: polar protic, dipolar aprotic and non-polar solvents. Although a previous study indicated increase the solvent polarity caused the increase of the extraction yield, the increase was mainly caused by the increasing amount of the co-extractives that being extracted out instead of wax. Therefore the non-polar solvent hexane, as the most selective solvent that gives 70% weight yield of wax compared to total extract,[24] was chosen and employed in the extractions.

1.6 Constituents of straws

Cuticle wax only accounts for a small fraction of the weight of the straw, generally the major constituents of straw consist of cellulose, hemicelluloses and lignin.[28]

In straws cellulose and xylan hemicellulose are the predominant components. Few pectic compounds and few mannans are also present. Ethanol extracts contain the low molecular weight sugars, fructose, glucose, sucrose, arabinitol and mannitol.

Biomass is essentially a composite material constructed from oxygen-containing organic polymers. The major constituents of straw consist of cellulose (a polymer glucosan), hemicelluloses, lignin, organic extractives, and inorganic minerals,[28] and these constituents make straw a potential renewable energy source.

1.6.1 Cellulose

Cellulose is a high molecular weight (10^6 or more) linear polymer of *β*-(1-4)-D-glucopyranose units in the $^4C_1$ conformation (

Figure **7**). The fully equatorial conformation of *α*-linked glucopyranose residues stabilises the chair structure with minimised flexibility. After the removal of water from glucose, the formed glucose anhydride is polymerized into long cellulose chains that contain 5000-10000 glucose units. Two glucose anhydride units constitute the cellobiose unit, which is the basic repeating unit of the cellulose polymer.[28]

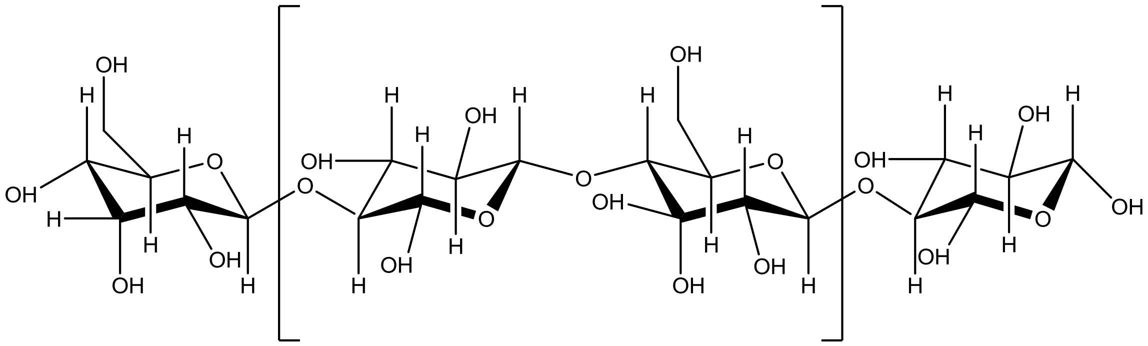
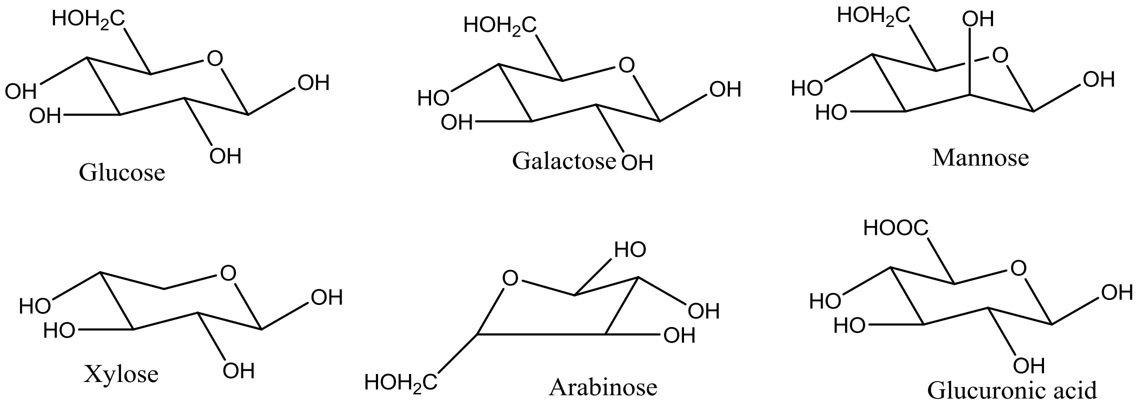


Figure 7 Chemical structure of cellulose

1.6.2 Hemicellulose

Hemicelluloses, the second most abundant natural polysaccharides after cellulose, comprise roughly one-fourth to one-third of most plant materials, and this amount will vary according to the particular plant species, such as for barley straw is 33-36%. The hemicelluloses are usually defined as the polysaccharide part of plant tissue, which is accessible to the action of dilute acids and alkalis. They are chemically complex and comprise a mixture of sugar monomers. For instance, besides glucose, sugar monomers in hemicellulose also including arabinose, galactose, mannose, and xylose, etc. Hemicelluloses exhibit lower molecular weights with little strength than cellulose.[29]



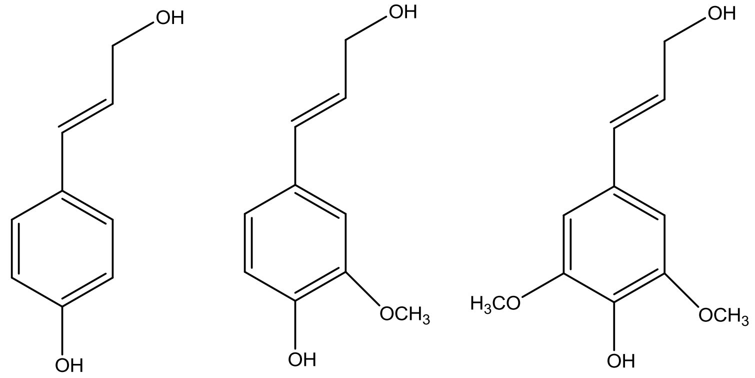
Glucose Galactose Mannose

Xylose Arabinose Glucuronic acid

Figure 8 Main components of hemicellulose

1.6.3 Lignin

Lignin is the third most abundant natural polymer present in nature after cellulose and hemicellulose. It is an amorphous cross-linked resin with no exact structure. It is the main binder for the agglomeration of fibrous cellulosic components while also providing a shield against the rapid microbial or fungal destruction of the cellulosic fibres. Lignin is a three-dimensional, highly branched, polyphenolic substance that consists of an irregular array of variously bonded "hydroxy-" and "methoxy-"substituted phenylpropane units. These three general monomeric phenylpropane units exhibit the *p*-coumaryl, coniferyl, and sinapyl structures (Figure 9). In lignin biosynthesis, these units undergo radical dimerisation and further oligomerization, and they eventually polymerize and cross-link. The resonance hybrids of the radical formed on oxidation of coniferyl alcohol illustrates the positions where radicals dimerizations occur during lignin formation.[28]



p-Coumaryl alcohol Coniferyl alcohol Sinapyl alcohol

Figure 9 p-Coumaryl, coniferyl, and sinapyl structures

A number of studies have been reported in the literature on pyrolysis of lignin. The pyrolysis generally leads to the formation of a volatile product and a solid residue, i.e. char.[30] The relative distribution of products is dependent on pyrolysis conditions.

The char was prepared by pyrolyzing lignin at atmospheric pressure and temperatures ranging from 150 to 550°C.

1.7 Pyrolysis

Pyrolysis is one of the most promising thermo-chemical conversion routes to recover energy from biomass.[31] Fossil fuel shortage and severe environmental problems have attracted great attention on the exploitation of clean renewable energies. The utilization of biomass resources will play an increasingly important role in the future.[32]

Pyrolysis chemistries are important for thermochemical conversion technologies. It is the thermal decomposition of materials in the absence of oxygen or when significantly less oxygen is present; less energy is used than required for complete combustion, and always results in a better yield. Normally for a lingocellulosic biomass, torrefaction happens in the temperature region of 150-300°C, pyrolysis in the region 300-600°C, and gasification is in the 600°C to greater than 1000°C region.[33]

Pyrolysis converts the biomass to liquid, gaseous and solid fractions. However it is difficult to precisely define its meaning, especially when applied to biomass. The older literature generally equates pyrolysis to carbonization, in which the principal product is a solid char. Today, the term pyrolysis often describes processes in which oils are preferred products. The time frame for pyrolysis is much faster for the latter process. This is also a clean synthesis as bio-oil, char and bio-gas are all valuable fuels.[28] Unlike fermentation to ethanol, pyrolysis is amenable to a smaller-scale with the potential for farm scale operations.[34] This could complement an integrated barley-to-ethanol plant fed by barley farms with distributed pyrolyzers to convert the straw and hulls to the fuel intermediates, and the biocharcoal could be returned to those farms, for use as a soil amender and to prevent soil erosion.[35]

The process parameters such as pyrolysis temperature, heating rate, holding time, particle size, pressure and reactor configuration, as well as addition of catalysts, have a great effect on the pyrolysis product yields and their chemical compositions.[36] The main product is bio-oil together with by-product char and gas.

1.7.1 Microwave pyrolysis

Additional to the numerous advantages stated for pyrolysis, microwave pyrolysis, as a modern technology, has the superiority over the traditional pyrolysis. As one of the researches focuses recently, it had obvious advantages over electric heating pyrolysis.[36b] During microwave heating process, energy transfer occurs through the interaction of molecules or atoms. Compared with conventional heating methods, more uniform temperature distribution can be achieved and the undesired secondary reactions may be avoided.[37] As a result, better control of the process and more desired products will be obtained such as heating rapid and uniform, more valuable products obtained.[36b] Due to the advantages of microwave pyrolysis mentioned above, it attracts more and more attentions. Recent research showed it has been employed to pyrolyze wood block and rice straw. [38]

1.7.2 Bio-oil

Bio-oils, also known under the names of pyrolysis oils, pyrolysis liquids, and others, are usually dark brown, free-flowing liquids having an acrid or a distinctive smoky odour.[31] Bio-oils are corrosive as it contains more or less solid particles and hundreds of organic compounds that belong to acids, alcohols, ketones, aldehydes, phenols, ethers, esters, sugars, furans, nitrogen compounds and multifunctional compounds.[39]. Till now, over 300 organic compounds have been identified in different bio-oils, with molecular weights from 18g/mol(water) to as high as 5000g/mol or more(pyrolytic lignins). Most of the compounds are in low concentrations.[31,40] Owing to their chemical composition, bio-oils show a very wide range of boiling temperature. These properties result from the chemical composition of the oils, which is significantly different from that of petroleum-derived oils, which will result in the vast difference in the fuel properties between them. Bio-oils are multi-component mixtures comprised of different size molecules derived primarily from depolymerisation and fragmentation reactions of three key biomass building blocks: cellulose, hemicellulose, and lignin. Therefore, the elemental composition of bio-oil resembles that of biomass rather than that of petroleum oils.[41]

The chemical compositions of bio-oils are determined by many factors, such as biomass type, feedstock pre-treatment (particle size and shape, moisture and ash contents), pyrolysis conditions(temperature, heating rate, residence time, pressure, gaseous environment) as well as vapour filtration and condensation (filter type, condensing method and medium, cooling rate). Therefore, bio-oils produced from different materials and by different pyrolysis reactors may differ greatly from one another. As a result, the fuel properties of different bio-oils usually vary in wide ranges.[31]

The yield of barley bio-oil at the condition of pyrolysis temperature of 500°C is in the range of 42-45%. As char and bio-gas are also generated as by-products during pyrolysis, bio-oil’s yield cannot reach to 100%. The perfect yield should be in the 70% - 75%(including water).[35]

1.7.3 Char

Biomass pyrolysis produces tar (pyrolysis oil), char (charcoal) and gaseous fractions (fuel gases). Char is the residual solid residue that comprises carbon and mineral ash that is formed in the pyrolysis of most biomass.[42] Char can be a potential source of fuel or a valuable co-product. Char residues can be used for physical or chemical absorption and as catalyst support or base material for fertilizers.[42]

The char is believed to contribute to the formation of polycyclic aromatic hydrocarbons (PAHs) during biomass pyrolysis, particularly at low temperature.[30] The structure of biomass char is complex. Many studies on biomass char structure have showed that changing of pyrolysis conditions, for example changing fuel type, temperature, heating rate and pressure, have great impact on char's structure and reactivity.[43] And it was found that the physical and chemical characteristics of chars were governed more by pyrolysis temperature than the nature of the substrate.[30] As microwave pyrolysis was used in this experiment instead of conventional heating, less char material was obtained as product.[44]

1.7.4 Bio-gas

Numerous investigations on biomass pyrolysis have focused on bio-oil and char, few detailed studies were found in bio-oil analysis. The product gases from pyrolysis are usually burned to generate energy for the pyrolysis process in many designs. Although the most valuable product is usually the liquid bio-oil, gaseous product are also valuable. Bio-gas contains various gases, such as CO, H_2, CO_2, CH_4, C_2H_4, C_2H_6 and C_4H_{10}. The variations in the percentages of these gases were due to increases in reaction pressure. Although bio-gas is the by-product from biomass pyrolysis, it may be useful if the CO and H_2 content are high.[45]

While comparing the components of the pyrolytic gases, it was revealed that the microwave pyrolysis gas usually had higher H_2 and CO contents and lower CH_4 and CO_2 contents than those obtained by conventional pyrolysis at the same temperature.[44]

1.8 Analytical technique

The isolated wax and pyrolysis products need to be analysed for a better understanding their chemical composition. GC-MS and GC are good methods for examine the chemical components of wax and bio-oil, while FTIR is good for analysing structure for char and gas.

1.8.1 GC and GC-MS

A mass spectrometer creates charged particles (ions) from molecules. It then analyses those ions to provide information about the molecular weight of the compound and its chemical structure. There are many types of mass spectrometers and sample introduction techniques which allow a wide range of analyses. This discussion will focus on mass spectrometry as it's used in the powerful and widely used method of coupling Gas Chromatography (GC) with Mass Spectrometry (MS).

Gas chromatography and gas chromatograph coupled with mass spectrometer are the key techniques for identifying the chemical components in the product. GC involves a sample being vaporised and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid.[46] The compounds become separated as they interact with the column. The difference in the chemical properties between different molecules in a mixture will separate the molecules as the sample travels the length of the column. These separated compounds then immediately enter the mass spectrometer after they take different retention time to elute from the gas chromatograph. And this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately.[47]

The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass to charge ratio (m/z).[48] Electron Impact (EI) Ionization was applied to generate the charged particles/ions required for mass analysis. The gas molecules exiting the GC are bombarded by a high-energy electron beam (70 eV). An electron which strikes a molecule may impart enough energy to remove another electron from that molecule.[49]

EI Ionization usually produces singly charged ions containing one unpaired electron. A charged molecule which remains intact is called the molecular ion. Energy imparted by the electron impact and, more importantly, instability in a molecular ion can cause that ion to break into smaller pieces (fragments). A molecular ion may fragment in various ways, with one fragment carrying the charge and one fragment remaining uncharged.

Molecular ions and fragment ions are accelerated by manipulation of the charged particles through the mass spectrometer. Uncharged molecules and fragments are pumped away. The mass analyser can uses positive and negative voltages to control the path of the ions. Ions travel down the path based on their mass to charge ratio (m/z). m/z represents mass divided by charge number and the horizontal axis in a mass spectrum is expressed in units of m/z. Since z is almost always 1 with GC-MS, because the EI ionization always produces singly charged particles, the m/z value is often considered to be the mass. [47]

Therefore in GC-MS analysis using EI method, an ion's path will depend on its mass. If the (+) and (-) rods were ‘fixed' at a particular voltage ratio, then one particular m/z would travel the successful path shown by the solid line to the detector. However, voltages are not fixed but are scanned, so that ever increasing masses can find a successful path through the rods to the detector.[49] The molecular weight is then determined from the molecular ions and structure is determined from the fragment ions. The original component in the sample can then be identified by combining the mass and structure information.

In GC-MS, the gas chromatograph utilizes a capillary column which depends on the column's dimensions (e.g. length) as well as the phase properties. Recent year, the coupling of gas chromatography with mass spectrometry using fused silica capillaries has played an important role in achieving a high level of chemical analysis.[50] In this experiment, one of the products was tested with two different length columns in order to capture the different molecules.

Although GC/GC-MS has great advantages, for example provides a highly accuracy when analysing unknown components, it also has disadvantages. The application of GC and GC/MS is limited to substances which are volatile enough to be analysed by gas chromatography.[50] Therefore samples need to be volatile or be made volatile for analysis. The samples are also need to be thermally stable to prevent degradation when heated during operation. Problems can be encountered when injecting such small samples accurately. And when using temperature programming, column bleed could happen and results in high background noise from the detector, if the vapour pressure of the stationary phase is too high for the oven temperature used and cause the stationary phase slowly elute from the column.[51] The bleeding phenomena were happened in this research and interfered with analysis.

1.8.2 Kovats indexes KI

Methods based on relative retention times are inaccurate for reporting chromatographic data that is being used later for substance identification. One identification process frequently employed in analysing unknown peaks in GC-MS spectrum is Kovats indices,[52] and is most often applied to hydrocarbons. The standard n-alkanes are used as markers, and other hydrocarbons' retention time are recorded and then being assigned an index individually. These indexes are then compared to the indexes of nearby standard n-alkanes.

These indices are independent of column flow rate and column dimensions, but must be matched with the same column stationary phase. Originally, Kovats indices were limited to isothermal column conditions,

$$KI = 100\frac{\log t_i - \log t_x}{\log t_{x+1} - \log t_x} + 100x \quad ^{52}$$ **Equation 1**

Where

I = Kovats retention index,
n = the number of carbon atoms in the smaller alkane,
N = the number of carbon atoms in the larger alkane,
z = the difference of the number of carbon atoms in the smaller and larger alkane,
tr = the retention time.

Modifications have been adopted to the above equation.[53] The method is used to determine the retention behaviour of substances in temperature-programmed GC. In the case of temperature-programmed gas chromatography a similar value can be calculated utilizing direct numbers instead of their logarithm. Since both the numerator and denominator contain the difference of two values, here we can use the total retention volumes (times). The Kovats index equation employed is:

$$I = \left[\frac{t_{r(unknown)} - t_{r(n)}}{t_{r(N)} - t_{r(n)}}\right] \times (100 \times z) + (100 \times n) \quad ^{53} \qquad \textbf{Equation 2}$$

Where symbol I, n, *N, z* and t_r are still present the same.

Compound identifications are made with running a mix of straight chain hydrocarbons and comparing the calculated Kovats index of the unknown peaks to the standard n-alkanes.

1.8.3 FTIR

In the FTIR spectrometer, specific molecular components and structures are specified by the corresponding infrared absorption bands, which allow computerized data searches to be performed against reference libraries.[14a] The FTIR spectrometer allows to analysis compounds by comparing the wavenumber of each peak to the standard, including CH, CO, aromatic ring, OH and carboxylic acid group in the sample can therefore be recognized.

1.8.4 Green chemistry metrics

Straw as a renewable resource has the potential of becoming the alternative fuel. Therefore the extraction process when transferring straw to fuel should not be too complex or cause the environmental pollution. Green chemistry metric was used in the end of the experiment to calculate how environmental friendly this research is. E-factor and effective mass yield were used for this purpose.

The E-factor reflects the reality of process chemistry much more accurately than atom economy. It takes into account the actual mass of reagents and products and offers a comparison in mass balance, so makes a qualitative attempt at defining which components of the overall synthetic process are environmentally friendly.[54] It is the first general metric for green chemistry and it remains one of the best:

$$E\text{-}Factor = Total\ waste(kg)/Product(kg) \qquad \textbf{Equation 3}$$

2 Experimental procedures

In order to study the possibility of successful extraction of wax and pyrolysis reaction output, experimental and analytical technologies were applied. Initially, straw was extracted by SC-CO_2 and Soxhlet. As the result, wax was collected and analysed by GC-MS and GC. De-waxed straw was collected as by-product. The de-waxed straw together with original one was used separately for microwave pyrolysis on Milestone microwave. The product bio-oil and the by-product char were characterised by GC, GC-MS, and FTIR respectively. After analysing the difference between bio-oil from two straw types, de-waxed straw was selected as starting material for CEM microwave, the reasons behind such decision is discussed in section 4. Different temperature conditions were applied to CEM pyrolysis. Closed vessel system is modified to opened vessel to enable bio-gas capture, it was employed as soon as prevention of reaction occurred by excessive formation of bio-gas. Bio-oil collected from both closed and opened vessel system was investigated by GC-MS while char and bio-gas were analysed by FTIR.

2.1 Barley straw as starting material

Saffron barley feedstock collected after harvest in Selby in UK in August 2009 was used as primary raw material throughout wax extraction by both SC-CO_2 and Soxhlet and later microwave pyrolysis. The extracted wax from Saffron barley was tested in difference in compositions against another two wax obtained from separate barley sources. Milled straw which contained small amount of grain was extracted by Soxhlet provided one of the two wax comparison, and a wax received from Germany using large scale SC-CO_2 on barley straw provided the second wax comparison. These three barley wax's GC-MS spectra were analysed and discussed in later section.

To provide another reasonable comparison to Saffron barley wax, Carat barley, which had been harvested in 2008 from Selby, was wax-extracted by SC-CO_2 under same condition as Saffron barley and tested in a different GC-MS machine in previous work. Peaks in GC-MS spectrum of Carat barley and Saffron barley were related to each other to investigate the wax composition difference of different variety barley.

2.2 Yield and semiochemical comparison

The previous extracted waxes as long as Carat barley wax were used in this research for the wax yield comparison. Samples of column chromatography extracted wax of Carat barley, wheat and apple peel from previous work were re-analysed by GC-MS for further examine the semiochemicals in those samples.

2.3 Methodology

Different experimental processes and products analysis techniques used for this research were described in this section. Supercritical carbon dioxide extraction was applied for wax extraction. Soxhlet extraction as the most common technique for natural plant extraction was also used, and hexane was used as solvent due to its comprehensive selective solvent nature for wax. Relationship between the differences of two extracted product compositions was studied.

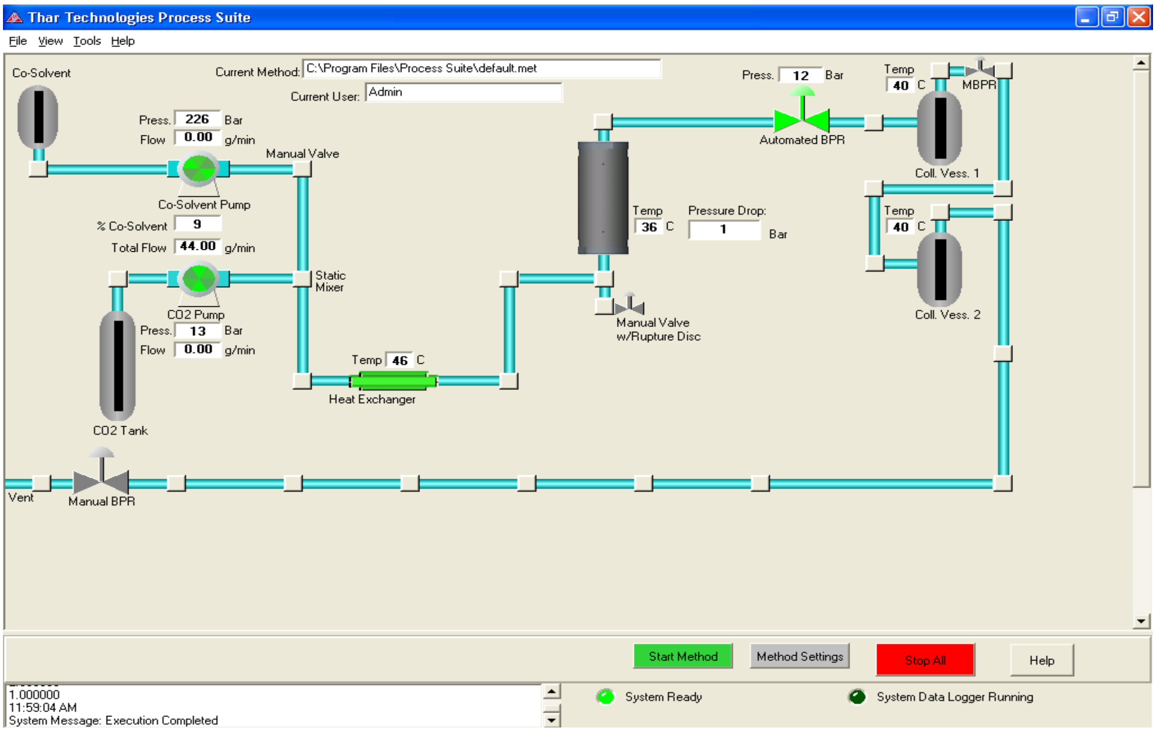
Two microwave machines were used, both the relative big scale Milestone microwave (50g) and the small scale CEM microwave (1g). The reason of using for this is to prove a more reliable research by comparing results from different microwave machines. Bio-oil was collected as main product while char was the by-product. Bio-gas was also collected as by-product when the reaction in CEM microwave moved to opened vessel system. Compositions of these products were analysed.

2.3.1 Pre-treatment

Straw used in this experiment was stored and dried in standard condition (room temperature) and roughly milled.

2.3.2 Supercritical CO_2 extraction

Milled barley straw (100g) was placed in the extraction vessel, using liquid CO_2 as solvent. Previous study has proven that with steady CO_2 flow rate under isothermal, wax extraction yield was enhanced by an increase in pressure. Thus this concept is applied to SC-CO_2 extraction in this research. Consequently, the reaction condition is set to isothermal of 50°C, isobaric of 350bar and constant SC-CO_2 flow rate of 40g/min. These reaction conditions can be monitored by the computer programmed flow sheet that is shown in Figure 10. SC-CO_2 was allowed to pass through the extraction vessel for 4 hours. Therefore the total amount of CO_2 for one extraction (100g) is 9.6kg.



Current Method: C:\Program Files\Process Suite\default.met

Current User: Admin

Press. 226 Bar

Flow 0.00 g/min

% Co-Solvent 9

Total Flow 44.00 g/min

Press. 13 Bar

Flow 0.00 g/min

Temp 46 C

Temp 36 C

Pressure Drop: 1 Bar

Press. 12 Bar

Temp 40 C

Temp 40 C

1.000000
11:59:04 AM
System Message: Execution Completed

Figure 10 Control mimic panel showing principle components

The SC-CO$_2$ program was run for 5 times and 500g straw was consumed in order to collect enough wax, the staring material for the later pyrolysis tests. Therefore, in later section, yield calculation was done by using the average yield of 5 wax extractions. The product wax was then stored in the sample tube until analysis. The average extract yield was determined by weighing the total wax collected and divided by the total weight of the starting material.

2.3.3 *Soxhlet extraction*

Wax was not just extracted using SC-CO$_2$ technique; it was as well as being extracted from Soxhlet extraction employing hexane as solvent. Hexane as mentioned before was the most selective solvent in Soxhlet extraction when apply to straw. Milled Saffron barley straw (11g) and unknown variety barley straw (11g) were extracted individually with 350 ml hexane in a Soxhlet extraction apparatus for 4 hours. Procedures were repeated for 3 times.

2.3.4 *Fatty acid Analysis*

Ester is one of the important compartments in wax. However esters with same chain length but different alcohol bindings always elute at the same retention time. In order to analyse the carbon number of fatty acid and alcohol ester group, the wax needed to be transesterified to free its fatty acids for further analysis of their components. 5mg of wax sample were transferred into a reaction vial followed by dilution with 1ml hexane. 50µl of sodium methoxide solution were added to the sample in hexane. The mixture was stirred for 2 hours. 5 minutes of incubation was allowed at room temperature before the sample top layer was separated for GC-MS analysis. By doing this, these waxes underwent methylation of the carboxylic acid function to form fatty acid methyl ester (FAME). This hydrolysis and methylation occurred at room temperature when reacted with 1N sodium methoxide. The FAMEs are identified and quantified under GC-MS.

2.3.5 Microwave

2.3.5.1 Milestone Microwave

Both extracted (by SC-CO_2) and un-extracted straw were used separately for microwave pyrolysis, and the impact in product of using two straw was identified. The pressurised Milestone system needed to be checked for leaks before the running of the experiment. Firstly, the glass vessel was fed with starting material and fit into Milestone microwave (illustrates by the right picture in Figure 11). 50g of straw together with 10w% water (5g, to reduce the static charge of straw) were used as staring material. After sealing the Milestone machine, the glass vessel was set to rotate while the vacuum pump was turned on; leaking from the system was check again. After checking the system, the cooling water was turned on and the cooler was set to maintain the temperature on 3°C. Then the reaction could be started using the program described below.

Figure 11 Milestone microwave used for the experiment

After several attempts, the microwave condition for operation was finally decided as,

- 1min, 400W energy, T1: 0°C, T2: 40°C (300mbar)
- 5min, 800W energy, T2: 120°C
- 10min, 1200W energy, T2: 200°C
- Leave the system to cool down for 30min, and the product can be collected

Most bio-oil formed during the reaction was transferred to a round bottom flask connected to the system (shown at the left part of the left picture in Figure 11). A small amount of bio-oil was left in the system and need to be washed off using diethyl ether. Bio-oil wash from different parts was collected and analysed separately. Bio-oil was identified as main product, char was collected as by-product, and small amount of water as waste was also collected.

2.3.5.2 CEM

CEM microwave was used to test bio-oil reproducibility which is capable of capturing bio-gas. CEM microwave has the advantage of running at a smaller scale and required less reaction time than Milestone. During the experiment, tests were carried on in both closed vessel and opened vessel to find relationship between the products yield and the reaction.

Unlike in the large scale Milestone microwave, only 1g of straw was used as starting material for the small vessel in CEM microwave. The reactions were carried out under constant energy input (energy 300W) and constant residence time (10min including cooling time). Various temperatures in closed vessel system were performed initially, and then experiments were conducted in opened vessel system.

Different reaction speeds were found at various positions along the reactant tube, where the upper part straw reaction had reached completion in contrast to the bottom part. In order to provide a more homogeneous heating process, straw was further milled to fine powder. Because of the un-equal heating of the straw, each sample was run consecutively two times under the same condition before collecting the bio-oil. Straw was evenly stirred after the first run and was put to microwave for a second run. Experiments were carried out under four different temperatures (100°C, 120°C, 140°C, 160°C) in the closed vessel system, and diethyl ether was again used to wash off the bio-oil.

When the reaction temperature increased to 160°C in closed vessel, the process was stopped soon after the starting. This was due to the large amount of gas being produced at higher reaction temperature which had caused a rise in pressure in the vessel. The machine was then modified to opened vessel system; gas was collected using a syringe with its needle penetrating the sealing rubber of the test tube (Figure 12). Reaction temperature 200°C and 250°C was performed on the opened vessel system, and bio-gas was collected for both temperatures. The gas in the syringe was then quickly injected to a transparent glass tube to be analysed by FTIR.

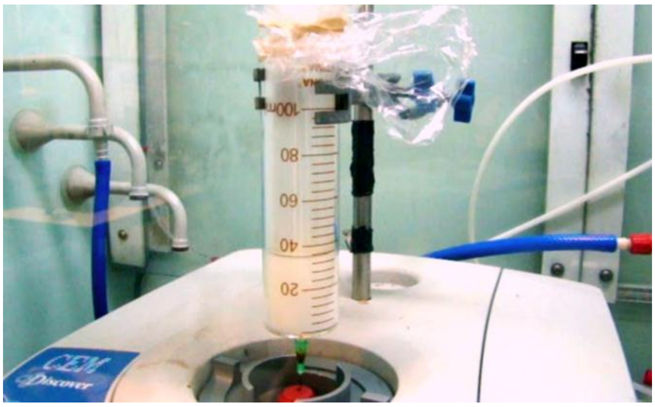


Figure 12 Bio-gas collected from CEM in open vessel (picture was taken during reaction process)

2.4 Chemical composition characterization

Wax, bio-oil, char and bio-gas were obtained as products by using above methods, and were subsequently analysed by using different techniques that explained in this section.

GC-MS: Two program conditions were designed for GC-MS analysis. Firstly, wax and bio-oil were analysed by GC-MS using condition 1, peaks were analyse to understand the detailed chemical compositions. Due to a heavy ester group in wax was retained under condition 1, condition 2 was programmed with a length reduced column and a higher temperature, to ensure the required components would elute in wax. Wax sample and transesterified wax that formed fatty acid methyl ester (FAME) were analysed under condition 2. KI numbers were calculated for peaks in spectrum and compared to the standard.

GC: In order to obtain the percentage of different chemicals existed in wax and a bio-oil sample, GC was applied. Areas under the corresponding peaks were calculated as percentage.

FTIR: The chemical composition of char and bio-gas were characterized by FTIR, and in order to understand the structure of char, straw and de-waxed straw were also analysed by same condition.

2.4.1 Gas chromatography-mass spectrometry (GC-MS)

Wax and bio-oil samples were taken for analysis using Shimadzu GC17A gas chromatograph fitted with an autosampler and a DB5-HT capillary column (30m×0.25mm, film thickness 0.25µm). To perform further analysis, samples were prepared by adding toluene and ethyl acetate of ratio 1ml to 0.02g of specimen. The chromatographic conditions are set as, Condition 1:

- Oven temperature 60°C for 1min,
- Heated a ramp rate 8°C/min to 300°C,
- Held for 10min.
- Carrier gas helium at constant flow rate 1ml/min.

And then in order to observe the high molecular weight wax ester groups, sample were re-processed in a length reduced DB5-HT column (15m×0.25mm, film thickness 0.25µm). The GC-MS machine functioned with a split injector and a mass detector, working in electron ionization mode. The conditions chromatographic were,

Condition 2:

- Oven temperature 60°C for 1min,
- Heated a ramp rate 8°C/min to 400°C,
- Held for 25min.
- Carrier gas helium at constant flow rate 1ml/min.

2.4.2 Gas chromatography (GC)

In order to obtain each corresponding peak area of barley wax and bio-oil, product samples were analysed with Gas Chromatography. The condition was set very similar to the method employed in GC-MS.

Wax and the hexane extracts from other straw were performed using an Agilent 6890N GC model equipped with an autosampler fitted with a DB-5 capillary column (30m×0.25mm) and an FID detector. The FID detector was used at 350°C. Then 1µl of this solution was injected into a DB-5 capillary column by means of split injection at a temperature of 300°C and a split ratio of 1:50. The temperature program was:

- Oven temperature 60°C for 2min,
- Heated a ramp rate 5°C/min to 350°C,
- Held for 10min.
- Carrier gas helium at constant flow rate 2.2ml/min.

KI number can be used to help identifying each peak, based on the retention time of each peak from GC/GC-MS.

Numerous peaks were observed in GC spectrum representing every components of the analysed sample. With absorbance shown in Y axis and retention time in X axis in GC spectrum, using retention time corresponded peak area table generated by GC software enabled correlating peak areas to its component. Thus the concentration of each chemical substance was determined by dividing its peak area with the sum of total peak areas.

2.4.3 Fourier transform infrared spectroscopy (FTIR)

The structure of the solid residues obtained from pyrolysis (char), the original straw and the de-waxed straw were studied by FTIR spectroscopy. It was also used for investigating the composition of bio-gas. Fourier transforms infrared spectroscopy (ATR, Specac Golden Gate). The FTIR spectra were obtained using the VERTEX 70 FTIR spectrometer resolution and 128 scans between 4000 and 600cm^{-1}, with 4cm^{-1} resolution for solid sample and 2cm^{-1} resolution for gas. The solid residue sample was compressed to powder before being tested, and the gas sample from the pyrolysis were transferred to a transparent glass tube to run the test.

3 Results and discussion

After products have been collected and analysed by methods and analytical techniques mentioned above, their spectra were investigated and their chemical compositions were analysed and discussed below.

3.1 Wax from Barley straw

The wax extracted from both SC-CO_2 and Soxhlet extractions exhibited a light yellow colour and have a strong and pleasant herbal odour.

3.1.1 Barley straw yield

SC-CO_2 wax has a slightly higher yield than Soxhlet wax when same variety barley (Saffron) was used.

- SC-CO_2 wax - average 1.2g product were collected from 100g starting material and the yield is 1.2 %
- Soxhlet wax - average 0.11g product was collected from 11g starting straw after vacuum evaporation, with the yield of 1.05%.

3.1.2 Chemical composition SC-CO_2 extracted wax

The GC-MS chromatography of the SC-CO_2 extracted barley wax suggested a great diversity in composition. The GC-MS chromatogram of the SC-CO_2 extracted wax is shown below in Figure 13.

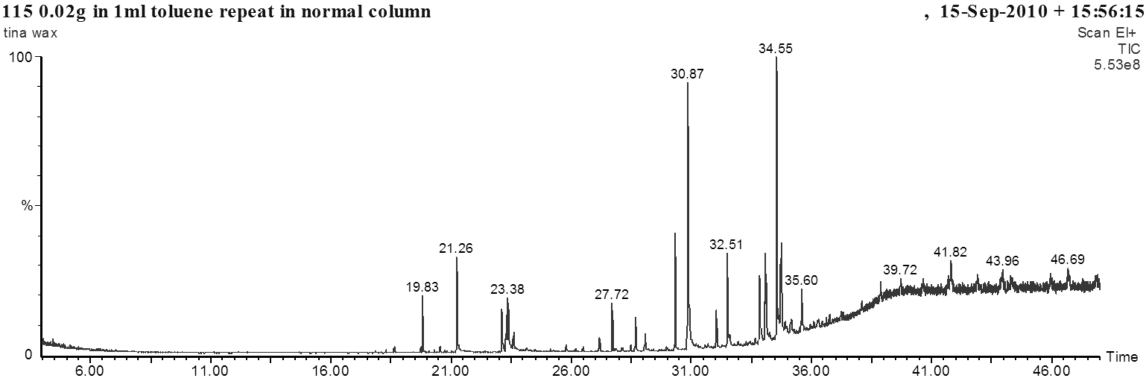


Figure 13 Barley straw wax (by SC-CO_2)

These peaks were divided into several main groups by their mass spectrum: long-chained alkane, aldehyde, free fatty acid, fatty alcohol, β-diketones, sterols and wax ester. The results were confirmed by previous studies.[25,55] Therefore the mass spectrum of each peak can be used to conclude of what chemical group it belongs to, rather than the precise chemical formula.

From the chromatography, most peaks were found concentrated between 18 to 36 min, as this is the region that hydrocarbon compounds elute from the GC-MS column; a chromatography in this range is shown in Figure 14 with peak details in Table 3.

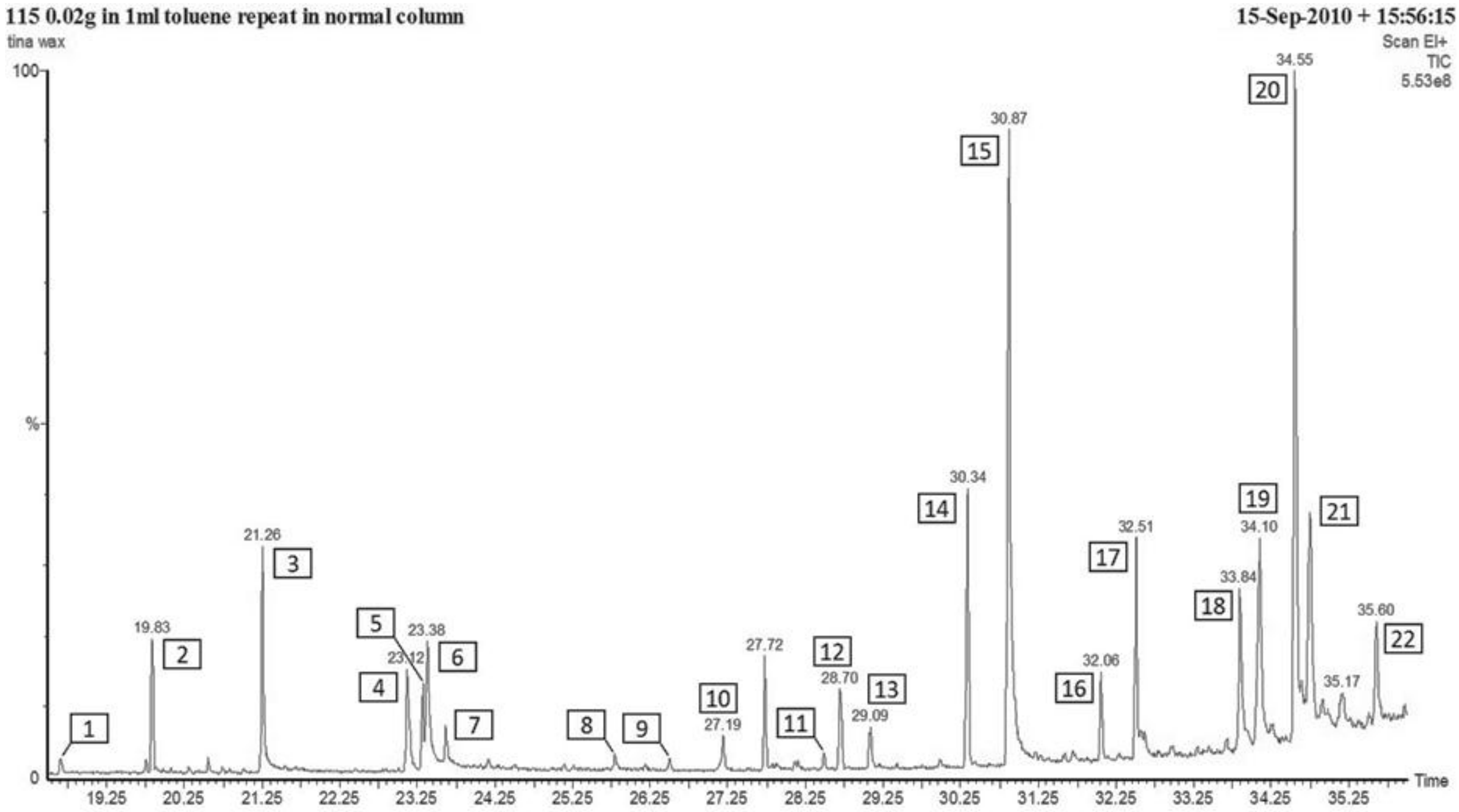


Figure 14 Detailed GC-MS Chromatogram of barley straw wax extracted by SC-CO_2

Table 3 SC-CO_2 barley wax components (✓: standard matched, N/A: not applicable, information cannot be found from literature or chemical database)

Peak	tr	KI	n	N	tn	tN	Compounds		Confirmed by		
									Mass spectrum	Expected KI	Standard
1	18.66	1755	18	20	19.23	21.75	tetradecanoic acid	$C_{14}H_{28}O_2$	✓	1770	N/A
2	19.83	1848	18	20	19.23	21.75	2-pentadecanone,6,10,14-trimethyl	$C_{18}H_{36}O$	✓	1845	N/A
3	21.26	1961	18	20	19.23	21.75	hexadecanoic acid	$C_{16}H_{32}O_2$	✓	1958	N/A
4	23.12	2119	20	22	21.75	24.06	phytol	$C_{20}H_{40}O$	✓	2114	N/A
5	23.32	2136	20	22	21.75	24.06	9,12-octadecadienoic acid	$C_{18}H_{32}O_2$	✓	2131	N/A
6	23.38	2141	20	22	21.75	24.06	9-octadecenoic acid	$C_{18}H_{34}O_2$	✓	2143	N/A
7	23.62	2162	20	22	21.75	24.06	octadecanoic acid	$C_{18}H_{36}O_2$	✓	2162	N/A
8	25.79	2362	22	24	24.06	26.19	eicosanoic acid	$C_{20}H_{40}O_2$	✓	2380	N/A
9	26.5	2431	24	26	26.19	28.16	docosanal	$C_{22}H_{44}O$	✓	2424	N/A
10	27.19	2502	24	26	26.19	28.16	pentacosane	$C_{25}H_{52}$	✓	2500	N/A
11	28.49	2636	26	28	28.16	29.98	tetracosanal	$C_{24}H_{48}O$	✓	2627	N/A
12	28.7	2659	26	28	28.16	29.98	squalane	$C_{30}H_{62}$	✓	2657	✓
13	29.09	2702	26	28	28.16	29.98	heptacosane	$C_{27}H_{56}$	✓	2700	✓
14	30.34	2842	28	30	29.98	31.7	hexacosanal	$C_{26}H_{52}O$	✓	2830	N/A
15	30.87	2903	28	30	29.98	31.7	1-hexacosanol	$C_{26}H_{54}O$	✓	2852	N/A
16	32.06	3045	30	32	31.7	33.3	octacosanal	$C_{28}H_{56}O$	✓	3032	N/A
17	32.51	3093	32	36	33.3	36.24	hentriacontane	$C_{31}H_{64}$	✓	3100	✓
18	33.84	3273	32	36	33.3	36.24	cholesta-3,5-diene	$C_{27}H_{44}$	✓	N/A	N/A
19	34.1	3309	32	36	33.3	36.24	stigmasterol	$C_{29}H_{48}O$	✓	3222	N/A
20	34.55	3370	32	36	33.3	36.24	beta-sitosterol	$C_{29}H_{50}O$	✓	N/A	N/A
21	34.74	3396	32	36	33.3	36.24	14,16-hentriacontanedione	$C_{31}H_{60}O_2$	✓	3357	N/A
22	35.6	3513	32	36	33.3	36.24	26-(Acetyloxy)-cholest-4-en-3-one	$C_{29}H_{46}O_3$	✓	N/A	N/A

3.1.2.1 Hydrocarbons

In barley SC-CO_2 extracted wax, the hydrocarbons were long chain alkanes. Studies showed the principal hydrocarbons of barley straw wax was comprised of carbon numbers 25, 27,29, 31 and 33.[55] By analysing the GC-MS spectrum of SC-CO_2 wax (Figure 14), the most abundant alkanes isolated were indeed pentacosan ($C_{25}H_{52}$, peak 10), heptacosane ($C_{27}H_{56}$, peak 13) and hentriacontane ($C_{31}H_{64}$, peak 17). The last two compounds had been confirmed by standard. The odd carbon chain number is a characteristic feature of the waxes of higher plants.

Below is the mass spectrum of peak 17, hentriacontane($C_{31}H_{64}$), this is a representative mass spectrum of alkane found in plant waxes.

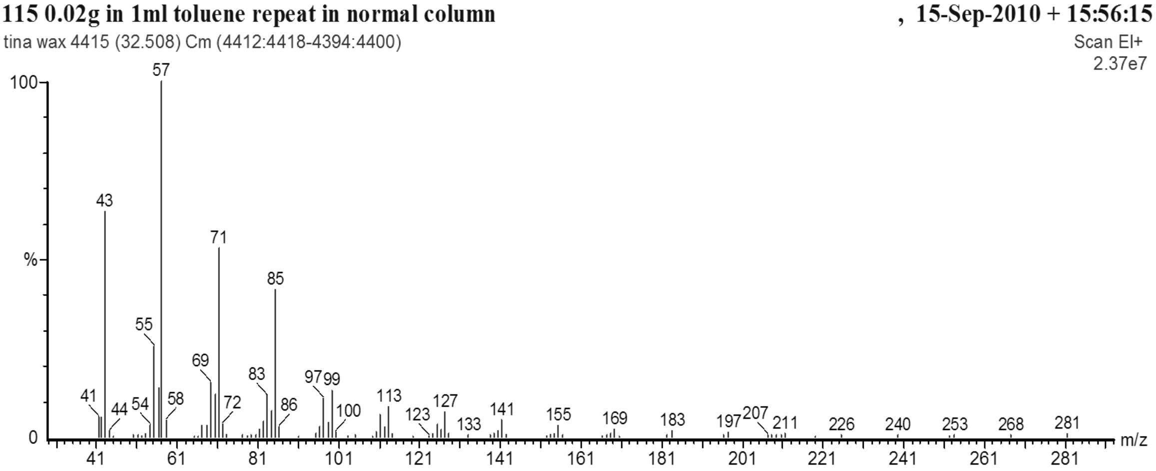


Figure 15 Mass spectrum of hentriacontane ($C_{31}H_{64.}$ Peak 17)

Alkane peaks are usually easy to identify as the fragment are often spaced by intervals of fourteen mass units. For examples in the above spectrum it is obvious to observe the fourteen intervals between most significant fragments: 43, 57, 71 and 85, corresponding to sequential loss of CH_2-groups. However, as alkane has high ionisation energy, the molecular ion of its peak is usually weak; the last fragment that can be identified for peak 17 in Figure 15 is 281, while $C_{31}H_{64}$ should have molecular weight of 436. It is therefore impossible and inaccurate to identify the precise chemical formula of the compound by using the m/z of the last fragment in its spectrum.

For a more accurate result, compounds in the wax are identified by matching both their KI and mass spectra to NIST library, standard compounds and published spectra. The KI of peak 17 is 3093, with the standard $C_{31}H_{64}$ has its KI of 3100. Together with its mass spectrum, peak 17 can be concluded as $C_{31}H_{64}$.

3.1.2.2 Aldehydes

Long chain aldehydes of plant were first reported as constituents of sugar cane and grape wax. Literatures suggested aldehydes have been detected chromatographically in the surface waxes of leaves or fruit of 18 out of 24 species investigated.[56] Aldehyde appears to be common constituents of plant waxes.

The GC analysis coupled to mass spectrometry confirmed the presence of four aldehydes in barley straw wax, which were decosanal ($C_{22}H_{44}O$, peak 9), tetracosanal ($C_{24}H_{48}O$, peak 11), hexacosanal ($C_{26}H_{52}O$, peak 14) and octacosanal ($C_{28}H_{56}O$, peak 16). Literature corroborated that hexacosanal ($C_{26}H_{52}O$) and octacosanal ($C_{28}H_{56}O$) are also exist in leaf and stem wax of triticales and wheat.[57,24] These two aldehyde are also the two most abundant aldehydes in barley wax. The KI number has the constant interval about 205 between the two adjacent aldehydes. (KI included in Table 3)

As aldehydes are unstable over period and can easily decompose to acids through oxidation, it is usually difficult to find their trace in mature plant straw. It was therefore surprising to find four aldehydes in barley wax not only survived the long term storage and the pressurised, high temperature SC-CO_2 extraction process, but two of them also generated a fairly strong peaks (peak 14 and 16, caused by hexacosanal (C_{26}) and octacosanal (C_{28})). The reasonably explanation for this could be these aldehydes have a very high concentration in barley straw. A typical mass spectrum of aldehyde is shown below.

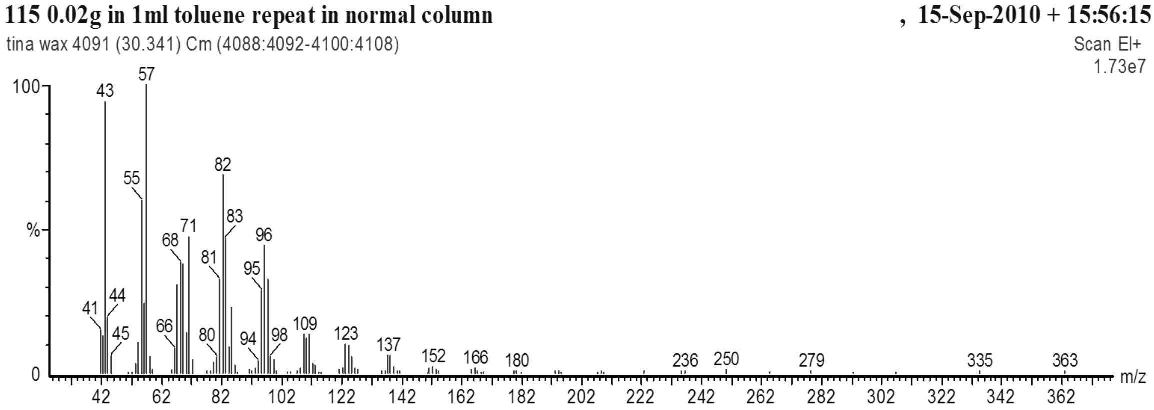


Figure 16 Mass spectrum of hexacosanal ($C_{26}H_{52}O$, peak 14)

3.1.2.3 Fatty acid

Fatty acid is commonly found in organic matter. Most naturally occurring fatty acids have a chain of 4 to 28 carbons. Previous literatures showed free fatty acid has also been found in plant surface wax. For instance, *ustilago maydis* contained fatty acids ranging in carbon number from C_{12} through C_{20}[58] , while palmitic acid (C16:0) and stearic acid (C18:0) are the two predominant saturated fatty acids in plants.

Among those fatty acid presence in Figure 14, four of them were saturated fatty acid (tetradecanoic acid (C14:0), hexadecanoic acid (C16:0), octadecanoic acid (C18:0) and eicosanoic acid (C20:0)) and two of them were unsaturated acid (9, 12-octadecadienoic acid (18:2) and 9-octadecenoic acid (18:1)).

Table 4 Retention time and KI number of free fatty acid in barley wax (relate to Figure 15)

Peak	tr	KI	Compounds		Confirmed by		
					Mass spectrum	Expected KI	Standard
1	18.66	1755	tetradecanoic acid	$C_{14}H_{28}O_2$	✓	1770	N/A
2	19.83	1848	2-pentadecanone,6,10, 14-trimethyl	$C_{18}H_{36}O$	✓	1845	N/A
3	21.26	1961	hexadecanoic acid	$C_{16}H_{32}O_2$	✓	1958	N/A
4	23.32	2136	9,12-octadecadienoic acid	$C_{18}H_{32}O_2$	✓	2131	N/A
5	23.38	2141	9-octadecenoic acid	$C_{18}H_{34}O_2$	N/A	2143	N/A
6	23.62	2162	octadecanoic acid	$C_{18}H_{36}O_2$	✓	2162	N/A
7	25.79	2362	eicosanoic acid	$C_{20}H_{40}O_2$	✓	2380	N/A

Hexadecanoic acid was identified with the highest concentration among the fatty acid and gave a strong peak (peak 3) on GC chromatographically.

Peak 4 and peak 5 represented 9,12-octadecadienoic acid (C18:2, also known as linoleic acid), and 9-octadecenoic acid(C18:1, known as oleic acid). They were the only two unsaturated acid presented in the extracted wax[59]. They are also common in the surface wax of other plants.[58]

The mass spectra of all the saturated fatty acid indicated the similar trend and their KI showed a constant 200 difference.

3.1.2.4 Fatty alcohol

Free fatty alcohols in straw waxes were found mainly n-alkanols with chain length ranging from C_{22} to C_{28}, for example in wheat straw wax the predominance aliphatic alcohol was 1-octacosanol[55]. The peak 15 was the second strongest peak in Figure 15, and its mass spectrum shown below the represented peaks at m/z 55, 57, 83, 97, 111 and 125, which are the characteristic peaks of fatty alcohol.[60]

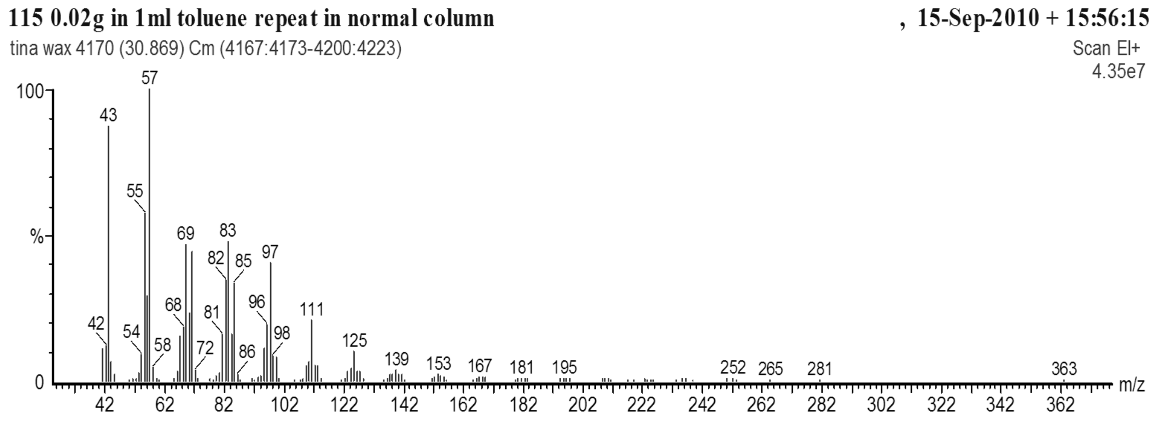
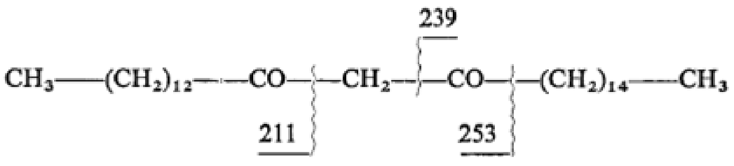


Figure 17 Mass spectrum of hexacosanol ($C_{26}H_{54}O$, peak 15)

With the exception of a fragment at m/z=31, caused by (CH_3O^+), the mass spectrum of a long-chain alcohol resembled the spectrum of an n-alkene. However, the chemical ionization mass spectrum of a long-chain n-alcohol is more specific. The last fragment of fatty alcohol is always caused by the loss of water molecule. Taking the last fragment of m/z 363 plus the molecular weight of water 18, the total molecular weight of the chemical should be 381, which is very close to molecular weight of 1-hexacosanol of 382. The difference may cause by the inaccuracy of the machine. The hexacosanol standard was also run and co firmed this peak was C_{26} alcohol.

3.1.2.5 β-diketone

The peak 21 in Figure 14 at 34.74 min with KI of 3396 was predicted to be hentriacontan-14,16-dione, also known as β-diketone.[55] Without any related chemicals in the wax/standard for comparison, the mass spectrum need to be related to its molecular weight and its structure to confirm this is the beta-diketone peak. The spectra of this peak showed a peak of 446 m/e (M-18) which is caused by the loss of water from the enol. Peaks were observed at m/e 211, 239 and 253, displaying the fragmentation shown on the structure (Figure 19). The prominent ion m/z 100 in MS of the diketone arises from two McLafferty rearrangements.[61] The peak at m/z 296 was also accounted by McLafferty rearrangement.



$$CH_3-(CH_2)_{12}-CO-\underset{\underline{211}}{\wr}-CH_2-\overset{\underline{239}}{\wr}-CO-\underset{\underline{253}}{\wr}-(CH_2)_{14}-CH_3$$

Figure 18 Structure of hentriacontan-14,16-dione

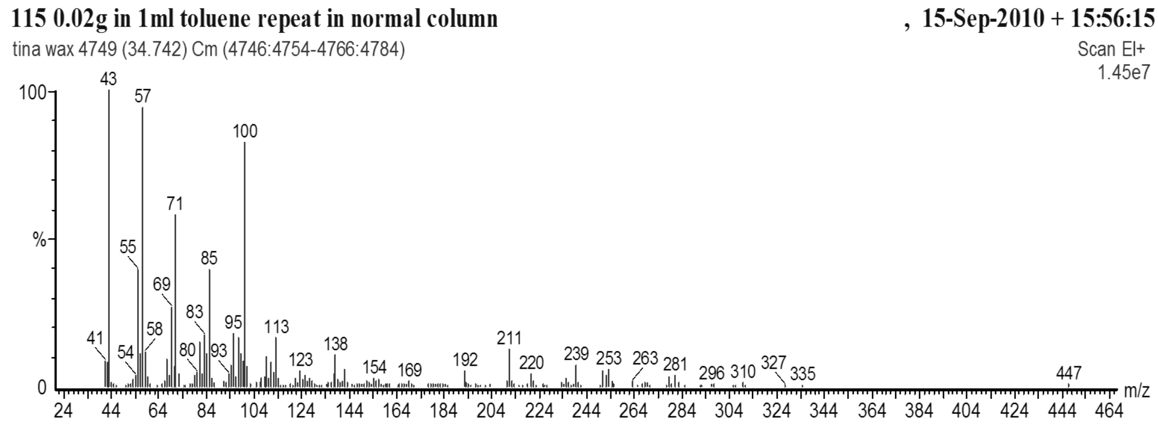
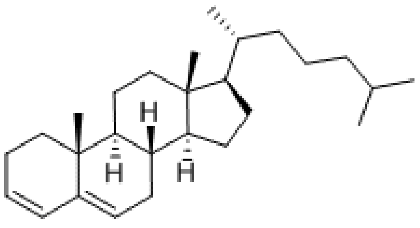


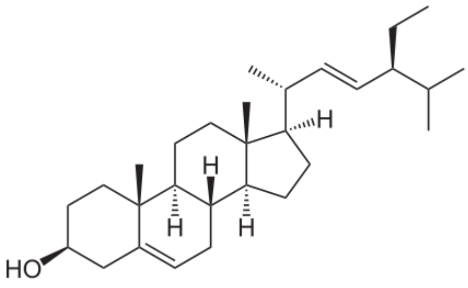
Figure 19 Mass spectrum of hentriacontan-14,16-dione

3.1.2.6 Sterols

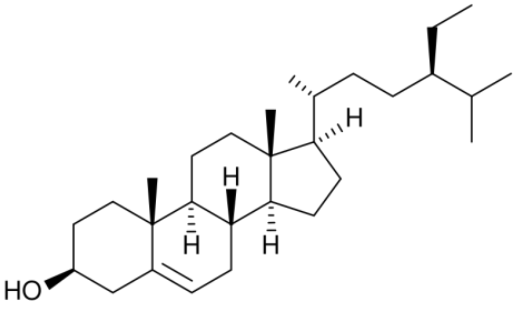
Sterols occur naturally in plants. Plant sterols include campesterol, sitosterol, and stigmasterol. NIST library showed the last group of peaks are almost all caused by different sterols, and the structure of sterols that appears in the wax sample is listed below.



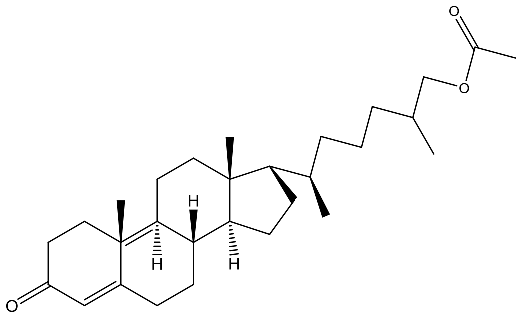
Cholesta-3,5-diene



Stigmasterol



β -Sitosterol



26-(Acetyloxy)-cholest-4-en-3-one

Figure 20 Structures of Sterols

The mass spectrum of peak 22 at retention time 35.6min suggested the peak to be cholesta-4-en-3-one. However the peak’s KI is calculated to be 3513, much greater than the cholesta-4-en-3-one published KI 3123. In addition, cholesta-4-en-3-one should escape from column before stigmasterol according to its chemical structure. Finally, this peaks was identified as 26-(acetyloxy)-cholest-4-en-3-one.

3.1.2.7 Other wax components

Majority peaks were analysed above, the remaining ones were peak 2 at 19.89min, peak 4 at 23.12min and peak 12 at 28.7min. 6,10,14-trimethylpentadecan-2-one, phytol and squalane were chemicals generating these peaks suggested by NIST library. The presence of squalane and phytol were proven by running squalane's standard in the lab, and having the phytol's KI and MS compare to the standard information in NIST webbook. Further evidence need to be provided to conclude that 6,10,14-trimethylpentadecan-2-one is the right molecule for peak 2.

3.1.2.8 Wax ester

Ester group is an important component of the wax, and wax ester occurs widely in nature in a variety of plant and marine species. They are usually found as mixtures of esters consisting of saturated or monoenoic fatty acids and alcohols, with the acid and alcohol groups varying in chain length from C6 to C34.[62] However, the heavy wax ester was retained with other wax components in normal GC-MS DB-5 column. A shorter column was needed, thus a length reduced DB5-HT column was required with operating temperature of 400°C, in order to ensure that the required components would elute. The last group of peaks (elute after 30min) represented the wax esters.

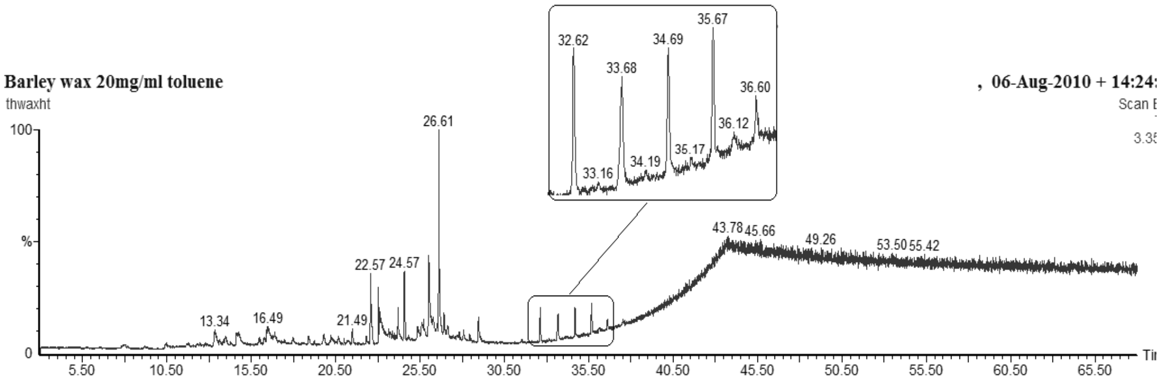


Figure 21 Wax ester peaks in GC-MS chromatogram

Figure 21 shows the fives ester peaks in the GC-MS chromatogram of barley straw wax, retention time range was set from 32.62 to 36.60min. The later spectrum was seriously interferes with evaporation of the liquid phase from the column, the so-called "column bleed", the reason for this was the vapour pressure of the stationary phase had gone too high for the oven temperature used and caused the stationary phase slowly eluted from the column. The bleeding is very common in GC-MS, particularly in programmed temperature operation,[63] and is one of the disadvantages of GC-MS.

A complex molecular ion was suggested by the mass spectrum of the peak at 32.62min (Figure 22). It contains the typical wax ester ions at m/z 229, 258, 286 and 314. These ions represented the four different fatty acid that contain varies carbon chain: ($C_{13}H_{27}CO_2H_2^+$), ($C_{15}H_{31}CO_2H_2^+$), ($C_{17}H_{35}CO_2H_2^+$) and ($C_{19}H_{39}CO_2H_2^+$). Molecules with the equal carbon number were discovered at the same retention time, caused by various alcohol bindings of fatty acids that have the identical chain length in total.

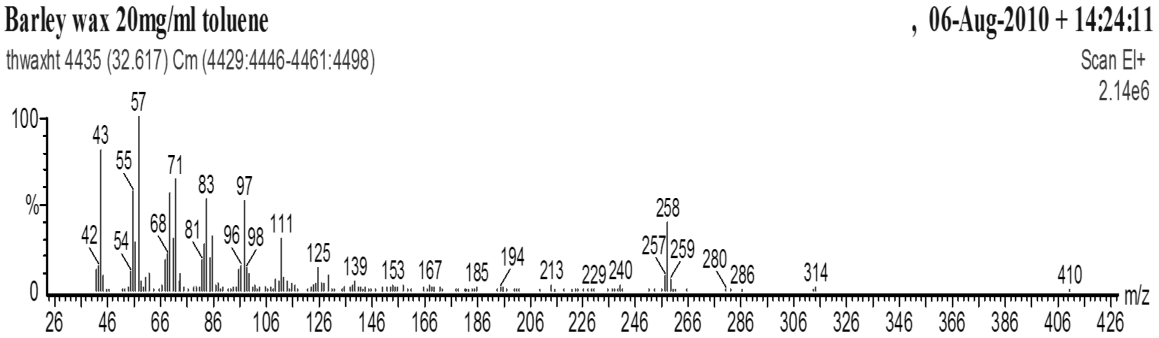


Figure 22 MS of the peak at 32.62min from SC-CO_2 extracted barley straw wax

In order to find the correct binding fatty acids, the total carbon number of such molecules was required, which could be obtained by comparing the KI of this peak to the standard.

Previous study showed the ester have carbon atoms number of 42 has KI of 4346 if utilising DB-1 column.[64] The first ester peak at 32.62min has the KI of 4381. As the DB-5 column is more polar than the DB-1 column, it generated a slightly higher KI, and this perfectly explains the first peak's higher KI of 4381. Therefore this peak is concluded contains 42 carbon atoms. As the previous section has been suggested the first peak contains tetradecanoic ($C_{13}H_{27}CO_2H_2^+$), hexadecanoic ($C_{15}H_{31}CO_2H_2^+$), octadecanoic ($C_{17}H_{35}CO_2H_2^+$) and eicosanoic($C_{19}H_{39}CO_2H_2^+$) by checking its mass spectrum, these fatty acids could combine respectively to octacosanol, hexacosanol, tetracosanol and eicosanol to make a 42 carbon atoms chain.

To support such conclusion, the chain length of the esterified fatty acids were determined by hydrolysis (waxes undergo methylation of the carboxylic acid function to form fatty acid methyl ester), and further analysed by GC-MS.

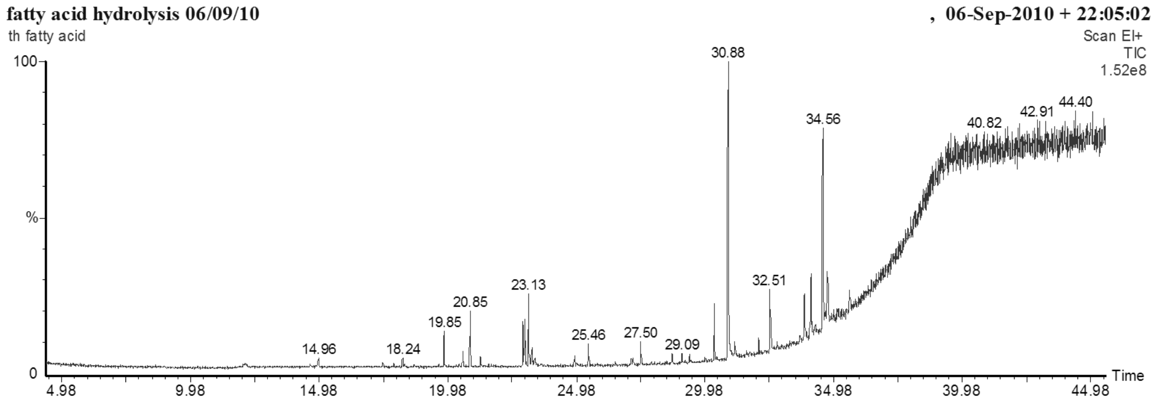


Figure 23 GC-MS Chromatogram of SC-CO_2 wax after fatty acid hydrolysis

By analysing Figure 23 using NIST library, peaks at 30.34 and 30.88 were found to be alcohols. Finally, octacosanol was discovered to be represented by peak at 30.88min.

In the mass spectrometry of methyl ester, the ionisation takes place at one of the free electron pairs of carbonylic oxygen. According to McLafferty rearrangement, the characterized peak for saturated and monoenoic fatty acids is at m/z 74. Therefore all peaks with mass 74 from Figure 23 were displayed at Figure 24 and their KI numbers were calculated. By comparing these peaks' KI to the standard methyl esters' KI on NIST webbook database, these peaks were concluded to cause by methyl undecanoate, methyl hexadecanoate, methyl octadecanoate, methyl docosanoate, methyl tetracosanoate and methyl hexacosanoate. (Table 5)

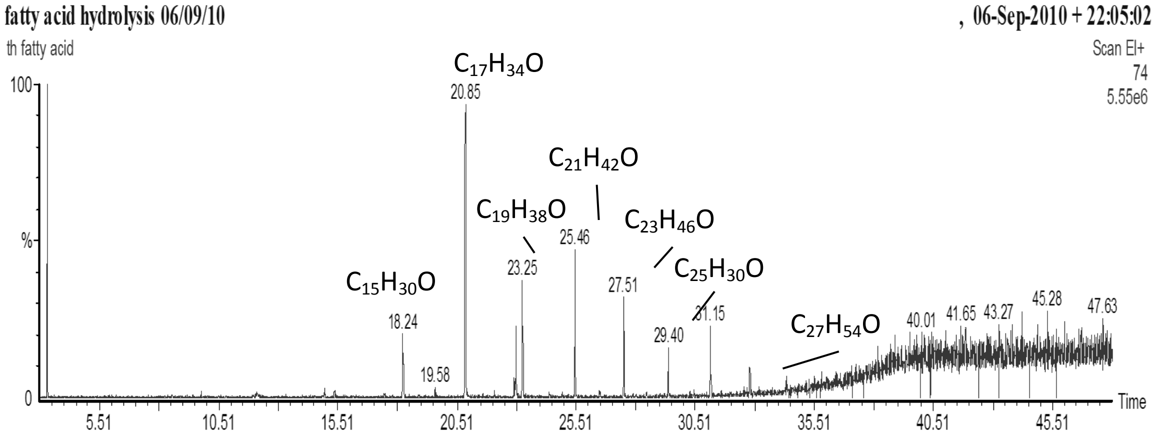


Figure 24 GC-MS Chromatogram of methyl ester

Table 5 Kovat index number of fatty acid methyl ester

peaks	tunknown	KI	Corresponding Chemical
1	18.24	1729	Methyl tetradecanoate
2	20.85	1922	Methyl hexadecanoate
3	23.25	2128	Methyl octadecanoate
4	25.46	2330	Methyl eicosanoate
5	27.51	2533	Methyl decosanoate
6	29.4	2734	Methyl tetracosanoate
7	31.15	2935	Methyl hexacosanoate

According to the calculated KI of the wax ester peaks, together with the wax ester part in Figure 21, the 5 different peaks presented a KI interval of 200, which represent the 2 $–CH_4$ difference, the wax ester peaks can be concluded as:

Table 6 Kovat Index number of wax ester

Peak	tr	KI	Formular	Confirmed by	
				Mass spectrum	KI
1	32.62	4381	$C_{42}H_{84}O_2$	✓	✓
2	33.68	4588	$C_{44}H_{88}O_2$	✓	N/A
3	34.69	4788	$C_{46}H_{92}O_2$	✓	N/A
4	35.67	4982	$C_{48}H_{96}O_2$	✓	N/A
5	36.6	5189	$C_{50}H_{100}O_2$	✓	N/A

3.1.3 Chemical composition Soxhlet wax

The wax obtained from performing Soxhlet extraction on barley straw was analyzed using GC-MS. The GC-MS chromatogram of the wax is shown below in Figure 25.

Peak 10 and 11 at retention time of 30.24min and 30.77min respectively were the strongest. They were caused by hexacosanal and 1-octacosanol. Most peaks in Soxhlet wax were also found in SC-CO_2 waxes, with exceptions of octacosane and 1-octacosanol. The difference between these two waxes is discussed in later section.

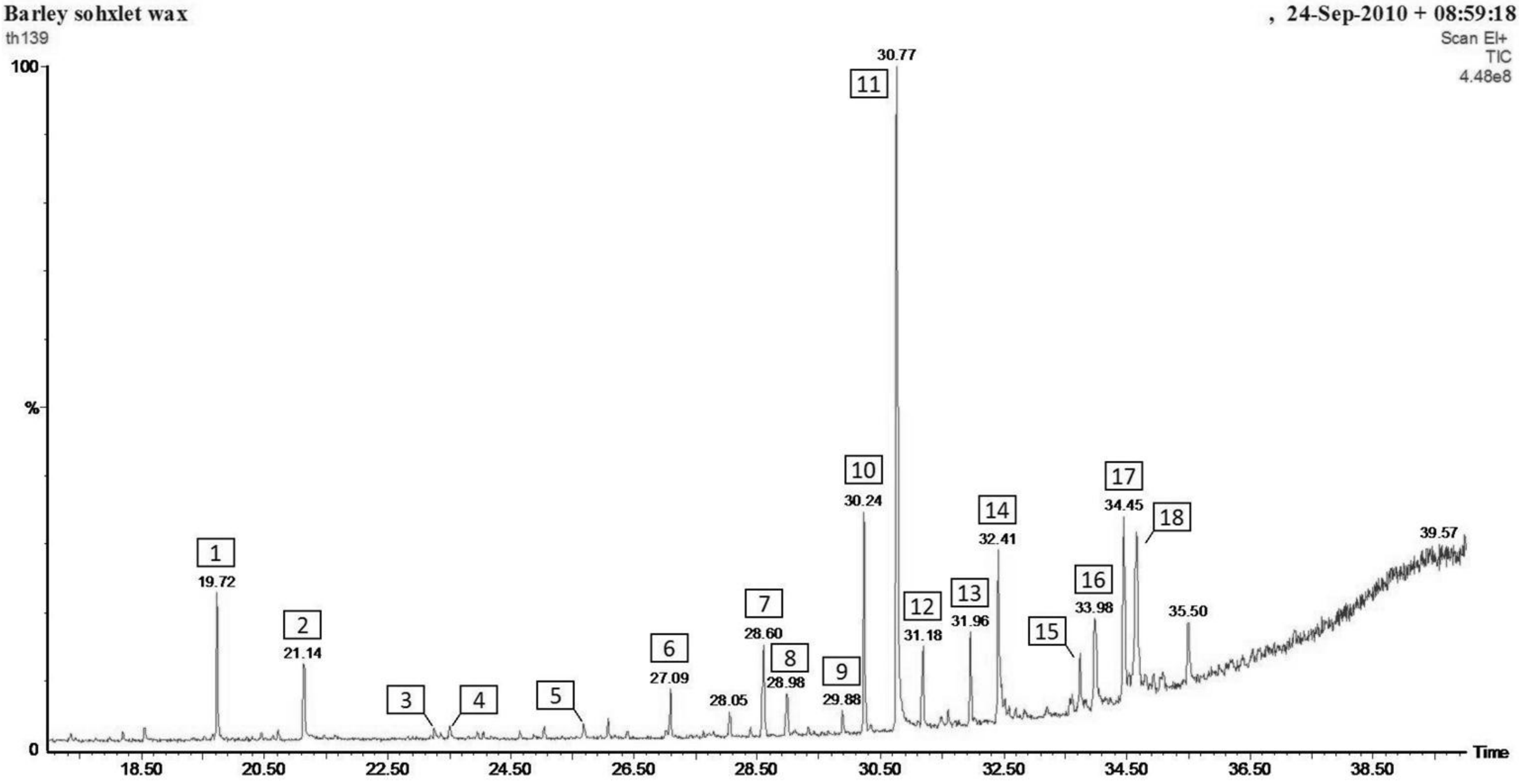


Figure 25 GC-MS chromatogram of the sohxlet extraction wax

Table 7 Peaks from sohxlet wax GC-MS (✓:standard matched, N/A: unavailable from literature or chemical database)

Peak	tr	KI	Compounds		Confirmed by		
					Mass spectrum	Expected KI	Standard
1	19.72	1848	2-pentadecanone,6,10, 14-trimethyl	$C_{18}H_{36}O$	✓	1845	N/A
2	21.14	1961	hexadecanoic acid	$C_{16}H_{32}O_2$	✓	1958	N/A
3	23.26	2141	9-octadecenoic acid	$C_{18}H_{34}O_2$	✓	2141	N/A
4	23.5	2161	octadecanoic acid	$C_{18}H_{36}O_2$	✓	2162	N/A
5	25.68	2363	eicosanoic acid	$C_{20}H_{40}O_2$	✓	2363	N/A
6	27.09	2504	Pentacosane	$C_{25}H_{52}$	✓	2500	N/A
7	28.6	2661	Squalane	$C_{30}H_{62}$	✓	2660	⍰
8	28.98	2702	Heptacosane	$C_{27}H_{56}$	✓	2700	⍰
9	29.88	2800	Octacosane	$C_{28}H_{58}$	✓	2800	⍰
10	30.24	2842	Hexacosanal	$C_{26}H_{52}O$	✓	2830	N/A
11	30.77	2903	1-hexacosanol	$C_{26}H_{54}O$	✓	2852	⍰
12	31.18	2951	1-octacosanol	$C_{28}H_{58}O$	✓	N/A	N/A
13	31.96	3045	Octacosanal	$C_{28}H_{56}O$	✓	3032	N/A
14	32.41	3102	hentriacontane	$C_{31}H_{64}$	✓	3100	⍰
15	33.74	3269	cholest-5-en-3-one	$C_{27}H_{44}O$	N/A	N/A	N/A
16	33.98	3304	Stigmasterol	$C_{29}H_{48}O$	✓	3222	N/A
17	34.45	3366	beta-sitosterol	$C_{29}H_{50}O$	✓	N/A	N/A
18	34.66	3393	14,16-hentriacontanedione	$C_{31}H_{60}O_2$	✓	3357	N/A

3.1.3.1 Wax composition difference

The following graph compares straws wax from different extraction technique and different origin. The differences in peaks among the spectrums were explained below.

Although the straws from three regions were processed using two techniques of Soxhlet and SC-CO_2, the resulting GC-MS spectrum possessed great similarities among each other. Composition differences between waxes are discussed by extraction methods and by origins respectively.

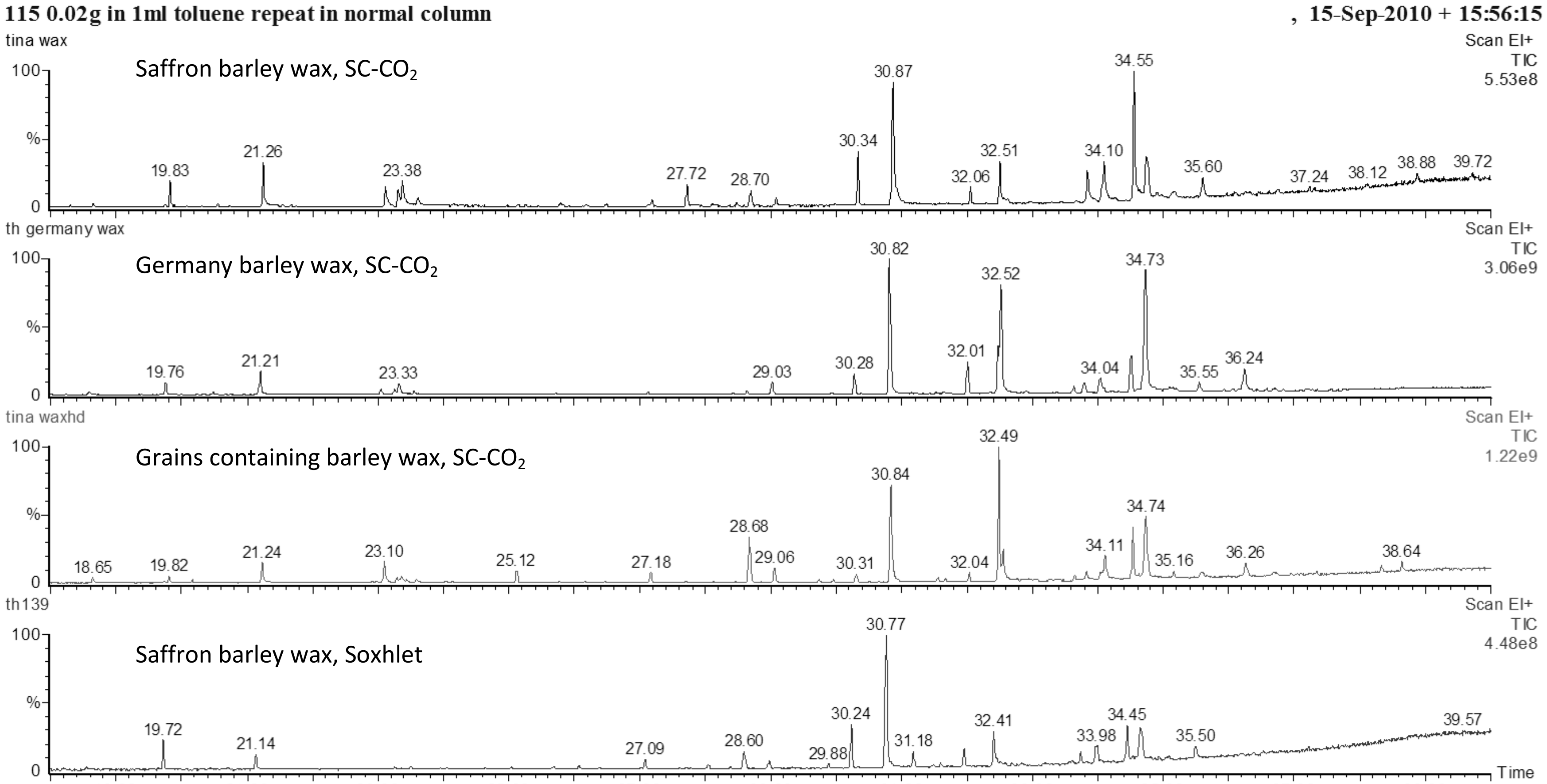


Figure 26 GC-MS of difference waxes

3.1.3.1.1 Composition difference by techniques

$SC\text{-}CO_2$ extracted wax was expected to show a profile different from hexane,[25] and this statement is corroborated by the results of this research. This was caused by non-polar property of hexane used in Soxhlet, because hexane did not participate in hydrogen bonding. In contrast, $SC\text{-}CO_2$ had quadrupole moment and thus was able to participate in hydrogen bonding. Subsequently, for $SC\text{-}CO_2$, there were contributions from dispersions, polar, and hydrogen-bonding interactions.

Although many peaks shared a great similarity in both waxes' spectrum, there were significant differences. For example, $SC\text{-}CO_2$ wax contained more diversions of fatty acid, aldehydes and greater percentage of sterols, but less alkanes; $SC\text{-}CO_2$ wax comprised seven different fatty acids and four different aldehydes, but Soxhlet wax only consisted of four fatty acids and two aldehydes; $SC\text{-}CO_2$ had a strong beta-sitosterol peak with KI at 3370, but Soxhlet wax only had a mild peak with the similar KI; In the meantime, octacosane and 1-octacosanol were found in Soxhlet wax but not in $SC\text{-}CO_2$ wax.

From these findings, different techniques can therefore be applied depending on the interest in different chemical groups. However the differences in chemical composition observed by using different variety barley straw or different extraction method were insignificant.

3.1.3.1.2 Composition difference by region

The Germany barley wax contained almost identical components to the Saffron wax, but the concentrations of each substance were slightly different. The concentration of hentriacontane, 1-hentriacontanol, octacosanal and 14,16-hentriacontanedione were hugely increased .

The spectrum of the grains containing straw has one unique peak at 25.12min which was caused by C_{23} alkane. And the concentration of beta-sitosterol at 34.74min is relatively low compare to other wax samples, makes the highest chemical content altered from beta-sitosterol or 1-hexacosanol to hentriacontane.

3.1.3.2 Relative concentration of chemicals in wax

The chemical composition of the wax layer is unique and characteristic feature of plants, the following figure shows the GC spectrum of the $SC\text{-}CO_2$ extracted Saffron wax.

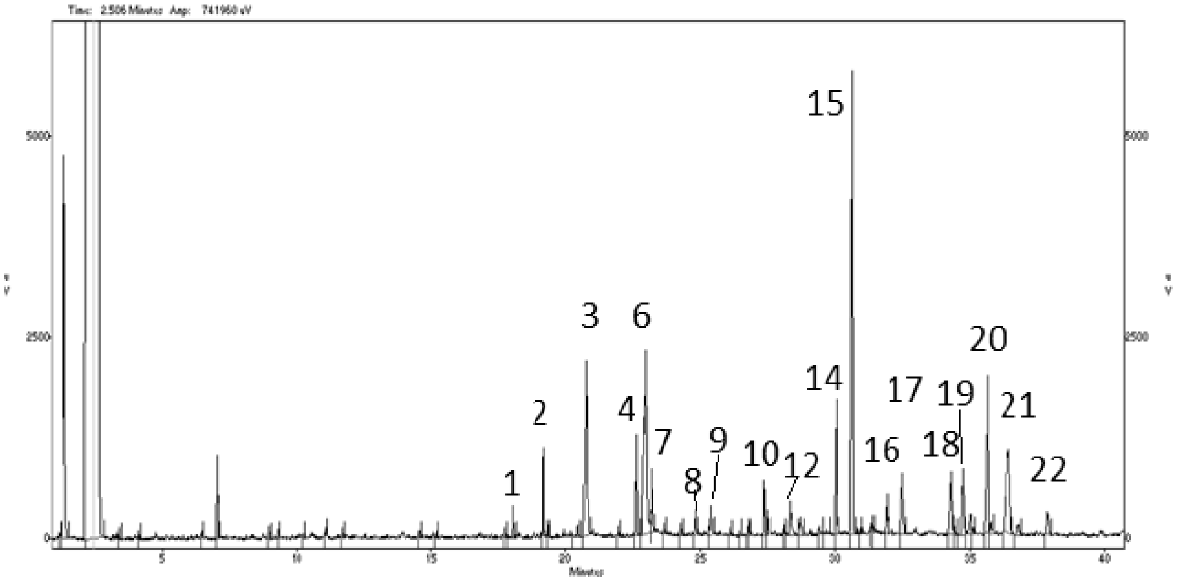


Figure 27 GC chromatogram of SC-CO_2 wax

From the figure, the quantities of the substances in the wax were not reflected due to the difficulty of calculating the area under each peak directly from the GC-MS program. Subsequently the sample was re-analysed using GC, and through which, the area under each peak was found using the method stated in section 2.4.2. The concentration was calculated by comparing areas under the individual peak to the total peak area of the sample.

Peaks observed at the left of peak 1 were caused by contamination, thus their areas were not accounted for the total peak area. It was not possible to determine peak 5, peak 11 and peak 13 in GC-MS (Figure 14), such that they were omitted from the peak percentage calculation. Instead of an appearance of a doublet in GC-MS spectrum, Peak 5 and peak 6 were single peaks in GC. Peak 11 and peak 13 were neglected due to their small areas. In order to investigate the potential of being as a raw material in cosmetic industry, it is necessary to fully understand the property of this SC-CO_2 extracted Saffron wax. The percentages of high concentrated chemical and chemical groups are tabulated in Table 8.

Table 8 GC-MS wax peak area

Chemical groups %	Peak	Chemical compound	GC Peak area	Percentage%
Fatty acids 32.5	1	tetradecanoic acid	1063	1.0
	2	2-pentadecanone,6,10,14-trimethyl	2537	2.3
	3	hexadecanoic acid	10432	9.6
	6	9-octadecenoic acid	17118	15.7
	7	octadecanoic acid	3249	3.0
	8	eicosanoic acid	929	0.9
Fatty alcohol 14.6	15	1-hexacosanol	3821	14.6
β-diketone 11.3	21	14,16-hentriacontanedione	12318	11.3
Sterols 17.3	18	cholesta-3,5-diene	4098	3.8
	19	stigmasterol	3598	3.3
	20	beta-sitosterol	9391	8.6
	22	26-(Acetyloxy)-cholest-4-en-3-one	1721	1.6
Aldehydes 6.0	9	docosanal	1330	1.2
	14	hexacosanal	3821	3.5
	16	octacosanal	1369	1.3
Alkanes 4.3	10	pentacosane	1449	1.3
	17	hentriacontane	3209	3.0
Stigmasterol 11.3	19	stigmasterol	3598	11.3
Other group	4	phytol	2748	2.5
Total peak area(all peaks in GC)			***108719***	***100***

The compounds listed in Table 8 accounted for 90.1 % of the total peak area in GC, this provided the confidence to conclude that most chemical components in the wax had been identified.

It is arguable that whether peak 6 was representing a single compound of 9-octadecenoic or, more likely, an overlapping result of 9,12-octadecenoic acid and 9-octadecenoic acid. The latter combination eluted at close retention time such that only a single peak was observed in GC spectrum instead of a doublet in GC-MS previously in Figure 14. Fatty acids were considered to a dominate group, which contributed to 32.49% of the total percentage. In addition, the SC-CO_2 wax sample also contained a great percentage of sterols (17.3%), followed by fatty alcohol (14.6%), and 14,16-hentriacontanedione (11.3%). As another constituent, aldehydes were, however, unstable and could easily decompose to acids during storage; so that aldehyde and alkane were the least in concentration among the groups in wax, only accounted for

6.0%, and 4.3% respectively. The chemical concentrations in the sample are re-illustrated graphically in the figure below for a clearer representation.

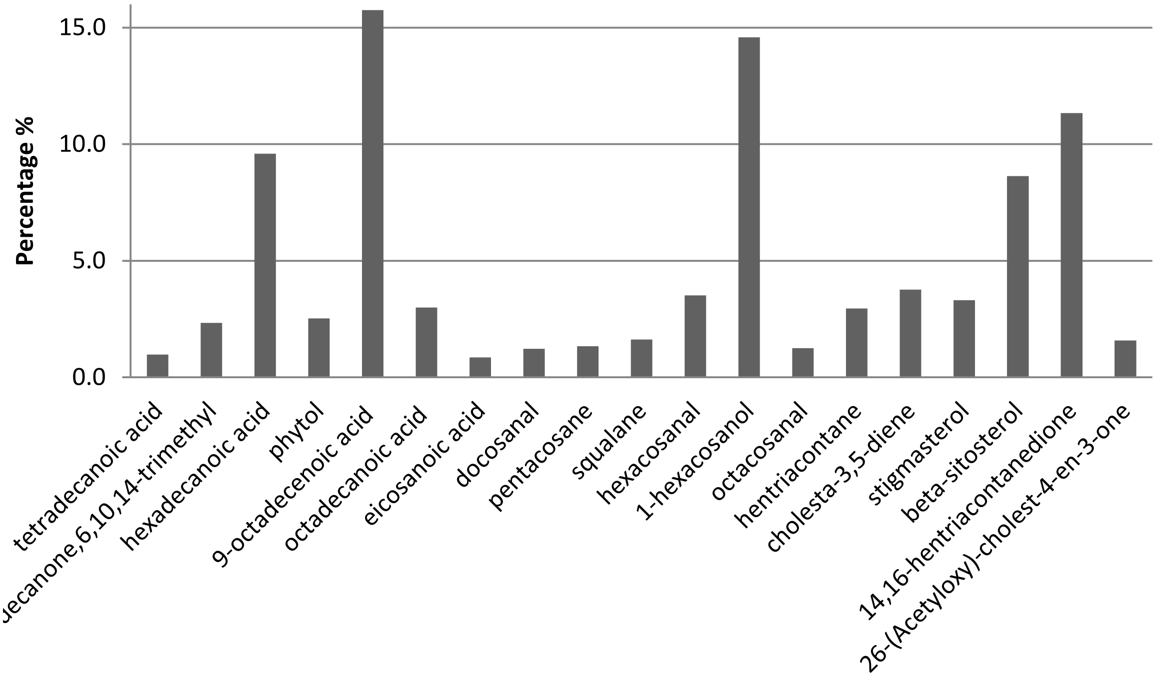


Figure 28 Percentage of chemicals in wax

3.2 Comparison of Saffron barley to other straw

With the aim of investigating the richness of the wax content on the straw surface, SC-CO_2 extracted Carat barely, oat and rape straw were analysed to evaluate their wax yields. The straws were processed using the identical extracting operation setting to allow fair comparison to Saffron barley straw. Peaks in Carat barley wax's GC-MS spectrum were related to the Saffron barley's wax in search of similarities in components.

3.2.1 Potential yield

An average of 1.2g (a yield of 1.2%) wax was collected from 100g Saffron barley. A higher yield of 2.1g (2.4%) was obtained from 87.7g Carat barley straw. In contrast, the oat straw generated a lower yield of 1.15 g (1.01%); and rape straw was observed to have the minimum yield (0.97%), only 3.0 g crude wax was obtained from 308g straw. These results are illustrated in Figure 29.

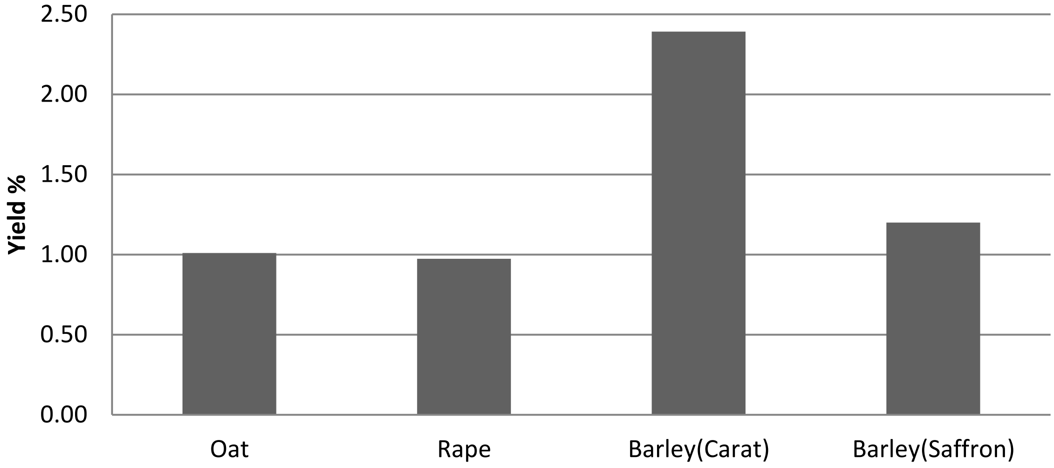


Figure 29 Comparison of SC-CO_2 wax yield

3.2.2 Wax components

The chemical compounds in Saffron barley straw wax were expected to be at presence in every variety of barley straw. To support the argument, Saffron wax was compared to a readymade variety Carat barley wax which had been previously tested in Rothamsted Agriculture Lab (shown in Figure 30). This analysis also offered an opportunity to investigate the reproducibility of the wax components from different variety.

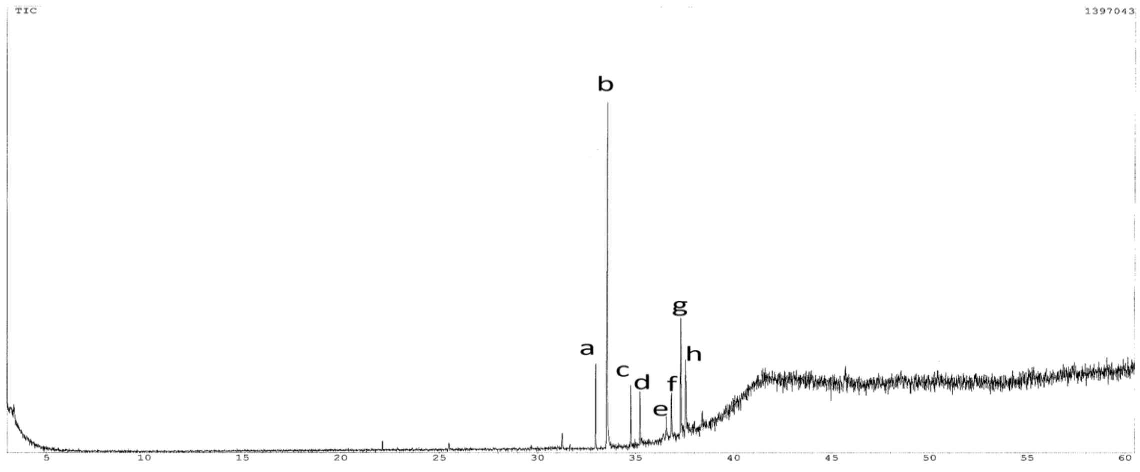


Figure 30 Carat Barley SC-CO2 crude wax GC-MS spectrum

The observed peaks was identified and tabulated below. The results are referred back to the Saffron GC-MS spectrum.

Table 9 Correlate Carat barley peaks with Saffron barley peaks (Figure 14)

Peak	Peak related to Figure 14	Compounds
a	14	hexacosanal
b	15	1-hexacosanol
c	16	octacosanal
d	17	Hentriacontane
e	18	cholesta-3,5-diene
f	19	Stigmasterol
g	20	beta-sitosterol
h	21	14,16-hentriacontanedione

From the table, a close correlation of peaks between variety of Carat barley wax and Saffron barley wax was found. Because two sets of wax were analysed using separate GC-MS machine under different conditions, there were significant differences in peak strength, but all peaks from Carat barley wax spectrum could be related to Saffron barley wax spectrum.

3.3 Semiochemicals

Literature reported that two of the hydrocarbons *n*-tricosane ($C_{23}H_{48}$) and *n*-pentacosane ($C_{25}H_{52}$) in ladybird footprint, are induced aphid avoidance.[1] Pentacosane was also found in Saffron barley wax, and previous study stated wheat straw wax comprises a similar blend of hydrocarbon compounds to ladybird footprint that consist of straight-chain and branched hydrocarbons[20]. No studies, however, have carried on other straw or fruit wax. It is therefore interesting to investigate if other straw or fruit wax comprises a similar blend of compounds, consequently causing the aphid avoidance.

Three kinds of wax extracted from wheat, Carat barley and apple peel (supplied by NATECO Germany) was analysed using column chromatography to test the existence of the valuable semiochemicals. The resultant extracts were retested by GC-MS and analysed in the following sections.

3.3.1 Carat barley extract

The GC-MS spectrum of the column chromatography extract obtained from Carat barley straw wax is shown below.

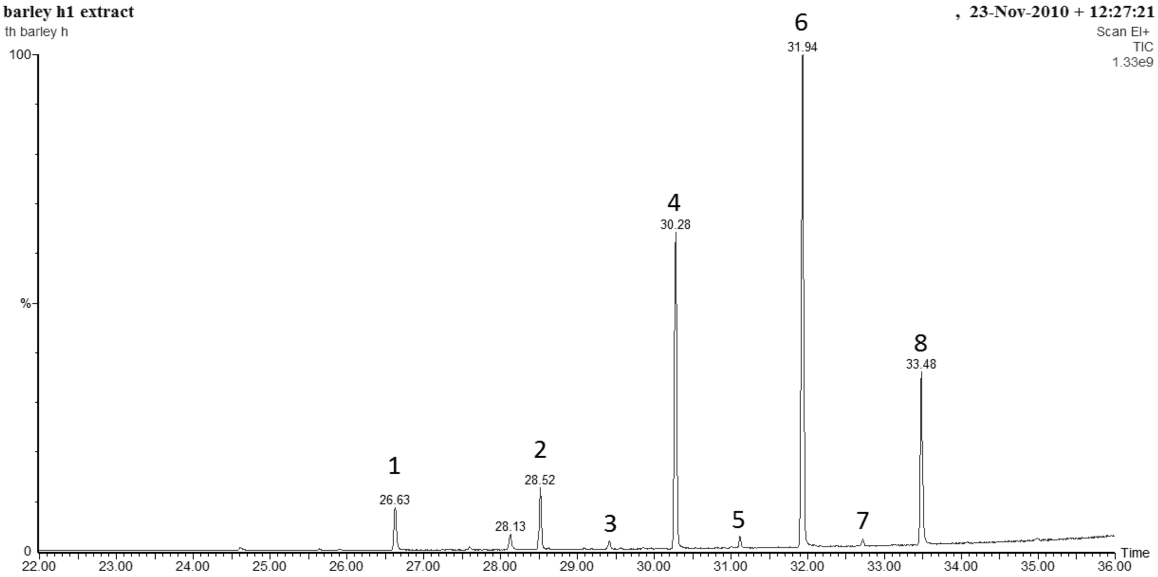


Figure 31 GC-MS of Carat barley wax hexane extraction

It should be noted that the peak at 28.13min in Figure 31 was contaminated by squalane residues in the GC-MS machine. This peak was also common to previous wax spectrum and later bio-oil spectrum. The remaining peaks demonstrated that the Carat barley extract mainly consist of heavy alkane compounds. Eight alkanes with carbon number ranging from C_{25} to C_{33} were identified from the spectrum; subsequently their KI were calculated below.

Table 10 Major components in barley hexane extract

peak	tunknown	KI	n	N	tn	tN	Compounds	
1	26.63	2502	24	26	25.63	27.59	Pentacosane	C25
2	28.52	2702	26	28	27.59	29.42	Heptacosane	C27
3	29.42	2800	26	28	27.59	29.42	Octacosane	C28
4	30.28	2908	28	30	29.42	31.01	Nonacosane	C29
5	31.11	2999	28	30	29.42	31.12	Triacontane	C30
6	31.94	3102	30	32	31.12	32.73	Hentriacontane	C31
7	32.72	3199	30	32	31.12	32.73	Dotriacontane	C32
8	33.48	3301	32	34	32.73	34.22	Tritriacontane	C33

Although the presence of *n*-pentacosane was not seen from Figure 30, it was confirm by column chromatography (peak 1). Pentacosane had also been found in Saffron barley wax, so did the hentriacontane. This proved again the similarity of wax from different barley variety.

Furthermore, between the retention time 18min and 30min in GC-MS spectrum, there were also many long-chain alkanes and branch-chain alkanes represented small peaks with relatively low concentration. Although these peaks had not been reported previously, and were not abundant in concentration, they were also being investigated for a thorough understanding.

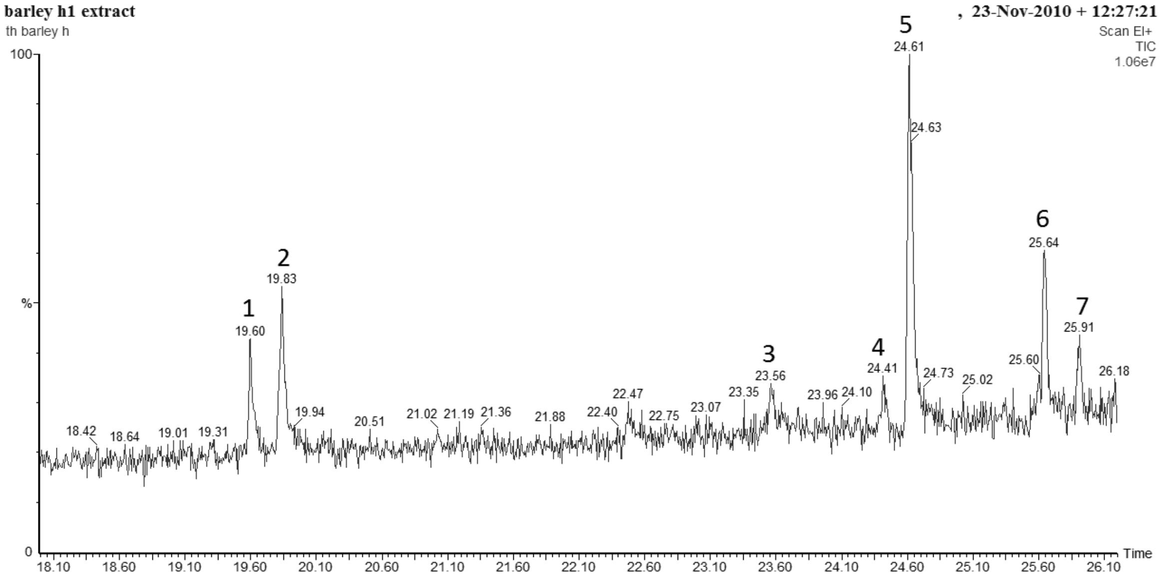


Figure 32 GC-MS of low concentration peaks in barley wax hexane extract

The KI numbers of these peaks were calculated, their mass spectra were investigated and their methyl-branch alkane's structures were predicted based on their KI and their spectra in Table 11.[65]

Table 11 Low concentration peaks of barley wax extraction

Peak	tunknown	KI	n	N	tn	tN	Compounds
1	19.6	1871	18	20	18.71	21.21	6,10-/6,18-dimethyl octadecane
2	19.83	1890	18	20	18.71	21.21	4,8-/4,16-dimethyl octadecane
3	23.56	2205	22	24	23.51	25.63	Docosane
4	24.41	2285	22	24	23.51	25.63	8,12,16-trimethyl tetratetracontane
5	24.61	2304	22	24	23.51	25.63	Tricosane
6	25.64	2401	24	26	25.63	27.59	Tetracosane
7	25.91	2429	24	26	25.63	27.59	10-/12-/14-/16-methyl tetracosane

Some of these small peaks were characterized as C_{22}, C_{23} and C_{24} long chain alkanes. Other peaks were predicted to be branch chain alkane of 6,10-/6,18-dimethyl octadecane, 4,8-/4,16-dimethyl octadecane, 8,12,16-trimethyl tetratetracontane and 10-/12-/14-/16-methyl tetracosane.[65]

3.3.2 *Wheat extract*

Seven alkanes were found in wheat extract, from C_{27} to C_{33}. C_{31} was the most abundant alkane from the hexane extract. The contaminated squalane peak was neglected.

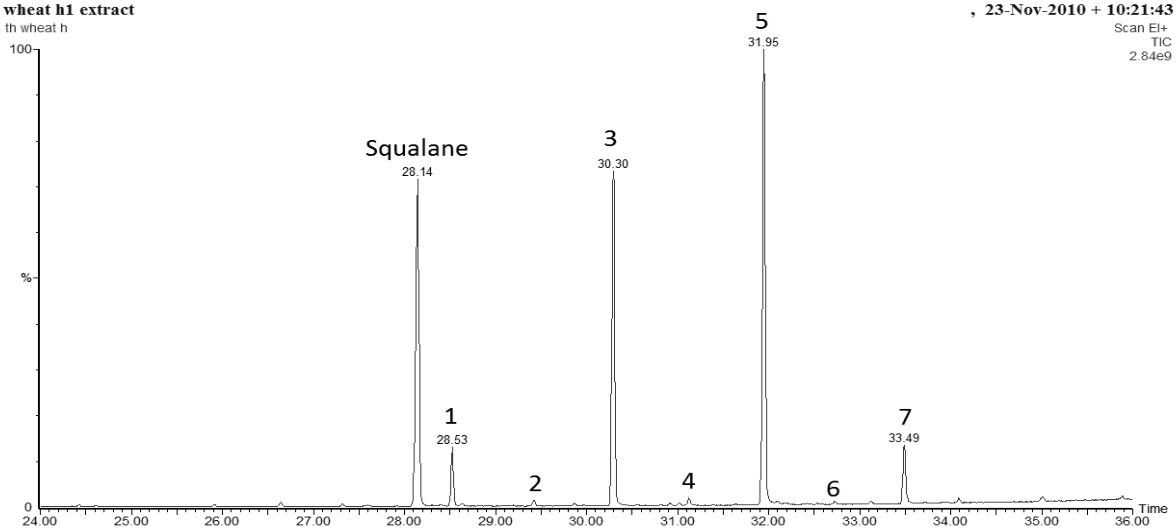


Figure 33 GC-MS of wheat wax hexane extract

Table 12 Major components in wheat hexane extract

peak	tunknown	KI	n	N	tn	tN	Compounds
1	28.53	2703	26	28	27.59	29.42	Heptacosane C_{27}
2	29.43	2801	28	30	29.42	31.01	Octacosane C_{28}
3	30.3	2911	28	30	29.42	31.01	Nonacosane C_{29}
4	31.13	3001	30	32	31.12	32.73	Triacontane C_{30}
5	31.95	3103	30	32	31.12	32.73	Hentriacontane C_{31}
6	32.73	3200	32	34	32.73	34.22	Dotriacontane C_{32}
7	33.49	3302	32	34	32.73	34.22	Tritriacontane C_{33}

Unexpectedly, the semiochemicals responsible for the aphid avoidance, n-tricosane (C_{23}) and n-pentacosane (C_{25}), was not seen as high concentration peaks from Figure 33, which indicated that the two compounds were not abundant. However previous research had proven that time spent by parasitoids on the wheat wax treated plant was reduced compared to normal plants.[20] To further prove the semiochemicals' existence, the low concentrated peaks in spectrum region from 20min to 28min were studied (Figure 34). It should be noted that peaks at 21.02min, 22.8min, 24.42min, 27.32min and 27.6min were cause by the contaminated siloxane compounds.

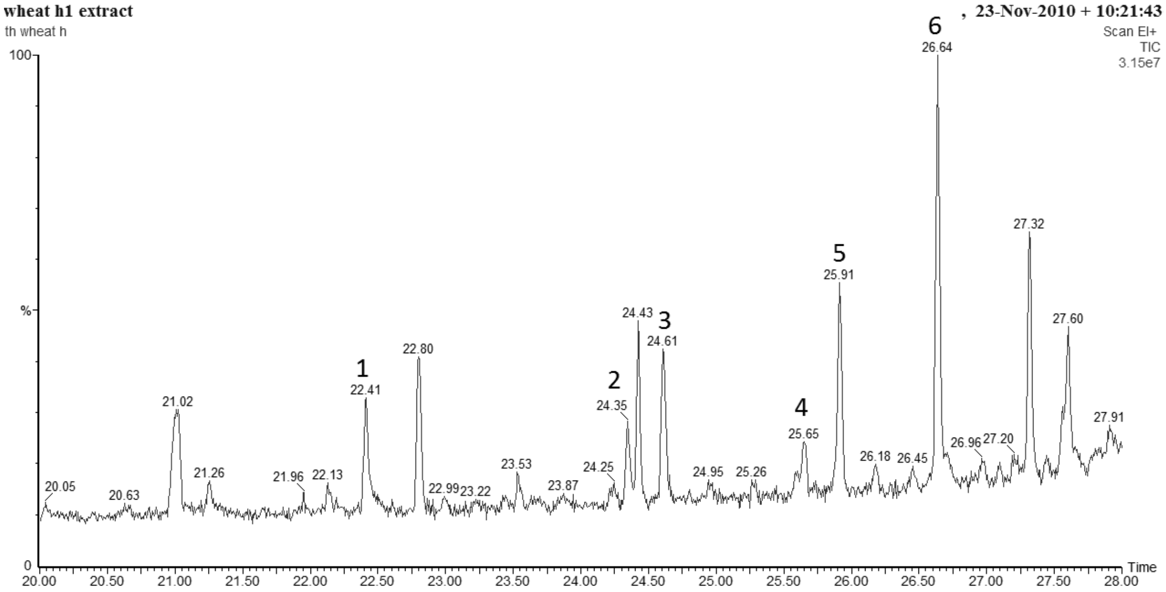


Figure 34 GC-MS of wheat wax hexane extract

Table 13 Low concentration peaks of wheat wax extract

Peak	tunknown	KI	n	N	tn	tN	Compounds
1	22.41	2104	20	22	21.21	23.51	heneicosane
2	24.35	2279	22	24	23.51	25.63	12,16,20-/10,14,18-trimethyl docosane
3	24.61	2308	22	24	23.51	25.63	tricosane
4	25.65	2402	24	26	25.63	27.59	tetracosane
5	25.91	2429	24	26	25.63	27.59	10-/12-/14-/16-methyl tetracosane
6	26.64	2502	24	26	25.63	27.59	pentacosane

The C_{23} and C_{25} were found as peak 3 and peak 6 respectively with C_{25} having a highest concentration in Figure 34. Wheat wax extract also contained C_{24} (tetracosane) and branch chain alkane 10-/12-/14-/16-methyl tetracosane.

3.3.3 Apple extract

As shown in Figure 35, six alkane peaks were included in apple extract, but only alkanes C_{27} and C_{29} possessed great strength.

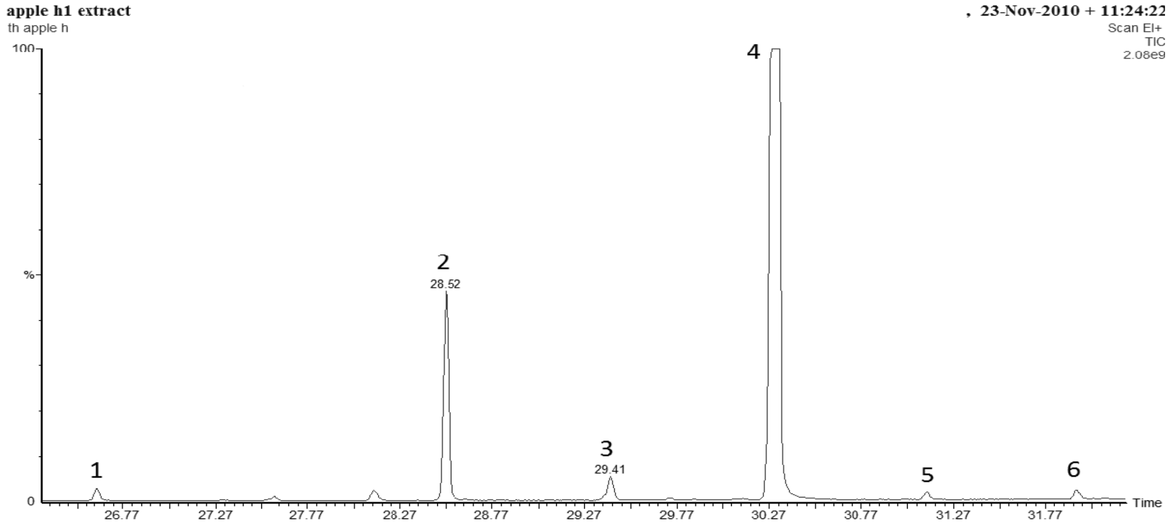


Figure 35 GC-MS of apple wax extract

Table 14 Major components in apple hexane extract

peak	tunknown	KI	n	N	tn	tN	Compounds
1	26.64	2503	24	26	25.63	27.59	Pentacosane C_{25}
2	28.52	2702	26	28	27.59	29.42	Heptacosane C_{27}
3	29.41	2799	26	28	27.59	29.42	Octacosane C_{28}
4	30.32	2913	28	30	29.42	31.01	Nonacosane C_{29}
5	31.13	3001	30	32	31.12	32.73	Triacontane C_{30}
6	31.94	3102	30	32	31.12	32.73	Hentriacontane C_{31}

C_{25} alkane also had a small concentration in apple wax, and C_{23} peak cannot even been seen. So the low concentrated peaks were analysed as indicated below.

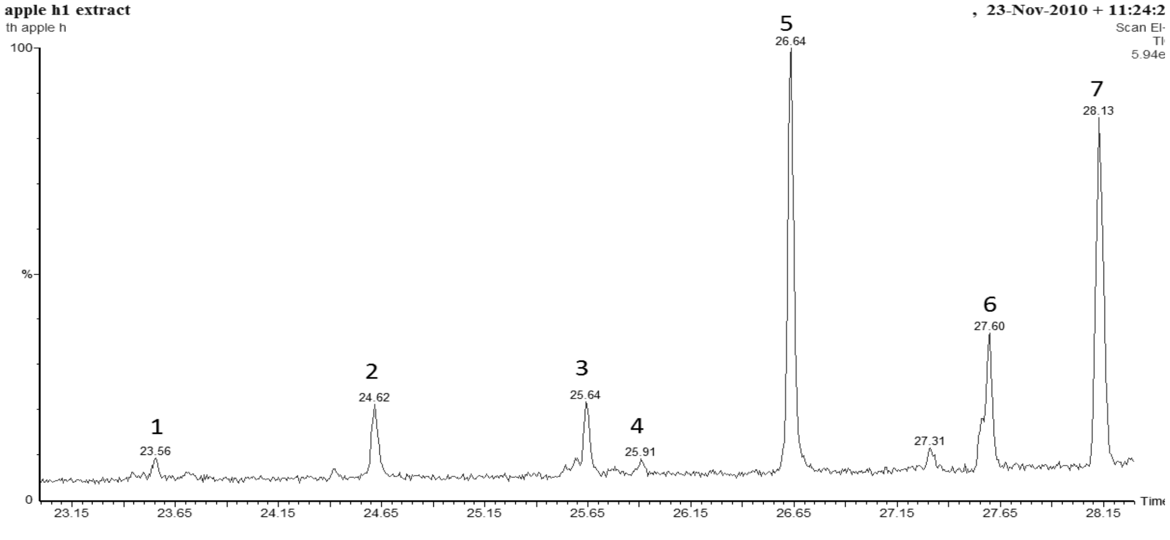


Figure 36 GC-MS of apple wax hexane extract

Table 15 Low concentration peaks of apple wax extraction

Peak	tunknown	KI	n	N	tn	tN	Compounds
1	23.56	2205	22	24	23.51	25.63	docosane
2	24.62	2305	22	24	23.51	25.63	tricosane
3	25.64	2401	24	26	25.63	27.59	tetracosane
4	25.91	2429	24	26	25.63	27.59	10-/12-/14-/16-methyl tetracosane
5	26.64	2503	24	26	25.63	27.59	pentacosane
6	27.6	2601	26	28	27.59	29.42	hexacosane
7	28.13	2659	26	28	27.59	29.42	4-methyl hexacosane

Peaks at 25.91, 27.31min were caused by siloxane. From Figure 36, C_{25} and C_{23} could be seen clearly. Rest peaks were caused by both C_{22}, C_{24} and C_{26} long chain alkane, and branch chain alkane of 10-/12-/14-/16-methyl tetracosane and 4-methyl hexacosane.

3.4 Pyrolysis Compounds

In this section, straw was further processed to explore its potential of being a renewable energy source. Bio-oil and char were first collected from Milestone microwave machine as products. Both the raw straw (straw without SC-CO_2 extraction) and the de-waxed straw were used as starting materials separately for pyrolysis production. The de-waxed straw after SC-CO_2 extraction had been traditionally regarded as waste, however, it was able to be used as reagent for pyrolysis. This not only saved the disposing costs of the straw but also avoided the expenditure of purchasing new reagents. The bio-oil product from both straws were analysed by GC-MS to examine their chemical compositions and differences, while the by-products bio-gas and char were analysed by FTIR.

The research was also focused on investigating the effect on product output and compositions when varying reaction temperatures. The Milestone microwave machine had drawbacks of large amount of starting material were required, long residence time and complex cleaning procedures after each pyrolysis. In addition, though improvements had been made on Milestone system to increase reaction efficiency, it was still unable to collect the bio-gas generated from during the pyrolysis. Another microwave machine, CEM, was therefore employed to work on the tasks that had not been accomplished by Milestone microwave.

In contrast to Milestone, CEM microwave has advantages of shorter residence time, less starting material required, more convenient when cleaning and, most significantly, being capable of collecting the bio-gas generated during the pyrolysis of straw. A moderate temperature (140°C) was tested on pyrolysis reaction initially, and the bio-oil collected from CEM microwave was analysed by GC-MS. The comparison of two bio-oil spectra from the microwave machines confirmed that the pyrolysis through CEM had been successful, as its bio-oil compositions were similar to the Milestone. Therefore the effect of changing temperature on pyrolysis products was tested confidently using CEM microwave machine. Without any disruption caused by biogas during the process, the pyrolysis was repeated with various temperature settings. The biogas generated during the reaction was collected and analysed by FTIR.

3.4.1 Bio-oil

Bio-oil being the main product of straw pyrolysis, It was collected from microwave as a dark brown fluid with a distinctive smoky odour.

3.4.1.1 Milestone bio-oil

An improvement was made to the Milestone microwave machine by adding a vacuum pump to its system, which increased the yield of the bio-oil from 4.5g bio-oil (50g starting material, yield 9%) to 5.5g oil (11%). Redesign the microwave had greatly increased the efficiency without having an effect on product quality. The microwave produced consistent pyrolysis results before and after the improvement was made.

3.4.1.1.1 Effect of wax on bio-oil

Bio-oil was extracted from raw straw and from de-waxed straw. Then oil samples form the two straws were separately analysed by GC-MS and the spectra in Figure 37 showed the differences in the oil produced.

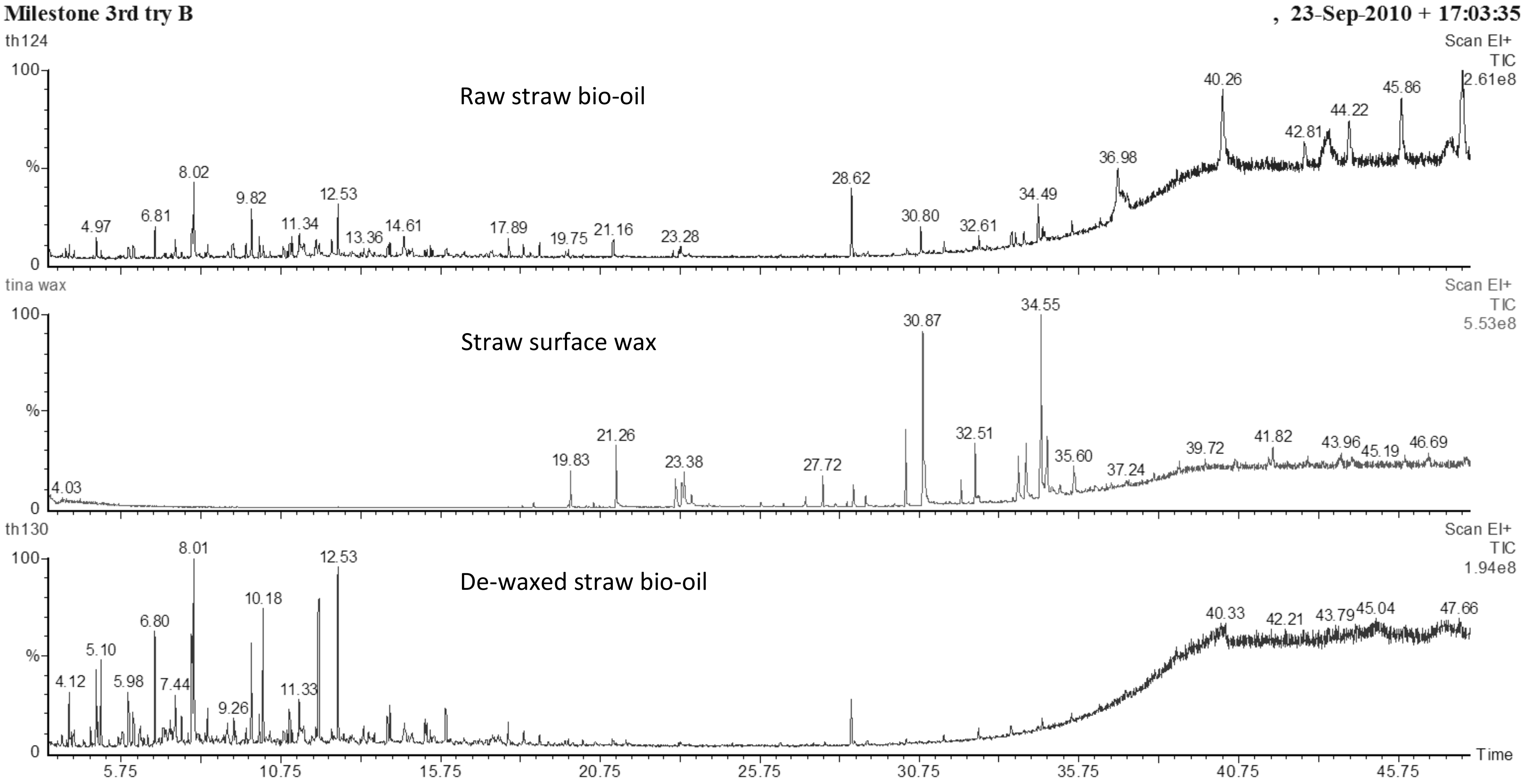


Figure 37 Bio-oil GC-MS spectra comparisons (raw straw, wax and de-waxed straw)

In the region between the origin and 18min in the spectra, raw straw oil and de-waxed oil were observed being alike patterns. Differences only arise after the retention time of 19min as seen in Figure 37. Peaks in raw straw oil began to share considerable similarities with the surface wax peak after retention time 19min; peaks with close retention times were observed from the two spectra. The de-waxed straw oil's spectrum, however, showed an almost blank region in this area. It was suffice to conclude that peaks in raw straw oil after 19min was only caused by the wax presence.

In the previous wax analysis section of chapter 3, study was mainly focused on the alkane peak appearances after retention time 19min (Figure 13). However, for bio-oil analysis, the majority of the peaks were shown before 18min (Figure 38). This represented that peaks in wax and in bio-oil were caused by different chemicals. The hydrocarbon compounds in wax elute from GC-MS column at different time to the bio-oil phenolic compounds.

The deviation of bio-oil from wax was caused by presence of aromatic organic molecules (such as phenol) instead of alkanes and fatty acid as dominating compounds. Phenolic compounds in bio-oil eluted quicker than the wax components, so the first 20 minutes of chromatogram was crucial for bio-oil analysis. The de-waxed bio-oil spectrum also presented a clearer pattern than the raw straw. As the difference between raw and de-waxed straws was only caused by surface wax, in order to simplify the analysis and have a more accurate result, de-waxed straw were decided to be used as starting material for the pyrolysis of bio-oil.

3.4.1.1.2 Peaks analysis

The de-waxed straw's bio-oil spectrum in Figure 37 was listed independently in Figure 38 for detailed analysis.

Peak patterns in the bio-oil GC-MS spectrum in Figure 38 were identified and tabulated in Table 16. In the de-waxed bio-oil GC-MS spectrum, presented substances of phenol and vanillin have been reported for the existence in bio-oil. Additionally, some of non-reported compounds as well shared the mentioned structure similarity. In this research, only 2-furanmethanol and 2-methoxy-4-vinylphenol were identified. According to previous study, the absence of furfural and 2-methoxy-4-methyl phenol[35] is likely to be caused by hydrolysis of bio-oil during the sample collection and storage due to its unstable property.

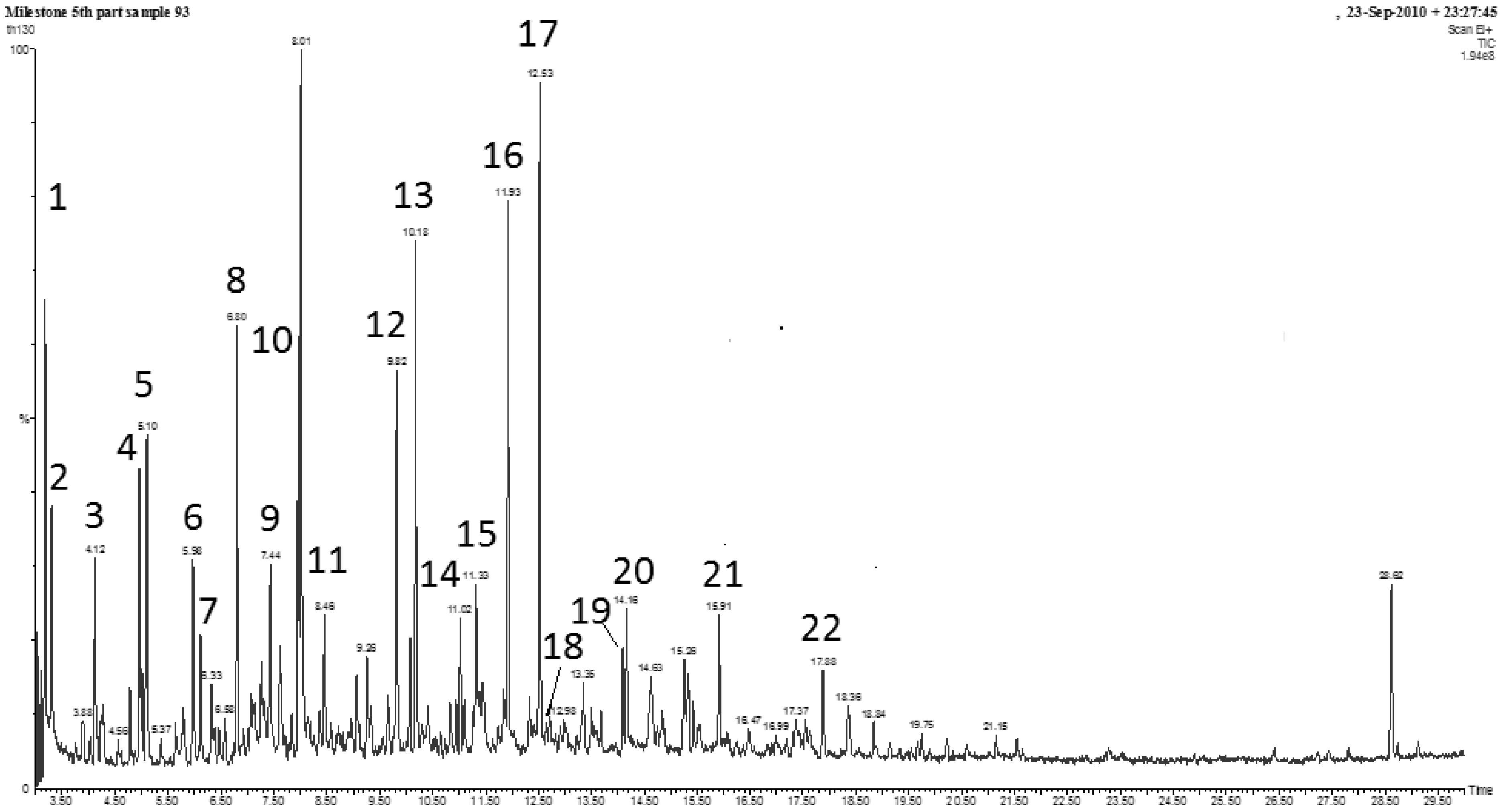


Figure 38 GC-MS of de-waxed straw bio-oi

Five compounds (phenol, 2-methoxy phenol, 2-methoxy-4-vinylphenol, 2,6-dimethoxyphenol, vanillin) were confirmed by matching both the MS and KI to literature, online database as well as the standards that had been run in the lab. The majority of the substances had matched their MS and KI to the references and the rest had their MS matched predictions but no records of KI were found.

Substances were identified from Figure 38 and tabulated in Table 16. The KI of each compound was calculated corresponding to their retention time. The tunknown and KI data obtained from the GC-MS spectra was checked against scientific database, and results confirmed the majority of the substances. Furthermore, pure chemicals as standard references were tested under identical condition and matched to the lab data to ensure correct identification of the chemical components in the bio-oil.

Table 16 Chemical components of bio-oil from wax extracted straw (✓: standard matched, N/A: unavailable from literature or chemical database)

Peak	tunknown	KI	Compounds	Mass spectrum	KI	Standard
1	3.17	828	acetic acid, (acetyloxy)-	✓	N/A	N/A
2	3.31	835	cis-1,4-cyclohexanediamine	✓	N/A	N/A
3	4.12	880	2-furanmethanol	✓	865	N/A
4	4.96	925	1h-imidazole,4,5-dihydro-2-methyl	✓	N/A	N/A
5	5.1	933	2-cyclopenten-1-one,2-hydroxy	✓	919	N/A
6	5.98	981	Phenol	✓	980	✓
7	6.12	989	Unidentified peak	N/A	N/A	N/A
8	6.8	1027	1,2-Cyclopentanedione, 3-methyl	✓	1043	N/A
9	7.44	1063	Furan, tetrahydro-2-(methyoxymethyl)-	✓	N/A	N/A
10	7.97	1093	2-methoxy phenol	✓	1090	✓
11	8.46	1120	2-cyclopenten-1-one,3-ethyl-2-hydroxy-	✓	1100	N/A
12	9.82	1197	1,2-benzenediol	✓	1197	N/A
13	10.18	1218	Benzofuran,2,3-dihydro-	✓	1226	N/A
14	11.02	1268	1,2-benezenediol,3-methoxy-	✓	1269	N/A
15	11.33	1286	Phenol, 4-ethyl-2-methoxy	✓	1286	N/A
16	11.93	1321	2-methoxy-4-vinylphenol	✓	1317	✓
17	12.53	1356	Phenol,2,6-dimethoxy-	✓	1357	✓
18	13.35	1405	vanillin	✓	1422	✓
19	14.09	1453	Benzenamin,2-methoxy-4-nitro	✓	N/A	N/A
20	14.16	1458	Phenol,2-methoxy-4-(1-propenyl)	✓	1438	N/A
21	15.91	1572	4-methyl-2,5-dimethoxybenzaldehyde	✓	N/A	N/A
22	17.88	1711	Phenol,3,5-dimethoxy-4-(2-propenyl)-	✓	N/A	N/A

Discussions were made for substances of significant interest or uncertain structure in the ascending order of their respective retention time (RT), connections between peaks were also mentioned.

RT of 6.12min: the substance which caused peak 7 was not identified. It was suggested the compound contained nitrogen group, it was likely to be originated from an amino acid. However, no exact match could be correlated to its MS.

RT of 7.97min: the KI of this substance at peak 10 was calculated to be 1093. The closest chemical to this substance was 2-methoxy phenol which had a KI value of

1090. The standard 2-methoxy phenol was analysed with GC-MS under the same condition as the bio-oil sample. After a same MS spectrum pattern was observed, the peak was indeed confirmed to be 2-methoxy phenol.

RT of 8.01min: the substance was not identified. It had a featureless spectrum comprised of m/z 41,43,44,57 which imposed difficulty for its identification.

RT of 8.46min: the substance at peak 11 was predicted to be 3-ethyl-2-hydroxy-2-cyclopenten-1-one. The calculated KI of 1120 was similar to its standard value of 1100. This chemical had an additional ethyl group compared to peak 5 of 2-hydroxyl-2-cyclopenten-1-one at 5.1min (KI 933). The ethyl group is a possible cause of shifting the KI by 187.

RT of 9.82min: the substance at peak 12 was identified to be 1,2-benzenediol after matching its MS spectrum and KI with standard data.

RT of 11.02min: the substance at peak 14 was predicted to be 3-methoxy-1,2-benezenediol by matching its KI of 1268 and MS spectrum to the standard data from online database. This chemical had an additional 3-methoxy group compared to peak 12 of 1,2-benzenediol with a KI of 1197. The methoxy group is a possible cause of shifting the KI to 1268.

RT of 11.33min: the substance at peak 15 was predicted to be 4-ethyl-2-methoxy by matching its KI of 1286 and MS spectrum to the standard data from online database. This chemical had an additional ethyl group compared to peak 10 of 2-methoxy phenol with a KI of 1093. The ethyl group is a possible cause of shifting the KI to 1286.

RT of 14.16min: the substance at peak 20 was predicted to be Phenol,2-methoxy-4-(1-propenyl) by matching its KI of 1458 and MS spectrum to the standard data from online database. This chemical had an additional 1-propenyl group compared to peak 10 of 2-methoxy phenol with a KI of 1093. The ethyl group is a possible cause of shifting the KI to 1458.

RT of 17.88min: the substance at peak 22 was initially suggested to be phenol,2,6-dimethoxy-4-(2-propenyl) which had a lower KI value of 1602 by machine program library, but it was determined to be phenol,3,5-dimethoxy-4-(2-propenyl). This decision was justified by the shielding effect which was caused by two bulky methoxy group at 2, 6 positions. The effect reduced hydrogen bonding formed between OH group in phenol and the stationary phase of the column, subsequently resulted in the lower KI value of 1602.

3.4.1.1.3 Bio-oil substance concentration

GC was used to determine the concentrations of different chemicals in the bio-oil, and the result is shown in Figure 39.

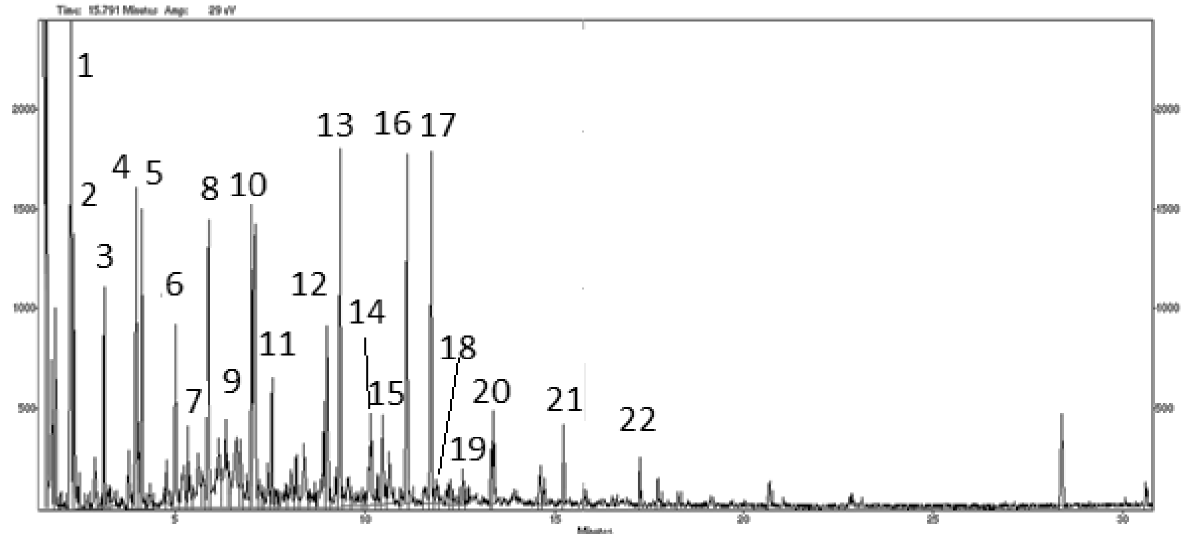


Figure 39 GC chromatogram of bio-oil

The KI values were used to relate the peak patterns to the substances identified in the previous GC-MS spectrum (Figure 38). Using the method stated in section 2.4.2, the area under the curve for each substance was tabulated in Table 17, and its relative concentration was calculated by dividing the substance peak area with the sum.

Table 17 Peak area and percentage in bio-oil

Peak	Chemical compound	GC Peak area	Percentage%
1	acetic acid, (acetyloxy)-	5828	7.0
3	2-furanmethanol	1786	2.2
5	2-cyclopenten-1-one,2-hydroxy	3308	4.0
6	Phenol	2135	2.6
8	1,2-Cyclopentanedione, 3-methyl	4327	5.2
9	furan, tetrahydro-2-(methyoxymethyl)-	2509	3.0
10	2-methoxy phenol	2815	3.4
11	2-cyclopenten-1-one,3-ethyl-2-hydroxy-	1342	1.6
12	1,2-benzenediol	2414	2.9
13	Benzofuran,2,3-dihydro-	3477	4.2
14	1,2-benezenediol,3-methoxy-	1860	2.2
15	Phenol, 4-ethyl-2-methoxy	1379	1.7
16	2-methoxy-4-vinylphenol	3696	4.5
17	Phenol,2,6-dimethoxy-	3823	4.6
18	Vanillin	415	0.5
19	benzenamin,2-methoxy-4-nitro	565	0.7
20	Phenol,2-methoxy-4-(1-propenyl)	941	1.1
21	4-methyl-2,5-dimethoxybenzaldehyde	696	0.8
22	Phenol,3,5-dimethoxy-4-(2-propenyl)-	433	0.5

The listed peak contributes 52.7% in bio-oil. Literature showed acetic acid were the most dominating peak (8.5%) in the bio-oils from barley straw[35]. In this research, acetoxyacetic acid showed the highest concentration in the sample, about 7%, followed by 3-methyl-1,2-cyclopentanedione (5.2%), 2,6-dimethoxyphenol(4.6%), 2-methoxy-4-vinylphenol(4.45%), benzofuran,2,3-dihydro-(4.19%), 2-cyclopenten-1-one, and 2-hydroxy (3.9%).

3.4.1.2 Bio-oil from CEM

To test the bio-oil reproducibility and investigate the effect of changing reaction temperature on bio-oil, CEM microwave was employed for its advantages of shorter reaction time, less starting material needed and more convenient on cleaning.

The bio-oil collected from CEM microwave strongly resembled the oil from Milestone in odour and colour, their chemical components were analysed. CEM machine was then used for testing the effect on pyrolysis production with changing of reaction temperature.

Reaction was initially tested on CEM microwave at temperature 140°C. The bio-oil formed in the pyrolysis was tested by GC-MS. In Figure 40, CEM bio-oil spectrum was listed with Milestone spectrum, and correlations between them were discussed.

The two spectra were not very similar from the first impression although they were run by the same GC-MS machine. One of the possible reasons for the difference was that they were tested at different period of time, and the retention time of the same substance was likely to shift by 1 to 2 min. Peaks' mass spectra were analysed, relationship between them was represented in Table 18.

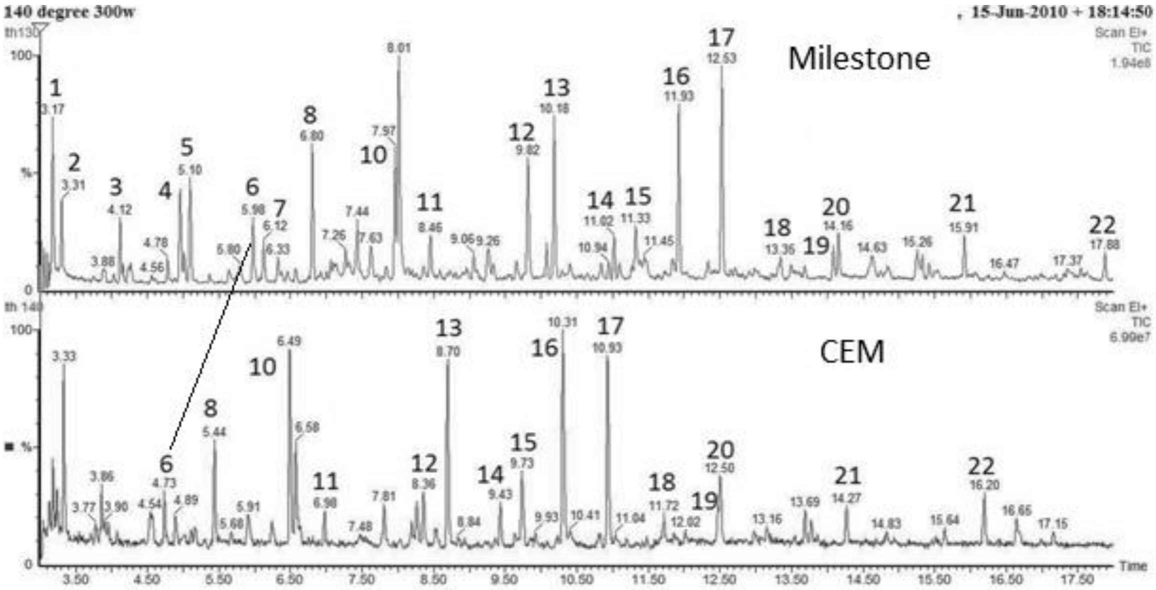


Figure 40 GC-MS spectrum of CEM bio-oil and Milestone bio-oil

It showed that peaks in CEM bio-oil spectrum had shifted about 1.2 min to the left than in the Milestone spectrum. Most peaks that existed in milestone bio-oil could be found in CEM bio-oil. The first peak that have been identified in both spectra was phenol (peak 6 in Milestone spectrum), appeared at 5.98min in Milestone spectrum and 4.7min in CEM spectrum in Figure 40. Peak 6 has been proved to be phenol in previous analysis (Table 16), and its mass spectrum from both Milestone and CEM GC spectra is listed below. The two mass spectra shared a great similarity and both presented a clear molecular ion of m/z at 94, demonstrated peak 6 was caused by phenol in both spectra.

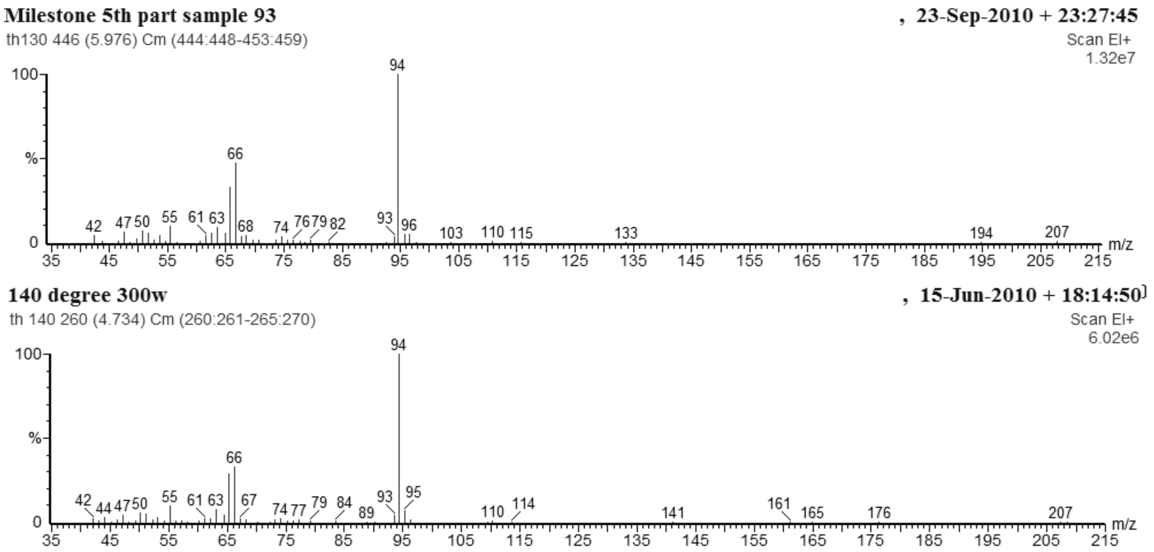


Figure 41 Mass spectrum of phenol from Milestone and CEM

Table 18 Relationship between Milestone peaks and CEM peaks in bio-oil

Peaks	Chemicals	Ret Time in Milestone/min	Ret Time in CEM/min
6	Phenol	5.9	4.7
8	1,2-Cyclopentanedione, 3-methyl	6.8	5.4
10	2-methoxy phenol	8.0	6.5
11	2-cyclopenten-1-one,3-ethyl-2-hydroxy-	8.5	7.0
12	1,2-benzenediol	9.8	8.4
13	Benzofuran,2,3-dihydro-	10.2	8.7
14	1,2-benezenediol,3-methoxy-	11.0	9.4
15	Phenol, 4-ethyl-2-methoxy	11.3	9.7
16	2-methoxy-4-vinylphenol	11.9	10.3
17	Phenol,2,6-dimethoxy-	12.5	10.9
18	Vanillin	13.4	11.7
19	benzenamin,2-methoxy-4-nitro	14.1	12.5
20	Phenol,2-methoxy-4-(1-propenyl)	14.2	12.5
21	4-methyl-2,5-dimethoxybenzaldehyde	15.9	14.3
22	Phenol,3,5-dimethoxy-4-(2-propenyl)-	17.9	16.3

However, when more and more peaks eluted, the time difference for the same peak appeared in CEM and Milestone spectrum increased from 1.2min to 1.6min, as seen in Table 18. To prove peaks related in both spectra were still caused by the same chemical, the mass spectra of peak 17 from both Milestone and CEM gas chromatograms which have the time shifting difference of 1.6min were selected and investigated in Figure 42.

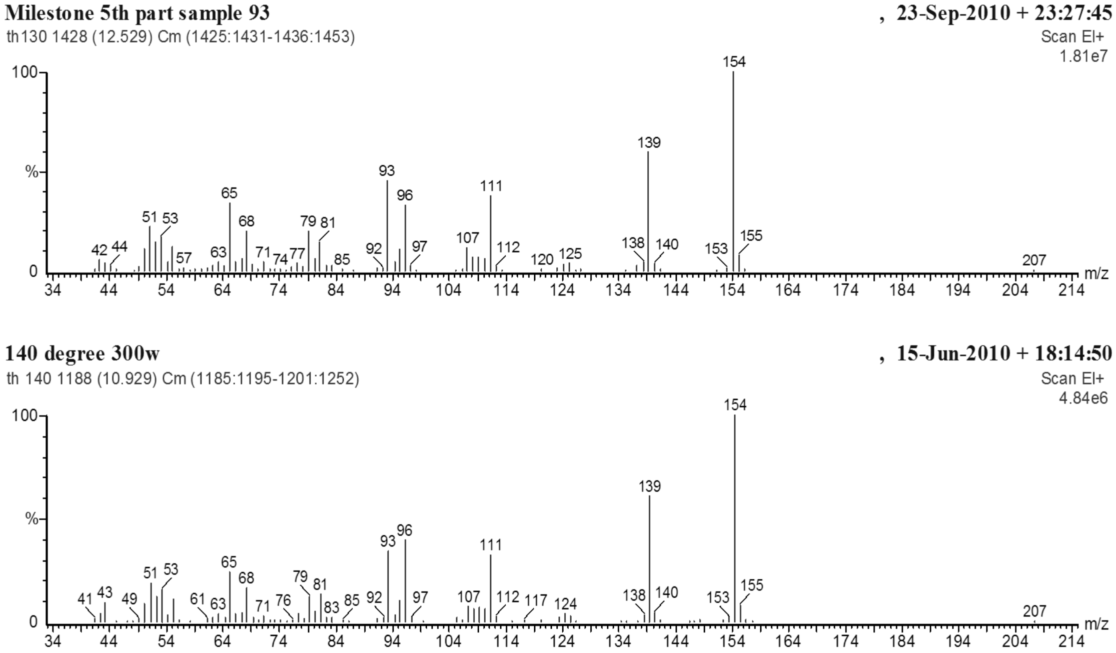


Figure 42 Mass spectrum of 2, 6-dimethoxy-phenol from Milestone and CEM

With the molecular ion of m/z at 154, it is very clear peak 17 was caused by the existence of 2, 6-dimethoxy-phenol. The insignificant m/z at 207 was caused by the contaminant as it has also been found in phenol mass spectrum.

Peaks 1 -5 in CEM spectrum before phenol could not correlate to peaks in Milestone easily. Peak 1 in Milestone oil was acetyloxyacetic acid, and was the most concentrated compound in milestone bio-oil. The same compound cannot be found in the CEM spectrum. However, there was a peak at 3.33min in CEM oil and was caused by 1-(acetyloxy)-2-propanone (confirmed both by KI and MS). Considering the similarity between them, it is reasonable to predict that 1-(acetyloxy)-2-propanone in CEM oil was the (acetyloxy)-acetic acid in Milestone oil. Conclusion of bio-oil extracted from different Microwaves contained similar components can be made. More testes were carried out using CEM to investigate the temperature effect on bio-oil chemical composition.

3.4.2 Bio-gas

Average 45ml gas was collected from 1g of straw under 200°C, and 42ml gas between 200°C and 250°C.

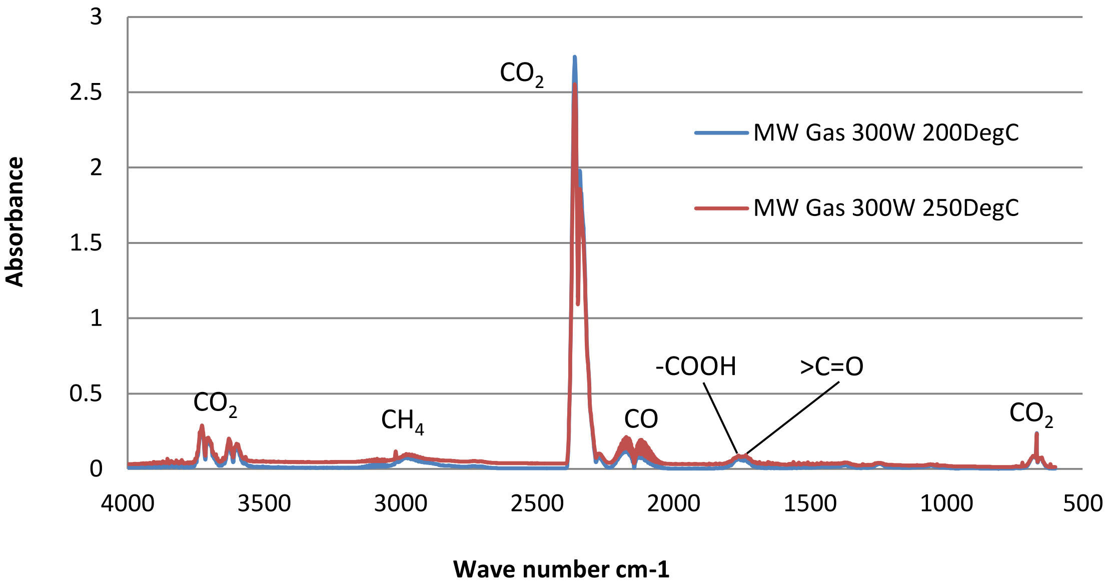


Figure 43 Isobaric bio-gas compositions

The bio-gas was collected in a syringe during pyrolysis. It was then injected to a glass vessel for FTIR measuring. In the FTIR spectrum, the bands due to the atmospheric contributions of water vapour and CO_2 have been subtracted in order to improve the spectral quality. The spectrum of bio-gas was shown above.

The two spectra overlapped each other and showed almost identical character. Unlike char, gas showed a simple and clear spectrum, only few obvious peaks were shown. The most significant and characteristic band at 2240-2402 cm^{-1} was caused by CO_2, so did the peak at 3730 cm^{-1}. Peaks at 2183 and 2127 cm^{-1} indicated the formation of CO. The absorption bands at 2850–3200cm^{-1} showed the presence of hydrocarbons, of which methane(3017 cm^{-1}), was the most abundant.[66] There were many tiny peaks in the bands between 900 and 1900cm^{-1}, indicated the release of some organics, including alcohols, aldehydes, acids, phenols, etc.[14a] But only -COOH (1789 cm^{-1}) and C=O bonding (1760 cm^{-1}) band can be seen clearly in this region.

The formation of CO below 400°C was mainly caused by the cracking and reforming of thermo labile carbonyl and ether groups.[14a] The evolution of CO started at around 230°C, increased significantly with rising temperature. Stronger peak at 2183 and 2127 cm^{-1} for 250°C reaction (represents by red line) were expected. However, only a slightly increase in intensity can be seen from Figure 43. One possible explanation for this might be that due to the unevenly heating, the average reaction temperature inside the microwave did not reach to 250°C, but around 230°C, so the evolutions of CO had only started, lead to a slightly increased intensity.

3.4.3 Char

Char was a dark solid with no obvious smell. The chemical composition of char from Milestone was characterized by Fourier transform infrared spectroscopy (FTIR). The bands due to the atmospheric contributions of water vapour and CO_2 have been subtracted from the spectra in order to improve the spectral quality. Char was the by-product of straw pyrolysis. In order to understand the structure of char, the structure of straw was analysed first.

3.4.3.1 Straw

As mentioned in introduction, the major constituents of straw are cellulose, hemicelluloses and lignin. A typical straw spectrum was shown below in Figure 44. This spectrum presents the pattern of both lignin and cellulose FTIR spectral characters: the left part of the spectrum (2500-4000cm^{-1}) resembled the lignin shape and the right part (500-2000 cm^{-1}) was more like a cellulose outline.[30,67]

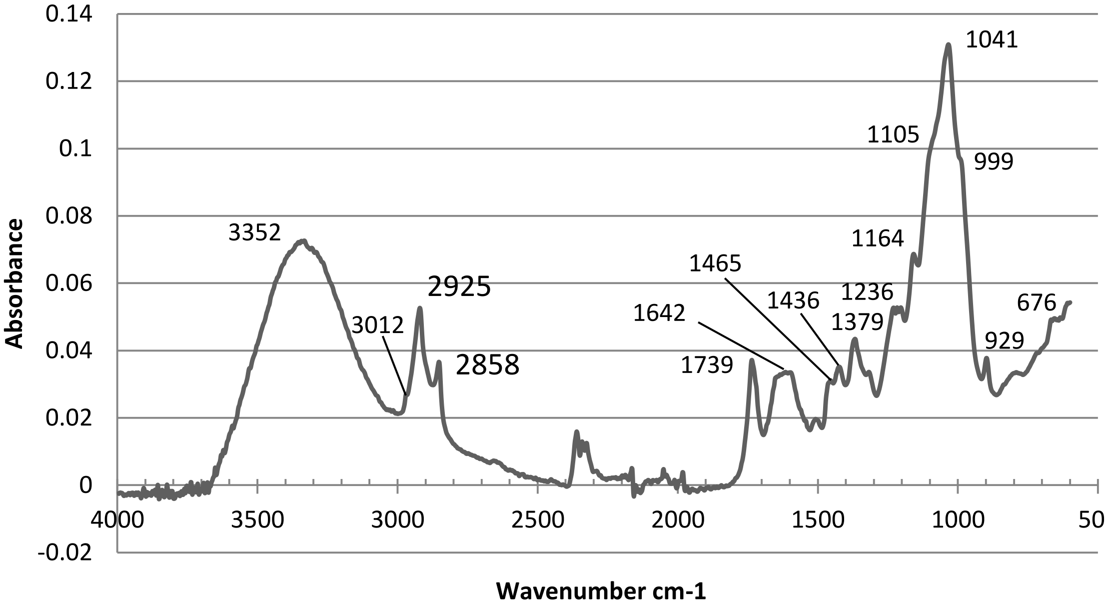


Figure 44 Raw straw FTIR spectrum

The FTIR spectrum contained several absorption bands that can be assigned to individual structural unit in lignin and cellulose as listed in Table 19.

Table 19 Absorption of peaks in FTIR straw spectrum

Region(cm^{-1})	Assigned to
3100-3600	O-H (stretching)
3012	CH (aromatic)
2860-3000	CH (aliphatic)
2858	symmetric CH_3 (methoxyl)
1739	C=O(carbonyl group)
1460-1470	CH (deformation and aromatic ring vibrations)
1310-1360	C-C and C-O (skeletal vibrations)
1379	Both OH and CH bending
1236	C-O (stretching in pyranose ring)
1164	C-O (anti-symmetric bridge stretching of C-O ester group)
1105	C-OH (skeletal vibrations)
1041	C–O (stretch for the O–CH_3 and C-OH)
999	C-O (secondary alcohols skeletal vibrations)

The 2858 cm^{-1} peak could be due to the symmetric CH_3 stretch of the methoxyl group and the band at 1041 cm^{-1} represented the C-O stretch for the O-CH_3 and C-OH. The position of C=O peak (1739 cm^{-1}) was usually used to predict the relationship of C=O groups to the aromatic ring. This peak appeared above 1700 cm^{-1}, stated the C=O group was conjugated with the aromatic ring. The band at 1379 cm^{-1} was attributable to both C-H in plane bending and CH bending, which the weak absorbance at 676 cm^{-1} was due to the out of plane OH bend. The most characteristic band of lignin peak in this spectrum occurred between wavenumber 1460 and 1470 cm^{-1}, because of the CH deformation and aromatic ring vibrations. The stretching of pyranose ring existed in cellulose also generated a peak at 1236 cm^{-1}.

The width and intensity of the bands between 1000 and 1100 cm^{-1} was dependent on presence of any sugars in the sample, while the bands for the hydroxyl group above 3000cm^{-1} were due to alcoholic or phenolic components.

3.4.3.2 De-waxed straw

De-waxed straw is the straw that had been extracted by SC-CO_2 to have their surface wax removed. As de-waxed straw was used as starting material for milestone pyrolysis, its structure was analysed by FTIR technology and the spectrum was overlapped to straws to compare the difference.

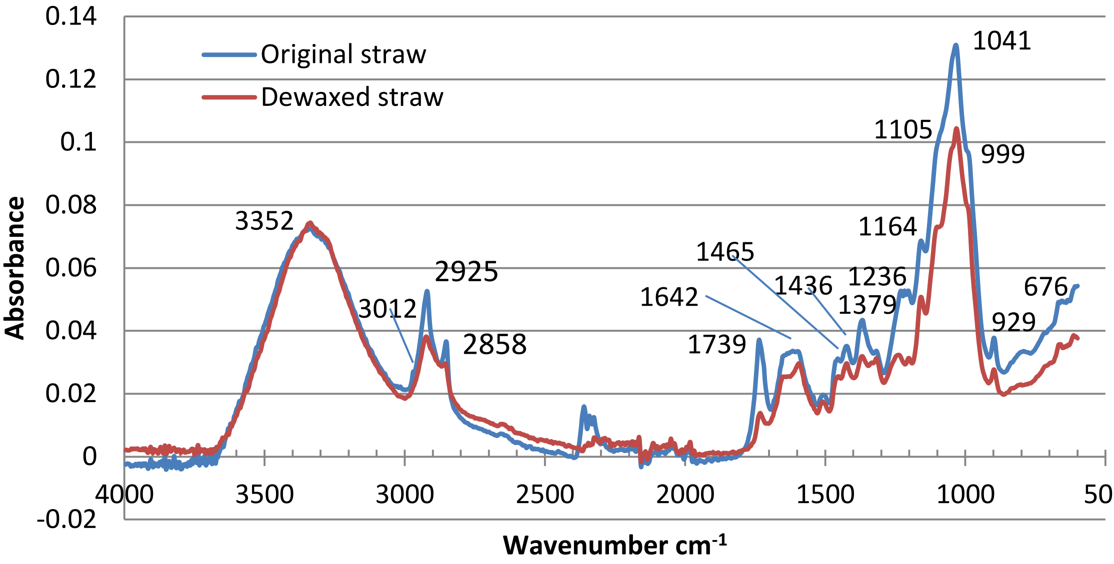


Figure 45 FTIR spectrum of straw and de-waxed straw

As investigated in the section before, the main component of wax was hydrocarbon, aldehydes, fatty acid, fatty alcohol, beta-diketone and sterol; therefore the lower intensity can be seen in almost all the peaks (expect OH band at 3352 cm^{-1}) compared to raw straw. For the lower intensity peak at 1739 cm^{-1}, it was likely to be caused by the removal of beta-diketone. As most waxes share similar spectral features[25], this also happened to other de-waxed straw (like triticale straw), when compared their wax extracted FTIR spectrum to original raw straw's.

3.4.3.3 Char from Milestone

The spectrum of char showed a similar trend to de-waxed straw but with much lower intensities in many peaks, as illustrates in Figure 46.

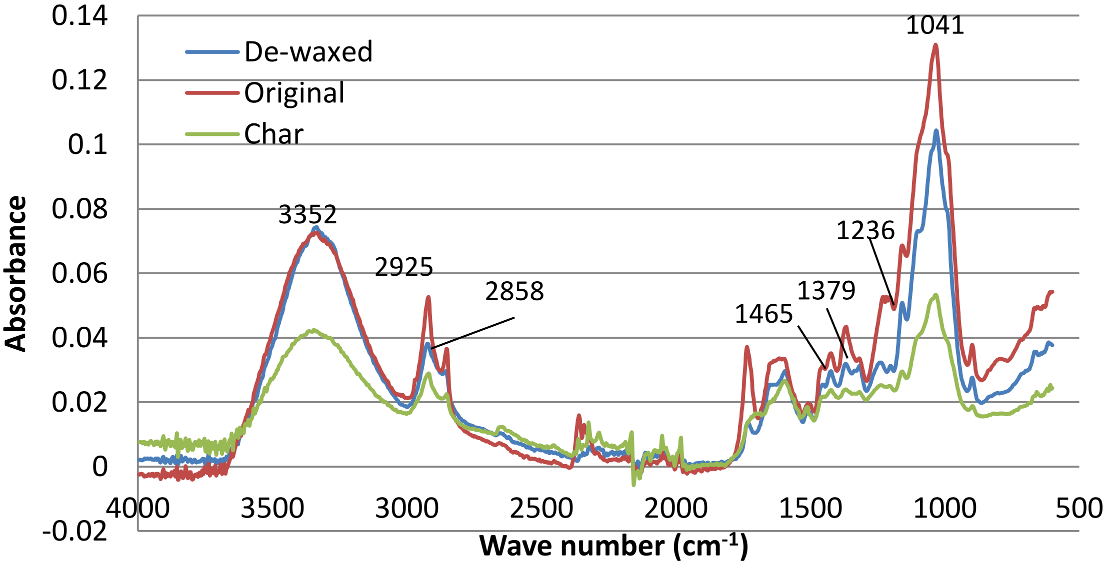


Figure 46 FTIR spectra of raw straw, de-waxed straw and char

The first indication of difference happened to hydrogen bonded OH stretching (3352 cm^{-1}), its intensity was much decreased compared to the de-waxed straw. The decrease may be due to the loss of phenolic or alcoholic groups (bio-oil) during the pyrolysis. This can be further proved as the band for the OH in plane bend (1379 cm^{-1}) was also decreased. The intensity of the peak at 2925 cm^{-1} for the aliphatic CH strength was also declined. Furthermore, the symmetric CH_3 stretch of the O-CH_3 group in char appeared at 2858 cm^{-1} had a decreased intensity, indicated that some CH_3 groups were removed from the substituted aromatic rings after the reaction. This was also happened to the peak at 1041 cm^{-1} that caused by the C-O stretch in the methoxyl group. Some of the pyranose structures of cellulose were still preserved, indicated by the C-O stretching in pyranose ring at 1236 cm^{-1}.

3.4.4 Effect of increasing temperature

3.4.4.1 Closed vessel system

Pyrolysis were run in closed vessel system using CEM with the same energy input and residence time but different temperatures (100°C, 120°C, 140°C and 160°C). As illustrated below, reaction residue decreased dramatically (from 82% to 27%) while the bio-oil yield increased slightly when increasing pyrolysis temperature.

Table 20 Bio-oil and reaction residue yield against pyrolysis temperature

Temperature/°C	char/g	oil/g	Yield of Residue/%	Yield of Oil/%
100	0.82	0.06	82.2	6.4
120	0.65	0.10	64.9	10.3
140	0.27	0.11	27.2	11.3
160	0.27	0.13	27.1	12.7

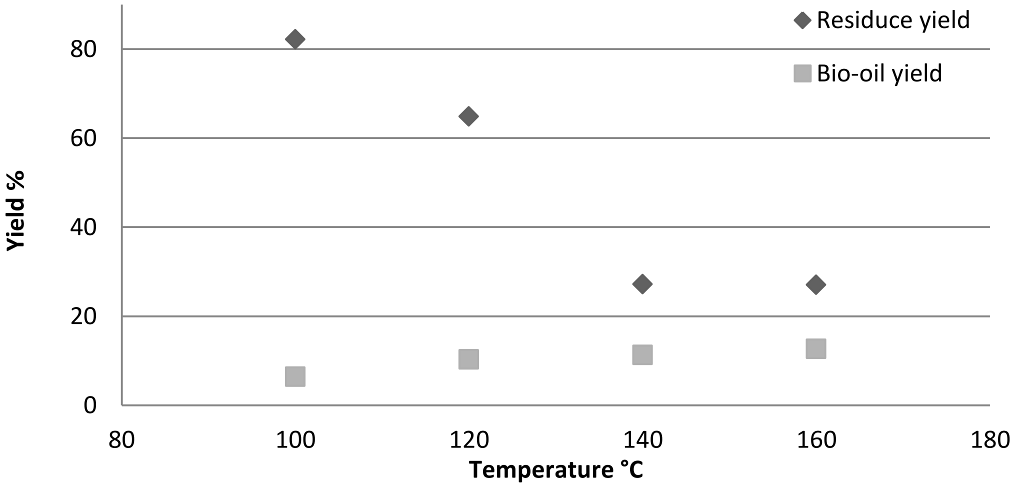


Figure 47 Bio-oil and char yield vs. reaction temperature

When reaction temperature rose to 140°C to 160°C, the decrease in residuce was not sinificant any more. It suggested the formation of char happened at 140°C and above temperature.

In order to identify the impact on the bio-oil composition caused by increasing pyrolysis temperature, bio-oil collected from different temperature were analysed. The bio-oil sample collected at 100°C was very dilute and therefore not included. Bio-oil collected from 120°C, 140°C and 160°C were analysed below.

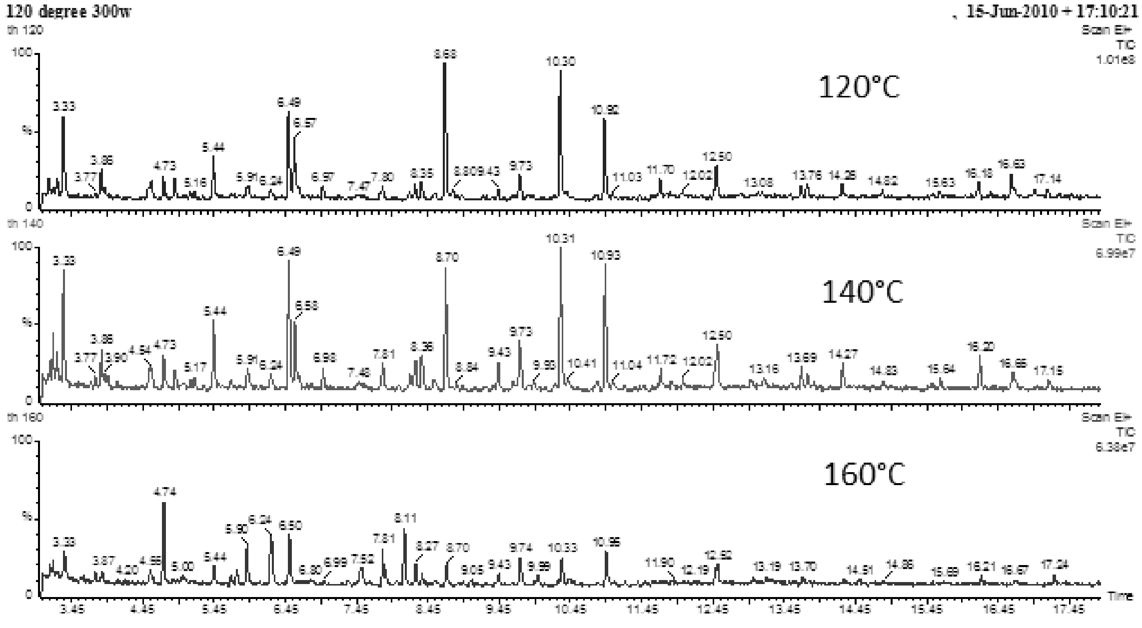


Figure 48 GC-MS of CEM 120°C, 140°C and 160°C bio-oil

The CEM 120°C and 140°C bio-oil shared a great similarity, but the 160°C spectrum showed different ratio of the components to the other two spectra. However, all peaks in 160°C spectrum were found in other spectra, represented the same chemical composition among all samples. This could lead to the conclusion of when the pyrolysis temperature increased, bio-oil being produced had a similar content but different concentration.

More gas was produced while increasing the reaction temperature, especially for the reaction temperature rose to over 160°C. The amount of gas being produced caused a high pressure in the closed vessel system and forced the reaction to stop after the reaction started for 17 seconds. It was therefore necessary to modify the system and move from the reaction from closed vessel system to opened vessel system.

3.4.4.2 Open vessel system

An opened system was therefore designed for CEM to allow reaction react smoothly and to collect bio-gas formed during the reaction. Reaction temperature of 200°C and 250°C were applied to reaction and bio-gas was collected at each temperature.

For reaction temperature at 200°C, the yield for char was 56% and for bio-oil was 6.5%. When the temperature rose to 250°C, the yield for char was 56% and for bio-oil was 7.1%.

The comparisons between CEM bio-oil collected at opened vessel system at 250°C and 200°C, and at closed vessel system at 140°C together with Milestone bio-oil were shown below.

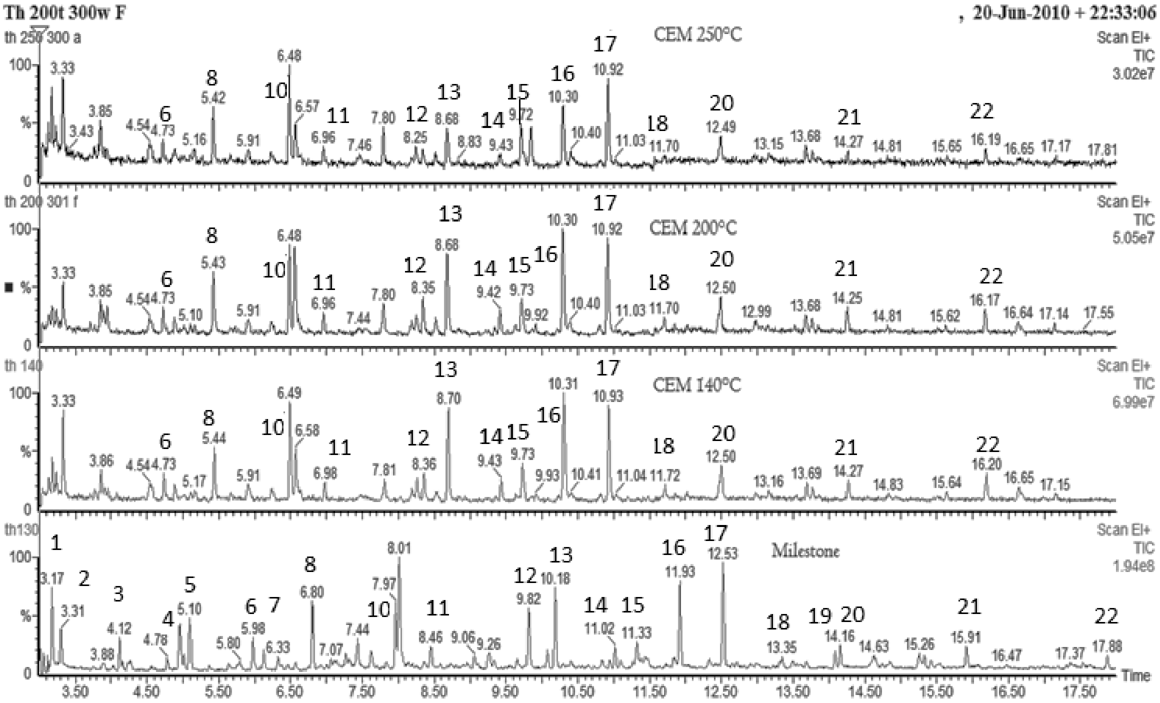


Figure 49 Bio-oil components comparison

As can be seen from the Figure 49, peak retention times in bio-oil from three CEM spectra were almost identical regardless of reaction temperatures. The exception was a small peak appeared at 9.85min for CEM 250°C bio-oil (next to peak 15). The peak was concluded to be benzoic acid, 2,6,-dimethyl-,methyl ester after analyse. Except of this, all other peaks observed from open vessel system existed in previous spectra.

The strong concentration peak at 8.01min in Milestone was shown again in all three CEM spectra at around 6.58min, with the same featureless spectrum of m/z

41,43,44,57. This peak also existed in CEM 120°C and 160°C spectrum. It was likely to be caused by the contaminated compound left in the GC-MS machine, and further investigation is needed for fully understanding of the peak.

3.5 Processes review and environmental aspects

The whole barley straw research consisted of two main processes, wax extraction by SC-CO_2 and thermal processing of microwave pyrolysis. The wax extraction was performed on 100g of barley straw each time, pyrolysis by Milestone microwave machine used 50g of de-waxed straw, whereas only 1g was used by the small scale CEM microwave every time. Consequently the processes were reviewed and later environmental aspects were investigated. This was based on the experimental data obtained from Milestone, as the small scale applied for CEM microwave can be neglected.

Scaling the Milestone experimental data from 50g starting de-waxed straw to 96g, which is the amount of de-waxed straw being produced after each wax extraction, the process flow sheet can now be illustrated quantitatively in Figure 50 using Table 21.

Table 21 Material quantities in experiments(unit: gram)

	Straw	**CO_2**	**Wax**	**Water**	**De-waxed straw**	**Water added**	**Oil**	**Char**	**Water**	**Gas**
Wax extraction	100	9600	1.2	2.8	96	-	-	-	-	-
Microwave pyrolysis	-	-	-	-	50	5	5.5	21	13	Un-known
Microwave scale up	100	9600	1.2	2.8	96	9.6	10.56	40.32	24.96	Un-known

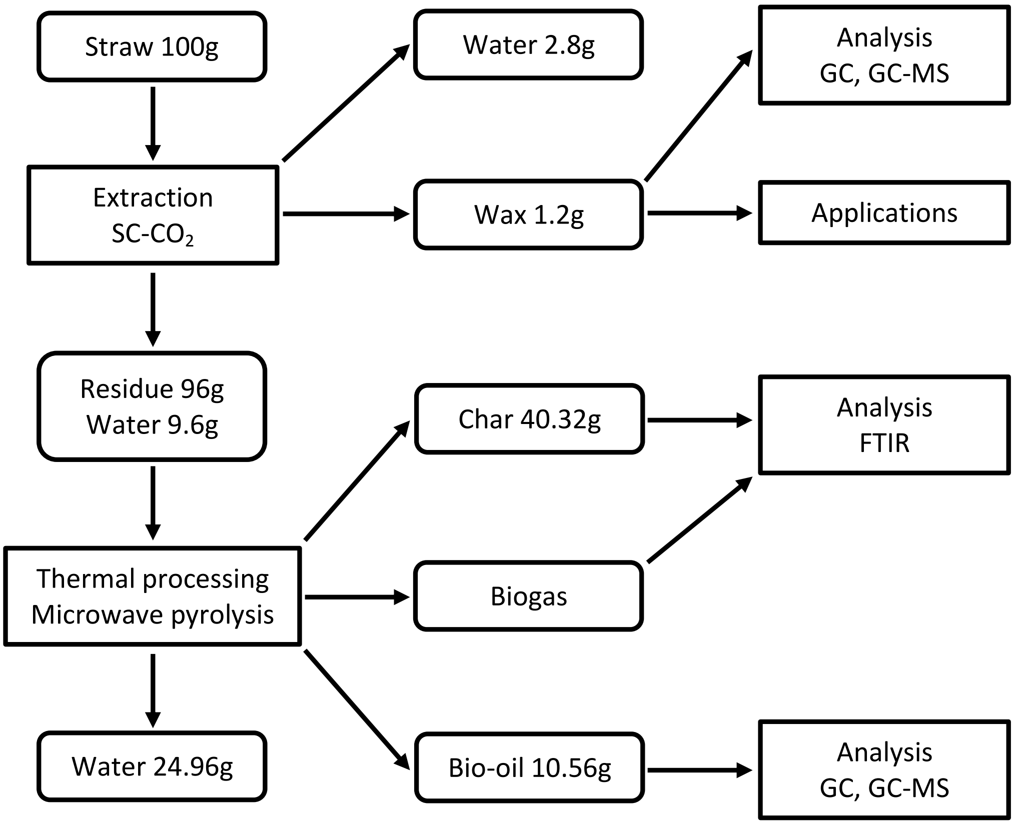


Figure 50 Integrated biorefinery process steps

By avoiding any mass usage of hazardous chemicals (only small amount of hexane was used for Soxhlet extraction), also applying 'green' techniques ($SC\text{-}CO_2$ and microwave) in the experiments, this research was generally considered as environmentally friendly. To quantitatively measure the environmental impact of each process, green chemistry metrics was employed.

Mass of every product/by-product as well as each reagent and none recycled solvent need to be known in order to perform the calculation for green chemistry metrics. Therefore the only unknown quantity of biogas produced during the microwave pyrolysis was now determined by applying mass balance. The stepwise calculation was shown below.

Mass accumulation = sum of mass in - sum of mass out - sum of mass generated

Material that enters a system must, by conservation of mass, either leave the system or accumulate within the system. There was no unreacted reagents, solvent nor product left in the neither the microwave machine nor any of the apparatuses, which meant no accumulation occurred. Therefore mass balance of this experiment is the following, 0 = (de-waxed straw/96g + water/9.6g) - (bio-oil/10.56g + char/40.32g + biogas + water/24.96g)

Bio-gas = 29.76g

3.5.1 *Environmental factor (E-factor)*

The E-factor calculation is defined by the ratio of the mass of waste per unit of product:

E-Factor = Total Waste (kg) / Product (kg)[68]

Although the E-Factor calculation generally excluded water for organic experiments (water as solvent), the usage of water for none-solvent purpose in this experiment should not be omitted.

During the wax extraction process, due to the massive consumption of CO_2 and lack of recycling process, for every 1.2g of wax produced, 9603g of waste was produced, which result in a E-factor of 98.8. And if considered extracted wax as the only interest product and de-waxed straw as the waste as well, this will cause the E-factor to rise by more than 7 times to 8082, which indicated the process as very mass-wise inefficient.

While bio-oil has potential of becoming fossil fuel substitute, the lack of fluid property results in relatively limited utilisation of char. Also difficulties are presented at biogas collection and transportation during microwave pyrolysis, such that it is necessary to consider the environmental metric on both keeping and discarding char and biogas as main products.

Finally, the E-factor for the combined wax extraction and pyrolysis process was determined. If solutions to CO_2 recycling, char and biogas problems could be found, the environmental impact would be significantly reduced. This could be represented by a new calculation of E-factor.

Every E-factor obtained from different process viewpoints are tabulated in Table 22 for easy comparison.

Table 22 E-factors for various process viewpoints

Condition	Waste		Product		E-factor
Wax extraction					
De-waxed straw as waste	CO_2, water, de-waxed straw	9698.8g	Wax	1.2g	***8082***
De-waxed straw as by-product	CO_2, water	9602.8g	Wax, de-waxed straw	97.2g	***98.79***
Microwave pyrolysis					
Char and gas as waste	Char, gas, water	95.04g	Oil	10.56g	***9.000***
Char and gas as by-product	Water	24.96g	Oil, char, gas	80.64g	***0.3095***
Overall process					
CO_2, char and gas as waste	CO_2, char, gas, water	9697.84g	Wax, oil	11.76g	***824.6***
CO_2 recycled, char and gas as by-product	Water	27.76g	Wax, oil, char, gas	80.64g	***0.3442***

The higher the E-factor, the more negative impact it brings to the environment. From the above table, it is clear to conclude that recycling of solvent (CO_2) is necessary for long term experiments and research. In addition, considerable amount of char and biogas was being produced during pyrolysis. In order to discount these from waste, wider applications such as coal substitute to power plant should be investigated. Therefore, the current processes have a great potential of improving.

4 Conclusion

The research discussed the additional value of the agriculture waste, barley straw, and its potential of being a renewable energy source. The straw was processed to extract valuable surface wax and was further pyrolysis to produce possible fossil fuel substitutes, bio-oil and char.

Wax was successively isolated from barley straw using SC-CO_2 and Soxhlet extraction. The SC-CO_2 extraction technique was proven to be superior to Soxhlet; it provided a product with not only higher yield but also a greater diversity of fatty acid as well as aldehydes and a larger percentage of sterols; whereas the Soxhlet product only surpassed the former technique in the variety of the alkane compounds.

The most available variety, Saffron barley straw, were found to provide little deviation in wax compositions and only a slightly variation in concentrations compared to Carat barley, the Germany originated barley and the grain containing barley.

90.1 % of the wax components were identified using the GC-MS technique. The analysis showed fatty acid was the most dominating group in Saffron barley wax, which contributed 32.49% to the wax sample; it was followed by sterols (17.3%), fatty alcohol (14.6%), and 14,16-hentriacontanedione (11.3%). The chemical composition in the wax layer was found to be unique and characteristic to a specific plant, which therefore can be used to identify its botanic species.

The wax produced from a variety of straws of a single species was differed in the yield quantity. Carat Barley had a higher wax yield than Saffron Barley. Between different species, the column chromatography extracts of Carat barley wax, wheat wax and apple peel wax was observed with little variation in their alkane distributions. Previous research showed that two hydrocarbons, C_{23} and C_{25}, were able to induce aphid avoidance. The hydrocarbons were found in extract samples of all three species in this study. Consequently, all formerly mentioned wax extracts possessed the potential of being used as pesticides. However, only by performing bioassays with aphid can the desired property of the wax be confirmed.

Using de-waxed straw for pyrolysis had great advantages. As de-waxed straw was treated as staring material instead of experimental waste, new starting material was not needed and disposal cost was reduced. In addition to the above benefits without the interference of wax components, a clearer bio-oil GC-MS spectrum was generate, demonstrated wax should be extracted from straw before pyrolysis.

Two microwave machines, Milestone and CEM, were used for pyrolysis and bio-oil was the main product. Similarities were presented in both bio-oil extracts despite of different temperature settings. This indicated a positive reproducibility of the valuable oil. Excluding insignificant small peak patterns due to their lack of strength in Milestone bio-oil spectrum, 52.7% of total peaks were analysed. Phenolic chemicals were the dominating group, whereas acetoxyacetic acid as a single substance had a highest concentration of 7%, followed by 3-methyl-1,2-cyclopentanedione(5.2%) and 2,6-dimethoxyphenol(4.6%). This study provided a fundamental base for the possibility of scaling up into industrial chemical plant.

Char and bio-gas was produced as by-product in pyrolysis. The decrease in absorbance observed in FTIR spectrum when comparing the starting material before the pyrolysis and their product char afterwards, indicated the loss of phenolic group. Because bio-oil was mainly composed of phenolic substance, it is confident to conclude the production and separation of bio-oil was successful.

The quality of the product produced is dependent on the reaction temperature. The bio-gas captured between temperature 200°C and 250°C contained CO_2, CO, CH_4, -COOH and C=O bonding. And char was mainly formed at 140°C or above. However, increasing temperature did not have significant impact on bio-oil's chemical composition, although its yield was slightly increased.

The environmental impact was investigated by calculating the environmental E-factor. The results showed that the processes are environmentally benign, and there are great potential for improvements. It is important to implement relative measures, such as recycling CO_2 during SC-CO_2 extraction and extending products applications of char and bio-gas into account, when scaling up the laboratory process into industrial plant, to minimize the environmental impact.

5 Further work

The results presented in this study suggested a future for producing wax and bio-oil product from barley straw. However, further improvements can be made to the research. In order to provide a more reliable spectrum for comparison, Carat barley straw wax should be collected under the same SC-CO_2 setting and run using the identical GC-MS condition as Saffron barely wax. Wax column chromatography of different straws or fruit peel should be repeated and followed by bioassay testing with aphid.

Thermal pyrolysis should be repeated on both de-waxed and raw straws in order to collect more bio-oil. With the aim of understand the bio-oil pyrolysis thoroughly and accurately, small peaks as well as the unidentified peaks in its GC-MS chromatogram should be investigated. For components in wax and bio-oil spectra with their KI or MS information missing from online database, their standards should be run for comparison. More analytical techniques, such as NMR and HPLC should also apply to wax and bio-oil to provide a better understanding in their composition.

The bio-oil yield in this experiment was about 11% (excluding water) and 25% (including water), but by previous repost the bio-oil yield can reach to 70% (including water) ideally at 500°C. The impact of changing temperature on the product of straw pyrolysis should be tested not just on CEM microwave, but also on Milestone microwave and other microwave machines to confirm the reproducibility of bio-oil. More operating conditions will be required, for example, to increase the reaction temperature to 500°C for further analysing the effect on products yield. Milestone should be re-designed to prevent the loss of bio-gas to the environment, and this will further lower the E-factor. GC-MS should be equipped to be able to analyse gas, so a more detailed analysis can be performed on bio-gas.

Other researching areas, like the digestion and fermentation of straw to produce ethanol should also be investigated to produce a complete mass and energy efficient flow diagram. There is a great economic potential presented in the straw bio-refinery processes.

6 Abbreviation List

Abbreviation	Description
EI	Electron impact ionization
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatography
GC-MS	Gas chromatography coupled with mass spectrometer
KI	Kovat Index
m/z	Mass-to-charge ratio
RT	Retention time
SC-CO_2	Supercritical carbon dioxide

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