BENEFICIATION OF SAWDUST: CHEMICAL FRACTIONATION OF LIPOPHILIC EXTRACTIVES

By

Zaakerah Badat

(B.Sc. Chemical Engineering)

University of KwaZulu-Natal

Submitted in partial fulfilment of the academic requirement for the degree of Master of Science in Chemical Engineering, at the School of Engineering, College of Agriculture, Engineering, and Science, University of KwaZulu-Natal,
Durban, South Africa.

Supervisor: Prof. Bruce Sithole

Co-supervisor/s: Prof. Anne Stark

Dr. Viren Chunilall

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PREFACE

The research contained in this dissertation was completed by the candidate while based in the School of Engineering, Discipline of Chemical Engineering within the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Howard College Campus, South Africa. The project was financially supported by the National Research Foundation (NRF) and CSIR Durban.

The work presented in this dissertation was performed at the CSIR laboratories in Durban.

This dissertation is submitted as the full requirement for the degree of Master of Science in Engineering (Chemical). All the work presented in this dissertation is original unless otherwise stated. It has not (in whole or in part) been previously submitted to any tertiary institute as part of a degree.

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Z. Badat

As supervisor of this candidate, I approve this dissertation for submission:

____________________	____________________	_________________
Prof. Bruce Sithole	Prof. Anne Stark	Dr. Viren Chunilall

DECLARATION 1 - PLAGIARISM

I, **Zaakerah Badat**, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.

2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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ABSTRACT

Lipophilic extractives are generally known to be those compounds in wood that cause pitch problems in the pulp and paper industry. However, these compounds are advantageous to the biorefining industries as they contain a selection of valuable components.

According to theory *like dissolves like* non-polar solvents dissolve the lipophilic compounds. In this study it was found that the non-polar solvent, cyclohexane, as a mixture with a polar solvent, acetone or ethanol, showed better extracting abilities as opposed to pure non-polar solvents. Many past researches looked at extraction from pine sawdust, but not specifically focussing on the lipophilic extractives and its composition, making it difficult to compare this work with others.

This study focusses on the viability of solid-liquid extractions on pine sawdust residues, of various ages, for the potential conversion of its useful compounds to value-added products. A potential product is the conversion of α-pinene to pine oil. Alpha pinene is the main terpene contained in pine wood sawdust, and pine oil is known to be a product with various uses in various industries. However, it is currently not locally produced.

When carrying out solvent extractions, it is important to know the composition of the extracts and not only focus on the total extractive contents. In this study, the terpene contents in the extracts were closely studied as it is seen to contain components that could be used to produce the most valuable compounds.

Experiments were conducted to determine the effects of operating variables such as; temperature, extraction time, extracting solvents, particle size as well as the effect of storage time on the composition of the extracts.

A preliminary study was conducted using a 6-hour extraction time. The results produced a maximum of 1,75% α-pinene, and a maximum of 3,7% of the total terpene content of the extractives, which is a significant but low quantity of terpenes. The ASE extraction method uses high temperatures and pressures and allows for extraction to occur at up to an hour. Results obtained indicated that shorter extraction times provide a marginally higher quantity of terpenes in the extracts. Thus, selecting time as a variable parameter was applied in Section 4.3 and the results validated the extraction over shorter time periods, for extracting more value from the sawdust residue. In Section 4.3, the extracting solvent and the particle size was also investigated. After various experiments, the optimised extraction conditions were determined as being: a 2-hour extraction time, 3:1 cyclohexane-ethanol (v/v) as the extracting solvent and 375-micron particle size. The temperature of the solvent was investigated in Section 4.5, the kinetic study. It is required that the temperature be close to the boiling point of the solvent, as when the temperature is lower, a slightly lower terpene content is noticed. On the other hand, when the temperature is greater than the boiling point, the terpenes are decomposed. 3:1 Cyclohexane-ethanol (v/v) as an extracting solvent, requires a maximum temperature of 80℃ after which components of the extract begin to decompose. The kinetic study enabled the development of a model equation that could determine concentrations at any time. The effect of storage time was investigated during the seasoning study. This however, was seen to have no significant impact on the overall extraction results.

Based on the various experiments conducted, the maximised terpene yield can be extracted at a 2-hour extraction, using 3:1 cyclohexane-ethanol (v/v) as the extracting solvent and grinding the sawdust residues to a 375-micron particle size.

ACKNOWLEDGEMENTS

I would like to extend my sincere gratitude towards the following people who have assisted me during my research:

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- The staff and colleagues at CSIR BIDF

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CHAPTER 1: INTRODUCTION

1.1 Background

The South African forestry industry is reliant plantation forestry using eucalyptus, wattle, and pine wood. Pine trees occupy 49.8% of South Africa's plantation area (Roger, 2017). When the plantations are ready they are felled and transported to sawmills for processing (Thaba Chweu Municipality . 2006). During the processing stage the lumber recovery rate is under 50% with the remainder of the log being unused residues such as sawdust and pulp chips (Thaba Chweu Municipality . 2006). The majority of this residue is then dumped in landfill sites with no use and cause serious environmental problems.

These residues are an important resource in the biorefining industries. These could be used in the production of value-added products; bio-materials, bio-chemicals, bio-fuels as well as other novel biorefining products.

The main constituents of lignocellulosic-based biomass are cellulose, hemicellulose and lignin. Apart from these main components, wood also contains an expansive range of low-molecular mass lipophilic compounds also known as extractives. These lipophilic compounds are predominant in softwoods than in hardwoods. Simple solvent extraction methods can be used to separate the extractives from the wood constituents by using polar and non-polar solvents.

The aim of maximising the use of wood for a range of products to beneficiate the wood more efficiently led to an increased interest in the study of its chemical components. Specifically, extractive compounds of pine wood are used in the production of industrial chemicals and health products (Routa *et al.*, 2017).

1.2 Problem Statement

The timber and lumber processors struggle with the disposal of large amounts of sawdust that is produced. Some of it is burnt for energy production and some is used as animal bedding and/or supplied to industrial plants for the manufacture of wood pellets. However, all this accounts for a very minute amount of the sawdust.

Hence, the vast quantities that remain are being disposed of into rapidly-depleting landfill sites which leads to various environmental problems.

1.3 Research Aims and Objectives

The main aim of this study is to investigate technologies to fractionate South African wood biomass via solvent extractions to increase their value, making them valuable assets to the forestry and wood processing industry.

The aim of this study was achieved by the following objectives:

1. Extracting the lipophilic extractives from pine sawdust and characterising the chemical components of the extractives using Py-GC/MS.
2. Investigating the various extraction parameters (extraction time, solvents used, sawdust particle size) on the lipophilic extractives yield and to evaluate the optimum conditions that yield maximum extraction of terpenes.
3. Determining the kinetic models that best describe the Soxhlet extraction process at different temperatures.
4. Determining the impact of seasoning (age of sawdust) on the composition of the extractives.

1.4 Thesis Statement

Pine sawdust contains a considerable quantity of extractives. These are highly valuable compounds and a potential product from the extractives is pine oil. Pine sawdust could thus be beneficiated as a source of a valuable product - pine oil.

1.5 Chapter Overview

Chapter 1 provides an introduction to the current utilisation of sawdust residues and the implications associated with the remaining unused residue. A possible use of the pine wood sawdust is thus proposed. The research aims, and objectives are clearly outlined.

Chapter 2 is a literature review on the extraction of lipophilic extractives from sawdust residue. The broader literary aspects of wood are discussed before progressively narrowing down to the specific work undertaken.

Chapter 3 is a description of the methods applied to conduct the different experimental sections of this work.

Chapter 4 entails the results and critical discussions of the comprehensive findings obtained from this study.

Chapter 5 provides an outline of the overall conclusions of the studies conducted including recommendations.

CHAPTER 2: LITERATURE REVIEW

2.1 Biorefinery concept

As economic development continues, the demand for both energy and physical matter, such as materials and chemicals is growing significantly (Banks and Schaffler, 2005). Historically, this demand has been satisfied mostly by exploitation of fossil resources. However, these resources are rapidly dwindling. South Africa has various renewable resources that could contribute to the substitution of fossil resources. The application of solar, wind, hydro, tidal and geothermal energies to provide a sustainable energy mix is well underway (Banks and Schaffler, 2005). Although biomass could also be used for the provision of energy, it is the only physically available non-fossil matter that can be harnessed to provide mankind with chemicals and materials, and should hence be used predominantly for this purpose. It offers opportunities to produce a wide range of new polymers as well as bio-products. Emphasis has now been placed on producing value-added products from biomass residue products for further use in various industries through the concept of the biorefinery. The biorefinery approach therefore aims at utilising the biomass efficiently thereby maximising the economic value of the feedstock used while minimising the waste streams produced (Suhag and Sharma, 2015). The forestry, timber, pulp and paper (FTPP) industry, especially, has been widely involved in biorefinery due to the decline in the demand for its conventional products. The generation of new and innovative products in the industry through biorefinery could bring about much needed growth and revitalization to the industry (Moodley *et al.*, 2017).

2.2 Sawdust

Sawdust is a by-product of the mechanical milling or processing of wood (timber) into various usable sizes. Sawdust is made up of the same chemical components as that of wood. The breakdown of these chemical components are: carbon (60.8%), hydrogen (5.2%), oxygen (33.8%) and nitrogen (0.9%) (Phonphuak and Chindaprasirt, 2015).

Sawdust has until now been perceived as a nuisance to both mankind and its environment, in particular since it results in coating of equipment as well as flooring, which requires additional cleaning and maintenance. Exposure to sawdust in plants create expansive problems, with equipment being damaged or destroyed due to fires and explosions (Hollamby, 2010).

In the past, sawdust was either dumped or incinerated to get rid of the residue (Canfield, 2008). Currently, only a portion of the sawdust is being utilised by mills, in furnaces and to run the kilns. Another small portion is used by dairy farmers as animal bedding and supplied to plants for the manufacture of wood pellets (Canfield, 2008).

2.3 Disposal methods and environmental concerns

Sawdust residues are continually produced throughout the year at various wood industries. These require marketing strategies, disposal methods as well as ways of utilisation to decrease the heaps of wood residue (Owoyemi *et al.,* 2016).

Currently the uncontrollable amounts lead to open air burning practices, dumping into water bodies and dumping at landfills. These constitute environmental pollution. Sawmills are thereby faced with the risk of fires and explosions due to the presence of the residues. Sawdust is inflammable, thus if ignited can cause fires, however due to the large volumes it can cause explosions.

Sawmill workers are regularly exposed to the wood residues and repeated inhalation leaves the workers in danger of respiratory infections. Over a period, more serious health conditions including asthma and emphysema begin to develop (Owoyemi *et al.,* 2016).

Often, wood treatment is conducted in the presence of chemicals, if the chemicals remain in the sawdust can cause dermatitis which may cause blisters. Frequent exposure can then lead to severe skin reactions.

During the burning of wood residues, harmful substances such as carbon monoxide, sulphur dioxide, nitrogen oxides and ash is released into the air. Unmethodical burning of the residues pollutes the air and the smoke causes scarring of the lungs owing to the fine particulate matter contained in the smoke. Polycyclic aromatic hydrocarbons (PAHs) and dioxins are also chemical known to be present in wood smoke however, these in particular are suspected to be carcinogens. Other effects of wood smoke are increased lower respiratory infections, abnormal lung development, depression of the immune system leading to breathing difficulties (Owoyemi *et al.,* 2016).

Heaps of sawdust left at landfills for long periods begin to decompose and emit a harmful greenhouse gas, methane. Dumping of the wood wastes also leads to environmental degradation, loss of aesthetic value, produces unpleasant odours and causes air pollution. These heaps then become breeding grounds for pests and disease carriers (Owoyemi *et al.,* 2016).

Wood residues that are dumped into water bodies eventually block drainage systems causing flooding during rainy periods. Dumping causes the loss of aquatic organisms and cause lakes and rivers to become toxic and clogging fish gills (Owoyemi *et al.,* 2016).

These concerns lead to the requirement of proper wood waste management practices.

2.4 Wood

Wood has many useful properties, amongst them are some remarkable properties such as it being renewable, recyclable as well as biodegradable (Rowell, 2013). It is due to these properties that it has remained an important substance for the past many decades (Rowell, 2013). Wood species can be divided into two groups, namely hardwoods and softwoods. Softwoods are gymnosperm trees (conifers) and hardwoods are angiosperm trees (flowering plants).

Wood contains carbon and hence is known as an organic material. There are three main chemical elements of woody matter, these show minor differences between different wood species in terms of distribution, i.e. carbon, oxygen and hydrogen. Wood contains trace amounts of nitrogen and some inorganic elements, which are materials tasked to aid in the "metabolism of living cells during wood formation and growth" (Herbert, 2006).

The major structural chemical components are displayed in Figure 2.1. Cellulose, hemicellulose, and lignin are the three main organic compounds that make up the cell wall (Chen, 2014). Low molecular-weight substances such as extractives and ash, are also be found in wood, in amounts depending on the wood species (Herbert, 2006).

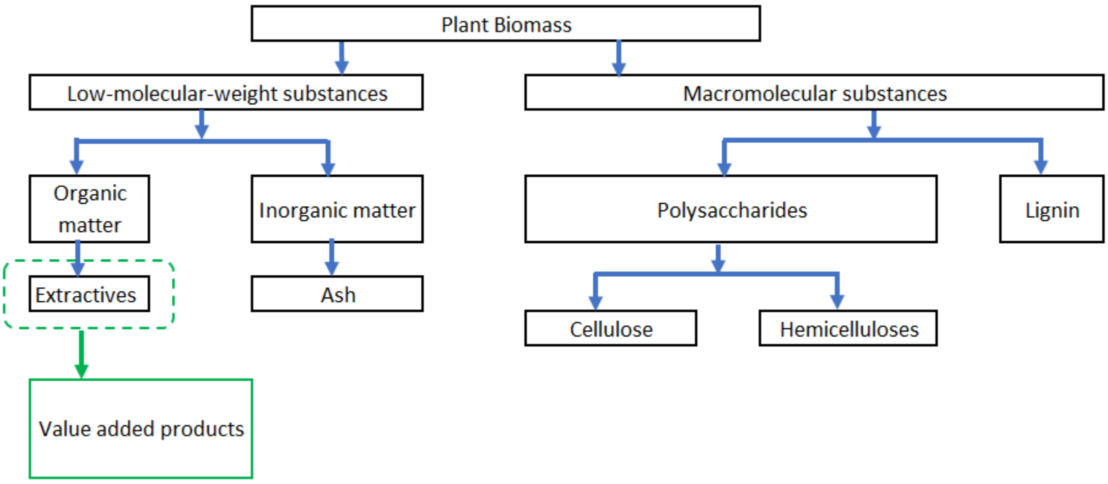


Figure 2.1 General components in plant biomass (Sun *et al.*, 2011)

South Africa has a forest plantation population of 1.1% of the country's land area, thus it is considered to be lightly forested. As seen in Figure 2.2, Mpumalanga encompasses the bulk of these plantations with KwaZulu-Natal following closely.

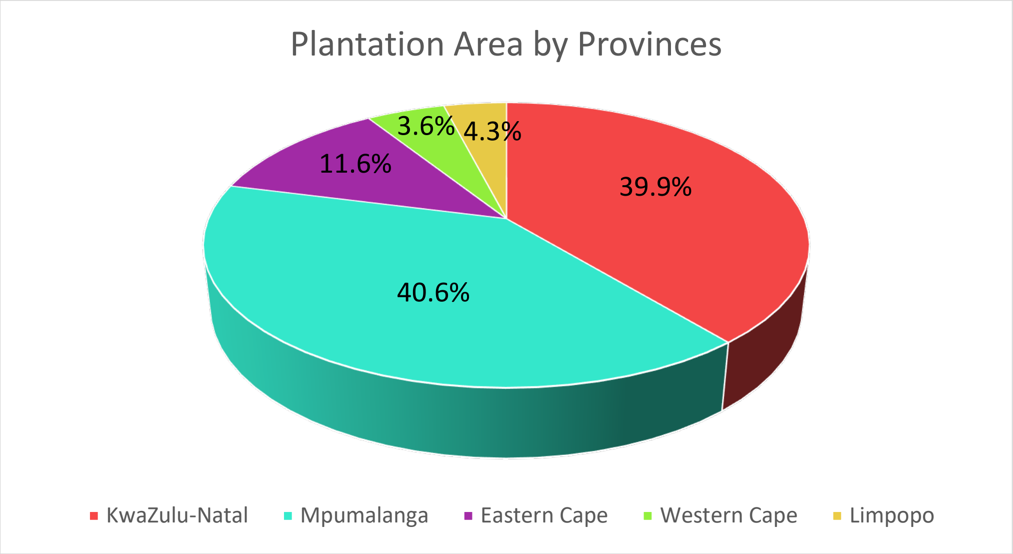


Figure 2.2 Representation of the provinces plantation area in South Africa (2016) (Roger, 2017)

In the South African Forestry Industry, pine and eucalyptus are the predominant species. For every log that is harvested, it is estimated that only 35% of it is being converted to useful products, while most it is waste. Wood wastes are made up of bark, slabs, off-cuts, shavings and sawdust. The solid wastes account for about 79% of the generated waste whilst sawdust accounts for 21% (Kwabena, 2009). Figure 2.3 shows that South Africa planted 49.8% pine trees followed by 42.7% eucalyptus in 2016.

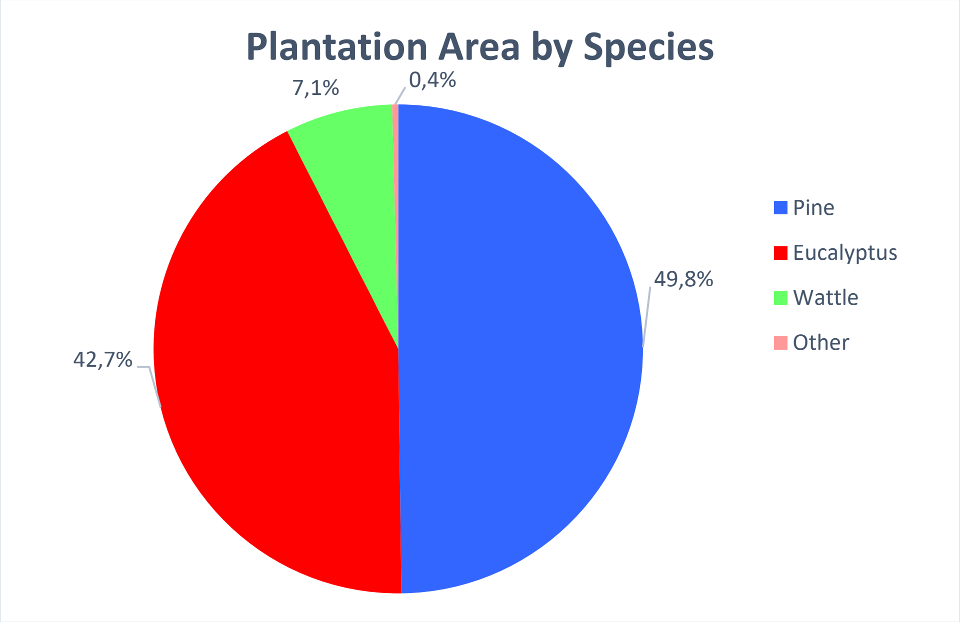


Figure 2.3 Use of plantation areas by species in South Africa during the 2016 period (Roger, 2017)

Lignocellulosic feedstocks are those plant fractions that are indigestible to humans and hence, are not require to be in competition with food production lines, and it is for this reason that they have a significant advantage over other biomass supplies (Isikgor and Becer, 2015). Large quantities of lignocellulosic biomass are annually piled up and require disposal. Disposal is thereafter taken care of by dumping in landfill sites or in soil. These biomasses would generally decompose but due to its huge volumes, raise a serious environmental concern (Isikgor and Becer, 2015).

Table 2.1 Composition of wood components (Pettersen, 1984)

Wood Components	
Primary Components (90-96%)	**Secondary Components (4-10%)**
Polyssacharide fractions • Cellulose (40-50%) • Hemicelluloses (25-35%) Polyphenolic fractions • Lignin (18-35%)	Inorganic Substances (ash) Organic Substances

2.4.1 Cellulose

Cellulose is the major component in wood and is made up of 40-50% of the dry wood mass. The cellulose polymer is a linear polymer of D-anhydroglucopyranose units linked together by β-1,4-glucosidic bonds (Wertz *et al.*, 2010). Two glucose anhydride units, called cellobiose, form the repeating structure of the cellulose polymer (Mohan *et al.*, 2006). The typical cellulose molecule is shown in Figure 2.4. The glucosidic bonds of the cellulose polymer enable it to assemble in its crystalline form (Fitzpatrick, 2011).

The strong tendency for intra and intermolecular hydrogen bonding allows for alternate highly ordered (crystalline) and less ordered (amorphous) regions to be formed from the bundles of cellulose molecules which aggregate to microfibrils. These microfibrils are further aggregated to fibrils and finally to cellulose fibres (Sjostrom and Alen, 1999).

Typical material properties which include high tensile strength and insolubility in most solvents, result from the tight fibre structure created by hydrogen bonds (Sjostrom and Alen, 1999). Due to the crystalline structure, cellulose is able to better withstand thermal decomposition as compared to hemicelluloses (Mohan *et al.*, 2006).

Cellulose is utilised in the production of paper and fibres. It can also be chemically modified to form substances such as cellophane and rayon. Cellulose products can also be used as adhesives, explosives, thickening agents for foods, and in moisture-proof coatings (The Editors of Encyclopaedia Britannica., 2018). Hence, isolation of cellulose is of great economic importance.

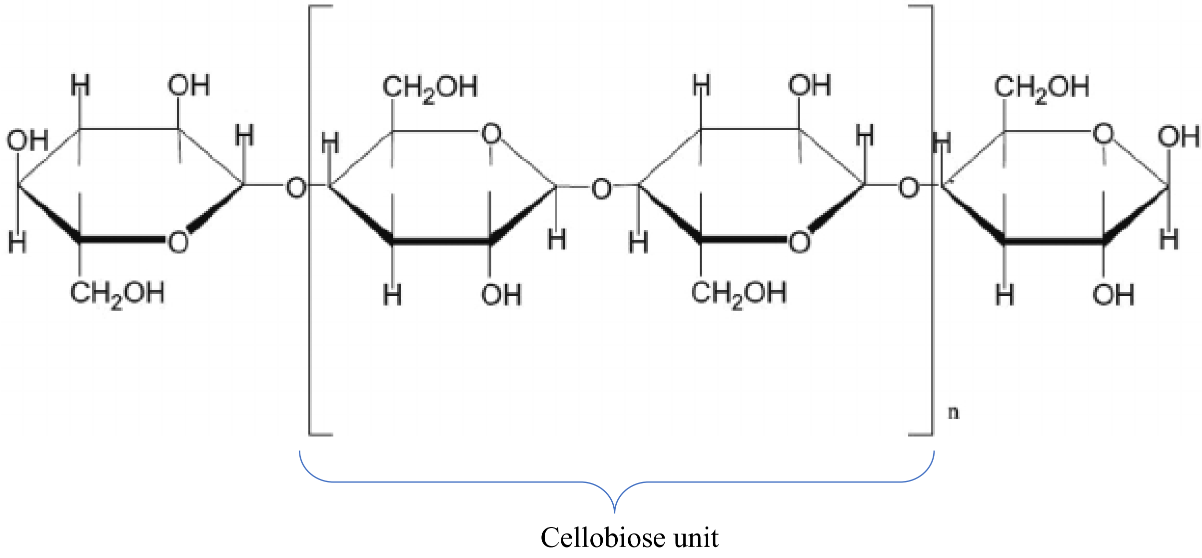


Figure 2.4 Chemical structure of cellulose (Thakur *et al.*, 2014)

2.4.2 Hemicelluloses

Hemicellulose is the second most abundant polymer class which accounts for 25-35% of the mass of dry wood (Mohan *et al.*, 2006). Hemicelluloses are classified as heterogeneous polysaccharides, amorphous polymers made up of branches with short lateral chains that can be easily hydrolysed (Fitzpatrick, 2011, Bajpai, 2016).

Hemicelluloses is comprised of various heteropolymers, namely, xylan, galactomannan, glucuroxylan, arabinoxylan, glucomannan and xyloglucan (Isikgor and Becer, 2015). A few of the heteropolymers are depicted in Figure 2.5. The hemicellulose composition varies in hardwoods and softwoods with hardwoods containing mainly xylan while softwoods contain main glucomannans (Isikgor and Becer, 2015).

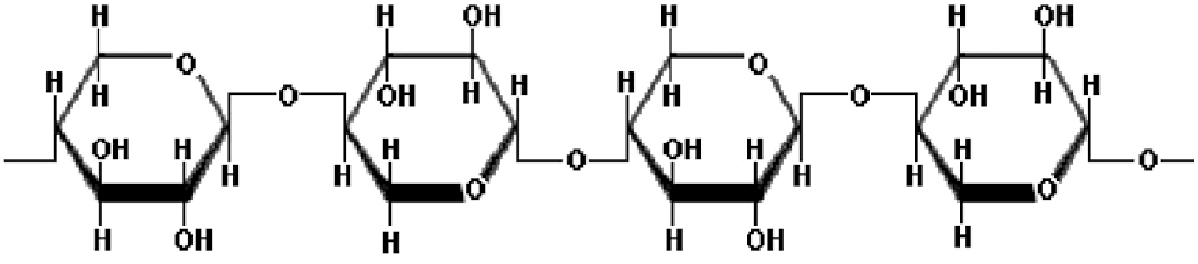
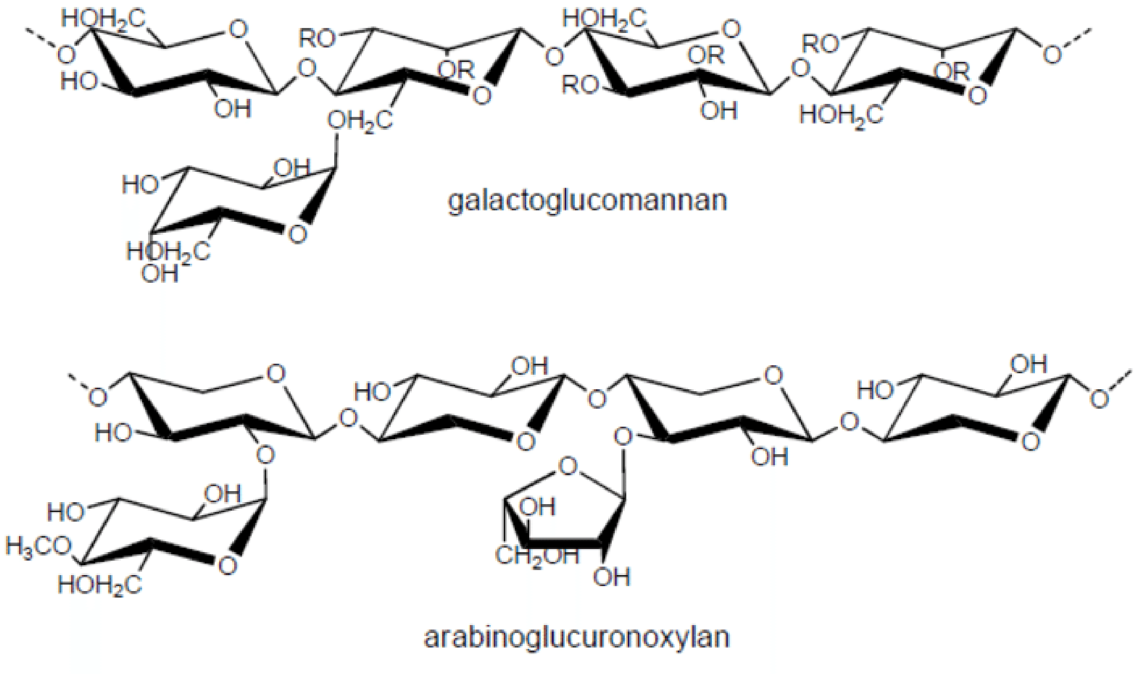


Figure 2.5: Structure of xylan (Costa et al., 2015)



galactoglucomannan

arabinoglucuronoxylan

Figure 2.6 Structure of hemicelluloses (Impola, 2016)

The monosaccharide building blocks of hemicellulose can be divided into 2 classes: hexoses and pentoses. Hexoses include glucose, mannose and galactose; and pentoses encompass for example xylose, mannose and rhamnose (Fitzpatrick, 2011). These sugar units are displayed in Figure 2.6. In addition to monosaccharide units, uronic acid residues and acetylated sugars are present. Uronic acids are made up of 4-O-methylglucuronic, D-glucuronic, and D-galactouronic acids (Bajpai, 2016).

The monosaccharide units of hemicelluloses also depend on wood type, with hardwoods containing mainly pentoses and softwoods containing mainly hexoses (Fitzpatrick, 2011).

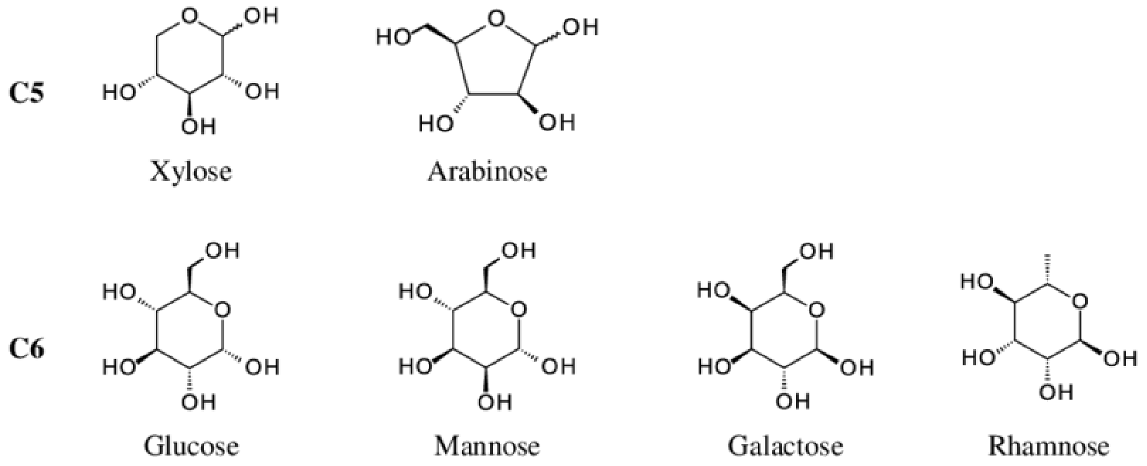


Figure 2.7 Sugar units (Vallejo *et al.*, 2016)

2.4.3 Lignin

Lignin is the third major component of wood. Lignin makes up 23-33% of the dry mass of softwood and 16-25% of the dry mass of hardwood (Sankir and Sankir, 2017). Lignin is a 3-D, highly branched, polyphenolic substance which is comprises of an irregular array of variously bonded hydroxy- and methoxy- substituted phenylpropane units (Ek *et al.*, 2009).

Lignin is considered to be the bonding agent which binds the individual wood fibres together to form a substance of strength and hardness. Lignin is also known to play an important role in transporting water and nutrients, as it has the ability to decrease the diffusion of water through the cell walls of the xylem (Ek *et al.*, 2009).

The structures of *p*-coumaryl, coniferyl and sinapyl are the general structures displayed by the three monomeric phenylpropane units (Mohan *et al.*, 2006). The structures of these three monomers differ in the number of methoxy groups attached to the ring as seen in Figure 2.7. These monomers are referred to as monolignols.

According to the content of monolignols, 3 main types of lignin are identified:

- Softwood lignin or guaiacyl lignin (G) - this type of lignin is present in softwoods, it contains mainly coniferyl alcohol and trace amounts of *p*-coumaryl alcohol with no sinapyl alcohol (Ek *et al.*, 2009).
- Hardwood lignin or syringyl-guaiacyl lignin (S) – this type of lignin is present in eudicotyledonous angiosperms and contains both coniferyl and sinapyl alcohols. The amounts of these two alcohols differ in proportion, in that at times they appear in approximately equal proportions whereas at other times, the sinapyl alcohol exist in three times higher levels. Some hardwood lignin contains trace amounts *p*-coumaryl alcohol (Ek *et al.*, 2009).
- Grass lignin or hydroxy phenol, guaiacyl, syringyl lignin (HGS) – this type of lignin is present in grass as well as in monocotyledonous angiosperms and contains all three monolignols and consists of higher contents of *p*-coumaryl alcohol than the other lignin types (Ek *et al.*, 2009).

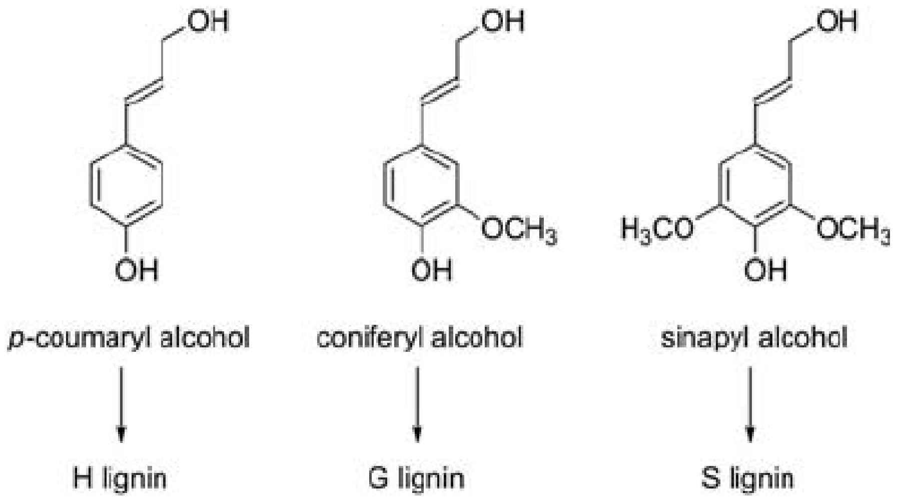


Figure 2.8 Lignin precursors (Stark *et al.*, 2015)

2.4.4 Extractives

Wood, along with its major components: cellulose, hemicelluloses and lignin, contains an extremely large number of low-molecular-weight compounds called extractives ((Herbert, 2006). Extraction of extractives from wood via the use of organic solvents results in terpenes, fats, waxes and phenols whilst extractions using hot water results in tannins and inorganic salts being extracted. Therefore, the yield and composition of the obtained extract are greatly impacted by the solvents as well as extraction procedures used. Contrary to cellulose, hemicelluloses and lignin, extractives composition varies substantially between tree types and species. However, the total amount of extractives is only a few percent of the wood (Ek *et al.*, 2009).

Wood extractives have largely been studied by the pulp and paper industries since they cause problems during the production process and hence also influence the paper properties. Lipophilic extractives, in particular, can be responsible for pitch, which are the dark spots found on bleached pulp and paper and also for the formation of sticky deposits on machinery (Back and Allen, 2000, Zhou, 2011).

Wood properties such as colour, basic density and durability are a result of the extractives that it contains, even though these extractives do not form a structural part of the woody tissue, they contribute to its properties (Zhou, 2011).

The main constituents of wood extractives can be divided into aliphatic compounds, terpenes and phenolic compounds (Ek *et al.*, 2009). The extractive content in hardwood is generally higher than that of softwood, even though different species contain different extractives contents. Terpenes, a lipophilic extractive of interest, are compounds mainly occurring in softwoods (Yang and Jaakkola, 2011). These compounds are rare in hardwoods. Hence, pine wood, a softwood, was chosen as the biomass sample of this study.

Pine tree species contain a total extractive content ranging between 4 and 10% (Pettersen, 1984). The terpenes are made up of a variety of mainly cyclic compounds. The basic units from which all terpenes are made up is the isoprene unit which consists of five carbons. The main terpene groups are: monoterpenes (C10), sesquiterpenes (C15) diterpenes (C20) and triterpenes (C30); their basic structures are shown in Figure 2.9 (Ek *et al.*, 2009). Softwood terpenes consist mainly of monoterpenes, sesquiterpenes and diterpenes as well as sterols, whereas, hardwood terpenes are mainly sterols, triterpenoids and higher terpenes (Ek *et al.*, 2009). Some examples of the terpenes of softwoods are illustrated in Figure 2.9.

The typical aroma of "pine forests" comes from the characteristic compounds found in the softwood resin, which are the monoterpenes and sesquiterpenes. Monoterpenes are volatile, due to this volatility they are not present in Kraft pulps (Ek *et al.*, 2009).

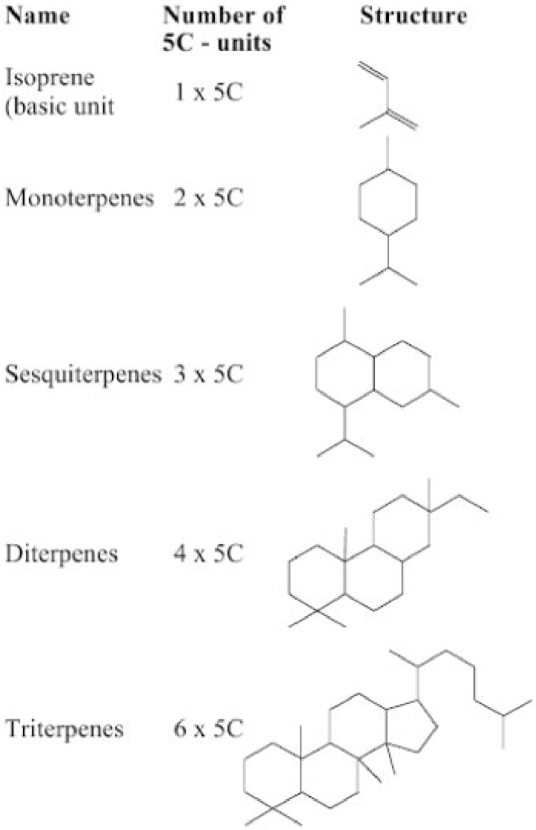
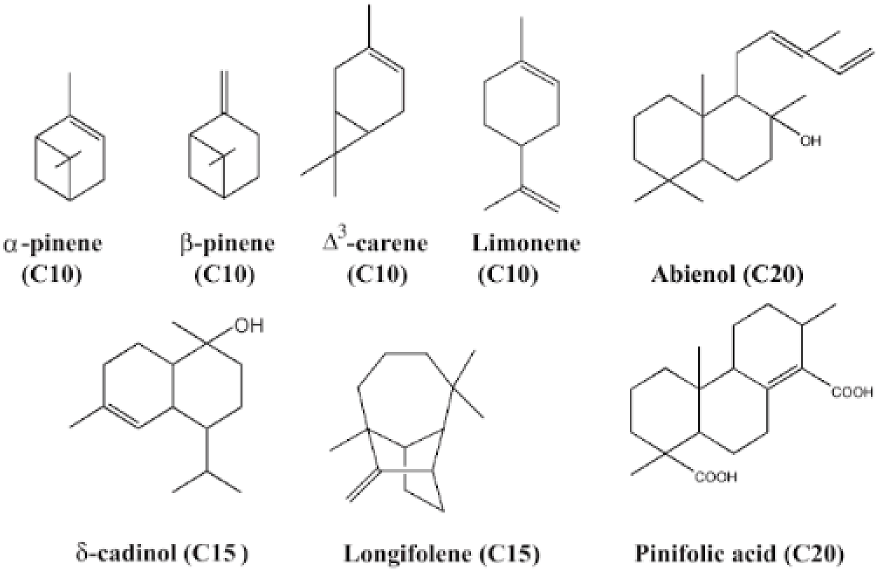


Figure 2.9 Basic structure of the various terpenes (Ek *et al.*, 2009)



α-pinene (C10) β-pinene (C10) Δ^3-carene (C10) Limonene (C10) Abienol (C20)

δ-cadinol (C15) Longifolene (C15) Pinifolic acid (C20)

Figure 2.10 Examples of mono-, sesqu-, and di terpenes (Ek *et al.*, 2009)

2.5 Pine oil

Pine oil plays a vital role in the manufacture of various perfumes, disinfectants, deodorants, etc. It is also used in polishes, insecticides, cattle spray, as a raw material for the production of perfumery grade menthol terpineol and camphorates, as a wetting agent in textile and paper industry (NIIR Board., 2003).

Pine trees contain a substance known as pine oleoresin, which used to be collected by a process called tapping. This pine oleoresin is then used to obtain pine oil. The pine oleoresin is made up of 20% spirits of turpentine (turpentine oil), 65% rosin (resin), 5-10% water and the remainder being bark and dust. Therefore its two major fractions are : turpentine – which is the volatile fraction, containing mono and sesquiterpenes, and rosin – the non-volatile fraction containing diterpenes (Fuller *et al.*, 2016).

The pine oleoresin is used as the raw material used in the manufacture of turpentine oil. The pine oil is later made after processing of the turpentine oil.

The world production of pine oil is dominated by China, Russia, Scandinavia, Italy, France, Spain, USA and Portugal (Sands, 2013). During the period 2000 – 2005, 330,000 tonnes of pine oil was either imported/ exported globally, with an expected growth rate of 7% annually (Sands, 2013).

Pinenes are generally found in the essential oils of coniferous trees. Essential oils are defined as “those products obtained from plant raw material by processes such as steam distillation, hydrodistillation, “dry” distillation or by mechanical processes for citrus fruit” ((Zuzarte and Salgueiro, 2015). Pinenes exist as optical isomers or enantiomers that do not overlap with each others mirror images and they differ only in their interaction with polarized light (Rivas Da Silva *et al.*, 2012).

Pinenes have two constitutional isomers: α-pinene and β-pinene. Both these isomers have enantiomers (non-superimposable mirror images) known to occur in nature as (-)-α-pinene, (+)-α-pinene and (-)-β-pinene and (+)-β-pinene (Rivas Da Silva *et al.*, 2012).

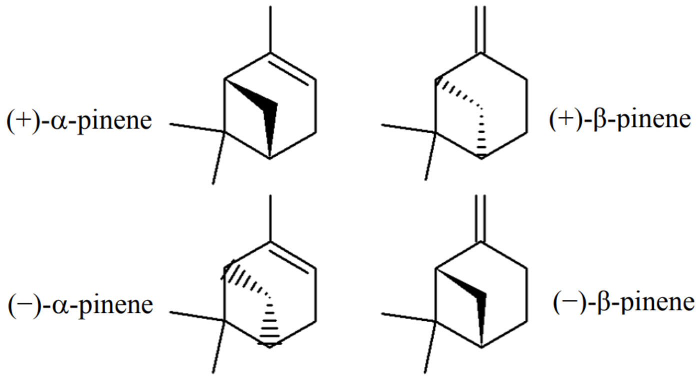


Figure 2.11 Structural formulas of α-pinene and β-pinene enantiomers (Rivas Da Silva *et al.*, 2012)

2.5.1 Turpentine production

Stainless steel batch stills are heated by passing steam through spirally coiled pipes. The pine oleoresin is initially charged into these stills to evaporate the turpentine, thereafter, the vapor produced, which consists of water and turpentine, enters the condenser. A decanter is then used to separate the water and the turpentine. After evaporation, there remains hot molten residue in the still, which constitutes the rosin (NIIR Board., 2003).

2.5.2 Pine oil production

Turpentine oil is reacted with sulphuric acid to produce pine oil.
A lead-lined reactor fitted with heating arrangements and a stirrer is used. Firstly, turpentine and acetone are fed into the reactor, then diluted sulphuric acid (30 - 40% by weight) is gradually added to the turpentine. The mixture is thoroughly mixed and stored at room temperature for 24 hours (NIIR Board., 2003).

The specific gravity of the product is continually checked to determine the end of the reaction. A specific gravity of 0.87 needs to be reached, thereafter the product is removed from the reactor. The next day the sulphuric acid is neutralised using a saturated caustic soda solution. A still with a condenser is used to recover the acetone. After the acetone is recovered, terpene alcohols are separated from other reaction products including unreacted turpentine oil with the use of steam distillation (NIIR Board., 2003).

2.6 Pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS)

Pyrolysis is the process by which heat is applied to large complex molecules in order to break them apart into smaller fragments in the absence of oxygen. This process can be used beneficially in analytical chemistry, for example when coupled to a gas chromatograph fitted with a mass spectrometer. In earlier days, the use of the simple GC/MS technique was restricted. However, with the aid of the pyrolyser being coupled with the GC/MS, a larger range of samples can be analysed which is fundamental for obtaining comprehensive qualitative and quantitative results. A fused silica capillary column is used to first separate the pyrolysis products and then using the mass spectra libraries, these products are identified. These results are recorded by an analyser which in turn provides a characteristic pyrolysis profile known as a *pyrogram*.

The use of the pyrolyser allows for the direct analysis of the solid polymer/ co-polymer sample as it eliminates the process of pre-treatment (Kusch, 2012). In the context of the analysis of lipophilic extractives, these are extracted by use of solvents such as, cyclohexane, ethanol or acetone (Sjostrom and Alen, 1999). These solvents allow for minimal extraction of lignans, which are a large group of polyphenols found in plants. The lipophilic extracts are then analysed using the Py-GC/MS analysis technique.

Pyrolysis techniques are used for the degradation of compounds by means of thermal energy. Temperatures used to conduct pyrolysis, affects the complexity of the pyrograms achieved. Lower temperatures cause less fragmentation and hence, smaller amounts of pyrolysis products, while higher temperatures cause more fragmentation and thus larger amounts of pyrolysis products (Sithole, 2000). The gas chromatography was designed to assist in the separation of the fragments for easier detection. The separated products are then detected using a suitable detector, in this case, a mass spectrometer (Sithole, 2000).

2.7 Accelerated Solvent Extraction

The Accelerated Solvent Extraction (ASE) instrument is used in this study to facilitate the extraction of lipophilic extracts from sawdust. ASE, also referred to as pressurized fluid extraction (PFE) and pressurized liquid extraction (PLE), is a technique that uses solvents at elevated temperatures and pressures (Luthria *et al.*, 2004).

This technique enables the use of temperatures greater than that of the boiling point of solvents being used, provided that the pressures used are adequately high enough. This allows solvents to remain in a liquid state during the extraction process. Pressure allows the extraction cell to be filled faster, thus forcing the liquid into the pores and otherwise unreachable sites, while keeping the solvent in liquid form at operating temperatures (Luque de Castro and Priego-Capote, 2010). The pressurized solvent is forced to penetrate the sample pores for more intimate contact with the target analytes, allowing for a much more efficient extraction than traditional extraction techniques that use atmospheric pressure operation. The high pressure facilitates better removal and higher yields/recovery of analytes. The system using less solvent reduces costs and pollution to the environment when being disposed of. The automation and reduced extraction time (typically between 20 to 40 minutes) allows for the extraction of multiple samples (approximately 24 in a standard ASE system) and allows less user interaction making it less labour intensive (Kaufmann & Christen, 2002). A schematic diagram of an ASE system is shown in Figure 2.11.

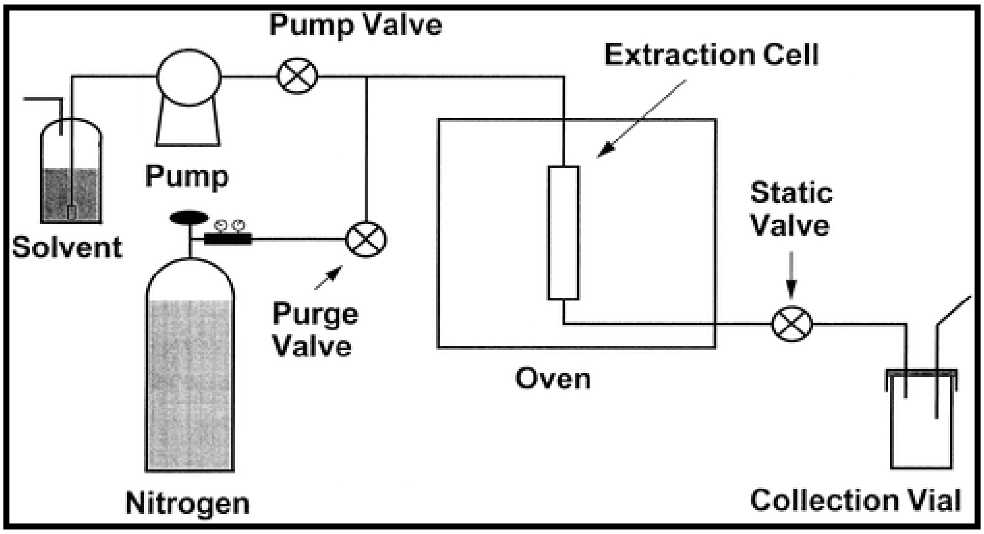


Figure 2.12 Schematic of accelerated solvent extraction (ASE) system (adapted from Kaufmann and Christen, 2002)

2.8 Soxhlet extraction

Soxhlet extraction is a method of semi-continuous solid-liquid extraction whereby a solid sample is brought into contact with an extraction solvent and the desired compound is extracted (Waksmundzka-Hajnos *et al.*, 2008).“It was originally designed for the extraction of a lipid from a solid material” (Donato *et al.*, 2013).

In conventional Soxhlet extraction, as depicted in Figure 2.12, a flask containing the extraction solvent is placed in a heating mantle. The solvent is allowed to heat until it vaporises, the vapours then run along the side arm until they reach the condenser (Waksmundzka-Hajnos *et al.*, 2008). Once the vapours have reached the condenser, the solvent drips into the thimble in the Soxhlet extractor. In this study a cellulose thimble is used, and it contains the solid sample that requires extraction.

An approximate time of ten minutes is assigned to each cycle, during this time sufficient solvent is evaporated and condensed in order to fill the Soxhlet extractor. Once it has been filled, the siphon unloads the contents back into the distillation flask, which now contain the extracted analytes in the bulk liquid (Luque de Castro and Priego-Capote, 2010).

Every time the siphon unloads its contents back into the distillation flask, this is regarded as one extraction cycle. It is advised that a minimum of six extraction cycles are observed to occur in an hour while allowing the entire system to run over a period of six hours (Gutierrez *et al.*, 1998). Hence, by the end of the six hours, the distillation flask contains a concentrated desired extract. This is when the extraction cycles are considered complete. If less than six cycles are observed in an hour this would then imply that the extraction cycles are not provided with sufficient time to extract the desired compounds.

The continuous character of the system is caused by the solvent being recirculated through the sample (Luque de Castro and Priego-Capote, 2010). After extraction, a rotary evaporator is used to remove the solvent, leaving behind the extract. The fraction remaining in the thimble is usually discarded.

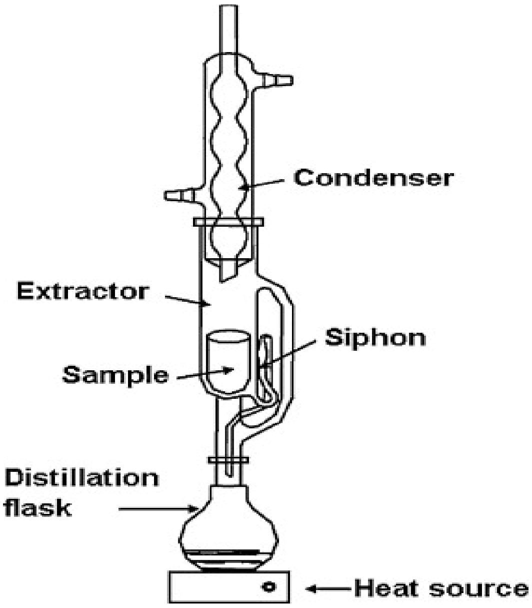


Figure 2.13 Conventional Soxhlet extractor (adapted from Castro and Priego-Capote, 2010)

Sithole *et al.* (1991) have compared Soxhlet and Soxtec extraction methods for the determination of extractives content. Several samples were tested using both methods and all results showed that lower extractives content were extracted using the Soxtec extraction method (Sithole *et al.*, 1991).

The Soxtec extraction method is a modification of the traditional Soxhlet extraction method. It used a 3-step procedure which includes: boiling, rinsing and lastly evaporation (Anderson, 2004). During the boiling step, the thimble, containing the sample, is placed in the solvents while ensuring the solvent level is higher than that of the sample in the thimble. Depending on the solvents used, the extraction times are generally between 20 and 40 mins (Anderson, 2004). Immediately after the boiling step, the rinsing step begins. The sample is suspended over the boiling solvent and the extractable material is flushed for approximately 10 minutes longer than the boiling step and is collected in the extraction cup. Lastly, in the evaporation step, the solvent is left to boil and evaporate, and the condensate is directed out of the condenser by means of an internal valve. Evaporation is complete when all the solvent has been directed out of the cup, leaving behind only a concentrated extract (Anderson, 2004).

Sithole *et al.* (1991) then modified the Soxtec extraction method, by doubling the time used during the rinsing step. This modification then lead to the extractive components from the Soxhlet extraction to be the same as well as being in similar amounts to those extracted by the improved Soxtec method (Sithole *et al.*, 1991).

2.9 Box-Behnken design

Traditionally, optimization studies were based on using the one-factor-at-a-time approach. This approach is time consuming and expensive since it allows for only one factor to be variable at a time while all others are kept constant. This approach poses difficulties in analysing results, in that, interactions between variables are not able to be evaluated hence, leading to deceptive conclusions being drawn. It is due to these drawbacks that the Response Surface Methodology (RSM) is useful as it allows for the evaluation of possible interactions between variables (Derkyi, 2010).

"Response Surface Methodology is a collection of mathematical and statistical techniques useful for the modelling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response" (Perincek and Colak, 2013).

The Box Behnken Design (BBD) is a type of Response Surface Methodologies, which is a collection of mathematical and statistical techniques, that is used to model and analyse problems where the response is influenced by multiple independent variables and thereby optimising the response (Perincek and Colak, 2013). Ensuring that the accuracy of optimisation of the experiments is not affected, implementing Box Behnken Design can significantly minimise the number of experimental sets (Qiu *et al.*, 2014).

Optimisation via application of a Box–Behnken design includes four main steps (Qiu *et al.*, 2014).
1) Provision of an experimental plan based on the specified conditions.
2) Perform statistically designed experiments.
3) Check the accuracy of the model and coefficients in the mathematical model.
4) Response analysis is performed to predict the optimal conditions, and these predictions are confirmed experimentally.
Box Behnken design is an independent three-level quadratic design as it does not contain embedded factorial or fractional factorial design. In this design, the combinations are treated such that they are located at midpoints of edges formed by any two factors (Leiviska, 2013).

2.10 Kinetic studies

Kinetic studies are conducted to improve efficiency and optimise the extraction process by analysing the variation of process yield with time and temperature (Rezazi *et al.*, 2017). The products generated from the extraction process are explained in this study by developing kinetic models (Aguilar *et al.*, 2002). According to the literature, a solid-liquid extraction process is best fitted by a second-order model (Gisila, 2018, Muhammad Hazwan *et al.*, 2013, Sayyar *et al.*, 2009). This implies that the extraction process takes place in two stages:

> Stage 1: when a major portion of the solute is extracted quickly caused by a driving force of the fresh solvent
>
> Stage 2: when the extraction rate is slowed down which is caused by a slow diffusion of the remainder solute

The rate of dissolution for the lipophilic extracts contained in the solid to solution can be described by the following second order kinetic equation.

$$\frac{dC_t}{dt} = k(C_S - C_t)^2 \qquad (2.1)$$

where k = the second order extraction rate constant (L g^{-1} min^{-1})
C_s = the extraction capacity (concentration of extract at saturation in g L^{-1})
C_t = The concentration of extract in the solution at any time (g L^{-1}), t (min)

By considering the boundary condition t=0-t, and $C_t = 0 - C_t$, the integrated rate law for a second order extraction was obtained:

$$C_t = \frac{C_s{}^2kt}{(1+C_skt)} \tag{2.2}$$

A linear form of equation 2.2, would be:

$$\frac{t}{C_t} = \left(\frac{1}{kC_s{}^2}\right) + \left(\frac{t}{C_s}\right) \tag{2.3}$$

The extraction rate can be written as the following:

$$\frac{C_t}{t} = \frac{1}{\left[\left(\frac{1}{kC_s{}^2}\right)+\left(\frac{t}{C_s}\right)\right]} \tag{2.4}$$

Then, when t approaches 0, the initial extraction rate, h (g L^{-1} min^{-1}), can be written as:

$$h = kC_s{}^2 \tag{2.5}$$

After rearrangement of equation 2.4, the concentration of the extract at any time can be obtained as:

$$C_t = \frac{t}{\left[\left(\frac{1}{h}\right)+\left(\frac{t}{C_s}\right)\right]} \tag{2.6}$$

The initial extraction rate, h, the extraction capacity, C_s, and the second order extraction constant, k, can be calculated experimentally by plotting t/C_t vs. t.

2.11 Previous literature studies

Studies have been conducted on extractions from pine wood, although not particularly on sawdust. They include studies by Bertaud *et al.* (2017), Harman-Ware *et al.* (2016), Smith (2011), Yuman (2011) and Zhou (2011). In the study by Harman-Ware *et al.* (2016) extractions of terpenoids from pine wood was conducted and the extracts were analysed by GC-FID: however, the extraction method was not stated. Bertaud *et al.* (2017) used gas chromatography with mass spectrometry detection to study pine wood extractives. Several extraction methodologies were used including Soxhlet extraction and Accelerated Solvent Extraction. The results showed that Soxhlet extractions yielded highest concentrations of volatile terpenes when using acetone – cyclohexane (1:9, v/v%) as the extracting solvent. Additionally, the ASE extraction method was not suitable for extraction of volatile compounds due to its use of high pressure. Zhou (2011) and Yuman (2011) studied extraction of pine wood: (Zhou, 2011) used a polar solvent whereas (Yuman, 2011) used a non-polar solvent. Zhou's work identified phenolic extractives whereas Yuman's study did not include a thorough composition of the extractives, however, indicated that extraction time was less influential than temperature on the efficacy of the extraction.

Kinetic studies on extractions from plant biomass are widespread and include first- and second-order kinetic models. For example, Aguilar *et al.* (2002), Conner (1983), Koostra *et al.* (2009) and Kusuma and Mahfud (2015) found that the first-order kinetic model best described their systems, whereas (Gisila, 2018, Krishnan and Rajan, 2017, Muhammad Hazwan *et al.*, 2013, Saxena *et al.*, 2012, Sayyar *et al.*, 2009) reported that the second-order kinetic model offered the best fit to the extraction systems.

This work covers the utilisation of pine sawdust, a 'waste' by-product from sawmills and other wood industries, for the extraction of valuable lipophilic extractives. Amongst these extractives is pinene that can be converted to pine oil, which is currently being imported to South Africa. Also, the seasoning (aging) of sawdust was studied to ascertain the effect aging has on the quantity of the extractives in the sawdust as it is well known that these extractives decrease after tree felling.

CHAPTER 3: MATERIALS AND METHODS

3.1 Solvents and samples

The solvents used in this study were cyclohexane (99.5%), acetone (99.5%) and ethanol (99.9%) and were all purchased from United Scientific SA cc. Pine chips and sawdust samples were obtained from a Hans-Merensky saw mill in KwaZulu-Natal, South Africa.

3.2 Sample preparation

Samples of the pine chips and sawdust obtained from 4 different chip piles at the mill. The ages of the piles were between 1 and 2 years old. These samples were labelled as Samples 1 - 4. These samples were used to conduct experiments of the preliminary soxhlet extractions as well as the accelerated solvent extractions. Fresh batches of sawdust collected and used for the seasoning as well as the kinetic studies.

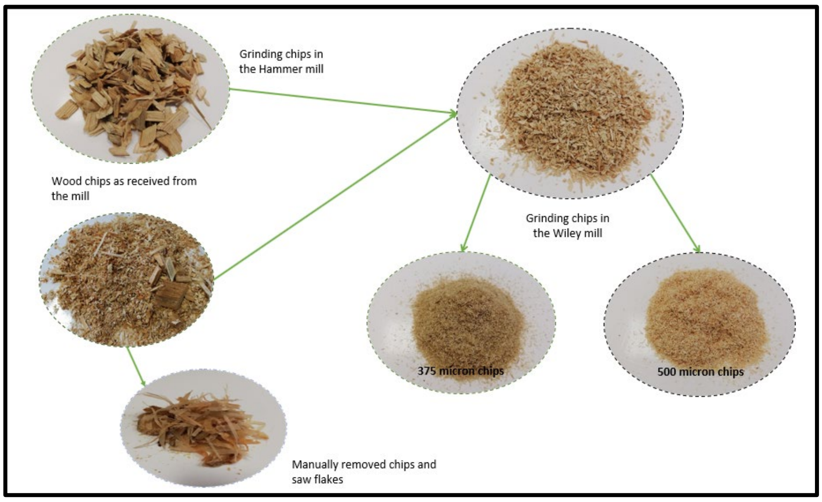


Figure 3.1 Schematic of sample preparation – wood chips to sawdust

Portions of the chips and sawdust were taken and comminuted into smaller particles using a hammermill and a Wiley mill. The ground samples were then sieved into different particle sizes using a series of screens that varied from 800 μm at the top to 355 μm at the bottom. According to the Tappi standard method T257 cm-85, material that passes through 40 mesh screens should be used for determination of extractives content. Therefore, particles closest to 40 mesh, namely, those screened through 500 and 355 μm were collected for analysis. Figure 3.1 is a schematic illustrating how the samples were processed.

3.3 Methodology

3.3.1 Soxhlet extractions

The sawdust was tested for moisture contents which were found to lie between 8 and 15%. Older pine wood residues are known to be at a risk of losing volatile components when left for large of periods of time (Routa *et al.*, 2017). However, this research aimed at determining the viability of using different aged sawdust residues to add value to the biorefinery industry for the extraction of lipophilic extractives. These results are presented below.

The extractives content is reported as a percent of dry mass and are determined using equation 4.1.

$$\%Extract = \frac{Mass\ of\ dried\ extract\ -\ Mass\ of\ blank\ residue}{Dry\ Mass\ of\ Sample\ (g)} \tag{4.1}$$

The mass of the blank residue was determined by adding 300ml of the solvents used in the extractions, to a pre-weighed round bottom flask, and thereafter removing all the solvent from the flask using rotary evaporation. The round bottom flask was then placed in the oven at 105 °C, for 2 hours and then weighed again.

A typical extraction is carried out over a period of six hours, to allow for a sufficient number of cycles. Thus, using two units simultaneously allowed for only two extractions to be carried out a day. These extractions were carried out using four solvents, two of which were pure solvents and the other two were mixtures and 4 sawdust samples. Extracts of pine wood consist of a majority of non-polar compounds; hence, the solvent choices were based on them having a low polarity. The solvents used were pure acetone and pure cyclohexane and the mixtures were a combination of cyclohexane, acetone and ethanol in a 3:1 (v/v) ratio with cyclohexane being the major solvent. The choice of the solvent ratios was determined following optimisation of extractions with various ratios that were tested. The sawdust samples are referred to, in this study, as sample 1, 2, 3 and 4, with sample 1 being the freshest sample and sample 4 being the oldest sample. Solvent extracts were analysed using Py-GC/MS.

Initial extractions were conducted using Soxhlet extraction. In this extraction procedure 4g of sample were weighed and placed into cellulose thimbles. Four different solvents were investigated: pure cyclohexane, pure acetone, 3:1 cyclohexane-ethanol (v/v) and 3:1 cyclohexane-acetone (v/v). 300ml of solvent was measured and poured into the round bottom flask. The extraction process was carried out for 6 hours to ensure sufficient extraction cycles occurred. The temperature of the heating mantle was set to ensure that sufficient boiling occurred for the required number of extraction cycles per hour.

On completion of the extraction process, the heating mantle was switched off and the solvent was left to cool, before the removal of the excess solvent via the use of rotary evaporation.

3.3.2 Accelerated solvent extractions

An automated extraction technique, i.e., the Dionex ASE 350 (Dionex Corp, Sunnyvale, CA) was used to carry out the accelerated solvent extractions. Approximately 8g of sawdust sample was weighed and added into stainless steel cells. Each cell was fitted with a glass fibre filter that prevented the suspended particles from falling out into the collection bottles. Pure cyclohexane was used as the extraction solvent for these experiments.

A Box Behnken experimental design was used to create an experimental plan for these extractions to determine the amount of extractives attainable factoring three independent variables which were desired temperature, static time and number of cycles. Each independent variable was evaluated at three levels: temperature at 80, 115 and 150 °C; extraction time at 10, 20 and 30 minutes; and number of extraction cycles at 1, 2 and 3 cycles.

Table 3.1 Coded values of variables used in Box-Behnken Design

			Levels		
Coded value	**Independent Variables**	**Units**	**-1**	**0**	**1**
x1	Temperature	°C	80	115	150
x2	Time	Min	10	20	30
x3	Cycles		1	2	3

Table 3.2 Box-Behnken experimental design for accelerated solvent extraction

Run	**Temperature (°C)**	**Time (min)**	**Cycles**
1	115	10	3
2	150	10	2
3	80	20	1
4	80	20	3
5	115	20	1
6	115	20	2
7	115	30	1
8	80	30	2
9	80	10	2
10	150	20	1
11	150	20	3
12	80	30	1

The extraction process encompassed first pumping the solvent into the cell that contained the sawdust sample, then pressurising the cell and thereafter heating the cell for the times stated above. Static

extraction periods occur between each cycle, during this static extraction period 40% of the fresh solvent was pumped into the cells over a period of 5 minutes, preparing it for the next cycle. Lastly, nitrogen gas was used to purge the solvent before it was collected for analysis.
The samples were then transferred from the collection bottles to round bottom flasks for the removal of the excess solvent.
For this set of experiments conducted a randomised design of experiment (Box-Behnken) was used.

3.3.3 Soxhlet extractions – Effect of extraction time, solvent type and particle size

This set of sawdust extractions were carried out using the Soxhlet Extraction method. The solvents used to extract the lipophilic material consisted of 3:1 cyclohexane-ethanol and 3:1 cyclohexane-acetone and 3:1 acetone-ethanol mixtures. The choice of these solvents was based on the results achieved from the preliminary extractions.

The system involved placing 4g of the sawdust sample into a cellulose thimble, which was then inserted into the Soxhlet tube. The round bottom flask, containing the extraction solvent, is placed in a heating mantle. The temperature on the heating mantle is adjusted to ensure that 6 siphon cycles occur per hour.

These experiments were carried out as a function of time; therefore, the total extraction times were 2, 3 and 4 hours. Once extraction time has been completed, the round bottom flask is cooled before the solvent is removed using a rotary evaporator.

The effect of extraction time, solvent type and sawdust particle size were chosen as the variables. The factors were chosen to determine the best combination of variables to achieve maximum extraction yield. Temperature of the solvent needs to be kept at a constant value to ensure that the boiling rate is sufficient for the cycles to occur.

3.3.4 Seasoning study

The prime objective of conducting seasoning tests, in this study, is to understand how the storage conditions and time affect the lipophilic extractive yield and composition on chips and sawdust stored on mill sites. Seasoning entails placing fresh wood chips or logs outdoors for a period of time (Farrell *et al.*, 1997) and this is an effective way of reducing lipophilic extractives before pulping of softwoods (Back and Allen, 2000). In this study, a seasoning silo was designed and utilised for this purpose (Sithole *et al.*, 2002). The seasoning silo was designed to allow for air to enter the silo and hence, air dry the sawdust particles. This method of seasoning of sawdust is not the commonly used operation carried out in a kiln, however this method has been successfully carried out by Sithole *et al.,* 2002.

The silo box was made using plywood with a polystyrene foam insulation as the lining. The polystyrene lid of the box was prepared with nine 10-cm diameter holes arranged around the circumference of a 60-cm diameter circle for ventilation purposes. The bottom of the box was also fitted with nine 10-cm diameter holes. Figure 2.13 shows top views of both these lids. The plywood cover of the box was placed in a manner so as to allow sufficient ventilation into the box. The volume of the box was $1 m^3$ and contained 50kg of sawdust (dry mass). The box was stored at room temperature.

It is commonly known that wood extractives are decomposed while left in the seasoning process (Farrell *et al.*, 1997). Various factors affect the extractives in wood and thereby cause its decline during seasoning, these factors are: respiration, emissions, auto-oxidation and fungal attack amongst others (Dai, 2001).

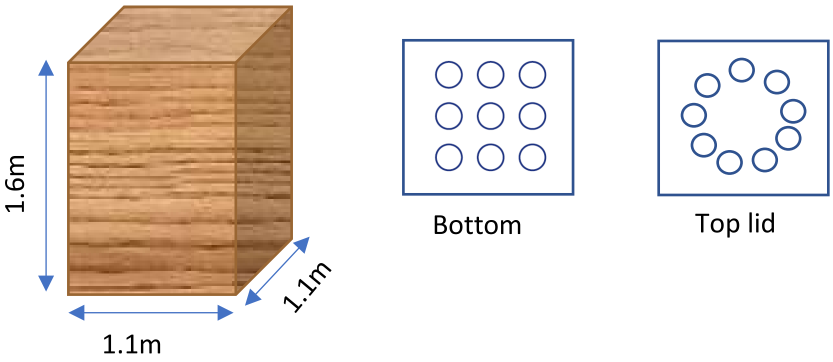


Figure 3.2 Schematic of seasoning silo (Sithole *et al.*, 2002)

Generally, it is not recommended to carry out the seasoning process on pine species when the intent is to recover tall oil and turpentine (Farrell *et al.*, 1997). It has been stated in the study by Farrell *et al.* that eighty percent of tall oil is lost during seasoning over a 30-week storage period, this shows the extent to which the decomposition of extractives occur during seasoning.

In this study, the seasoning process was conducted, over a 3-month period, to determine whether sawdust that has been stored at a mill, for several months after a tree has been cut, could be of value to the forestry and biorefinery industries. It has been stated that during the seasoning process, the extractive content initially decreases and then increases, which is as a result of the carbohydrate fraction in wood degrading (Dai, 2001).

The sawdust for this set of experiments were stored in the silo as mentioned in section 2.9. The sawdust was seasoned over a 3-month period, during which weekly samples were removed to conduct analysis. During the study of section 3.3.3, it was found that 2-hour extraction times and 375-micron particle size were most efficient in extracting maximum extractives and these conditions were used to carry out the seasoning study.

3.3.5 Kinetic Study

The kinetic study was conducted using Soxhlet extraction method with the round bottom flask placed in a water bath to control the temperature to which the solvent was heated. Four temperatures were tested, these were 70, 75, 80 and 85°C. The extracting solvent used in this section was 3:1 cyclohexane – ethanol (v/v).

The following method was followed for each temperature.
The temperature of the water bath was set, and left for approximately an hour before the correct temperature was reached. The Soxhlet extraction system was set up with the thimble containing the sample placed in the Soxhlet tube and the solvent in the flask. The flask was thereafter immersed into the water bath, and it was left until the solvent was heated. When the first drop of solvent reached the sample, the timer was started and after 30 minutes the solvent containing extract was removed. The experiment was terminated and the round bottom containing the solvent and extract was removed. This was then replaced with a round bottom flask containing fresh solvent and the experiment was resumed. This was repeated at each of the desired time intervals. The solvent was then removed using rotary evaporation.

The concentration of the terpenes in the extract was determined by first determining the mass of the terpenes in the extract, and dividing the value by the quantity of solvent used in litres. The concentration was thus presented in (g/l) as this was seen to be the concentration units used in majority of the kinetic studies conducted for extractions from plant biomass. Amongst these researchers were (Aguilar *et al.*, 2002), (Gisila, 2018) and (Koostra *et al.*, 2009).

At each temperature, 3 systems were set up and the method described above was followed. The extracts were thereafter all characterised using Py-GC/MS analysis and average values were reported.

3.4. Rotary evaporation

The rotary evaporation process is operated under vacuum pressure to lower the boiling point of the compound while at the same time heating the solvent by placing the flask in a water bath. A pump is thus used to provide the pressure. The flask containing the solvent is continuously rotated to increase the effective surface area thereby increasing the rate of evaporation of the solvent.

The temperature was set just below the boiling point of the solvent to allow all of the solvent to evaporate to almost complete dryness. When all visible solvent has been removed, the flask was placed in an oven for 2 hours to dry off the excess solvent and thereafter cooled to room temperature in a desiccator. The waste solvent is collected in a collection flask, and on completion of the rotary evaporation, the solvent is kept in a bottle to be disposed of accordingly.

The extract is then prepared for analysis by Py-GC/MS and the remainder of the extracts are stored in 10 ml vials and kept refrigerated.

3.5. Characterisation of extractives

The extracts were analysed by Pyrolysis Gas Chromatography-Mass Spectrometry (Py-GC/MS) using a Frontier PY-2020iD typed pyrolyser directly connected to a Shimadzu 6890 GC/MS equipped with a 30m x 0.25mm with 0.25 μm HP 5MS fused silica capillary column. Approximately 100 μg of extract was added to the sample cup and allowed to dry in the oven at 105°C. After drying, the sample cups were placed in a desiccator to remove moisture from the sample and to protect it against water vapour from the atmosphere. The GC/MS conditions were as follows: the oven temperature was held at 50 °C for 2 min and then increased up to 220 °C at 2 °C per min. The column was maintained at 220 °C for 20 min. The carrier gas used was helium with a controlled flow of 1 ml/min. The pyrolysis products were identified by comparing their mass spectra with the mass spectra of the Wiley and National Institute of Standards and Technology libraries.

Preliminary Soxhlet extractions were conducted in duplicate to assess the reproducibility of the results obtained. Minor deviations were noticed between the runs, indicating good reproducibility. All results obtained thereafter were assumed to have similar deviations.

CHAPTER 4: RESULTS AND DISCUSSIONS

This work was based on the extraction of lipophilic extractives from softwood sawdust, to ascertain their concentrations and chemistry of the components for possibility of beneficiation into valuable bio-chemicals and materials. When conducting studies on the extractives of wood, not only are the total extractive contents important, but knowing the composition of the extracts are also relevant.

The first section discusses the obtained results of the traditional Soxhlet method of extractions using a 6-hour extraction time. The second section discusses the use of an alternate method of extraction that makes use of a decreased extraction time. The third section focusses on the positive outcome of the ASE extractions to study the effects of using Soxhlet extractions as a function of time. The fourth section focusses on the seasoning study to determine the effect of aging of sawdust on the extractives obtained. The last section discusses the use of the optimised conditions on an old batch of sawdust samples to ascertain their effects on extractives obtained.

4.1 Soxhlet extractions

Results for the Soxhlet extractions, carried out over a 6-hour extraction period, are displayed in Table 4.1. The value of the extractives reported are the percentage of the dry mass sample, while the α – pinene and terpene contents are percentages of the extractives. The extractives content cannot be used as the only factor to determine the most efficient solvent as these do not correctly represent the extracts with the best composition of extractives. All the total extractives were found to lie within the 4-10% range as indicated from the literature value presented in Table 2.2. The α – pinene content as well as the total terpene contents, which are inclusive of the α–pinene, would be more effective as these increase the value of the extracts. Using sample 1, with 3:1 cyclohexane – ethanol as the extracting solvent, a high percentage of α – pinene was extracted. However, using the same sample and 3:1 cyclohexane – acetone as the extracting solvent, the α – pinene content decreased by approximately 40%. This α–pinene content, however, is still relatively high compared to the other results obtained. Pure cyclohexane is also shown to have extracted α–pinene in sample 1, but with a much smaller percentage, and a slighter greater percent of other terpenes. Sample 2 displayed α–pinene contents in extracts of 3 of the solvents tested. The fourth solvent had no α–pinene but did contain a small percent of other terpenes. A maximum α–pinene content was observed in the 3:1 cyclohexane-ethanol extract. Sample 3 had no α–pinene present in any of the extracts but did contain other terpenes in extracts of pure cyclohexane and 3:1 cyclohexane acetone (v/v). Sample 4 had a low α-pinene content but an overall significantly high terpene content. When 3:1 cyclohexane – ethanol was used as the extraction solvent, from Table 4.1 it is seen that 0,03 % was that of α – pinene and the total terpene percentage was 3,7%. The major terpene accounted for in this case was d-limonene, which made up 3,67% of the terpenes in the extract. Some uses of d-limonene are, as flavouring, to treat as well as to prevent cancer, and to treat bronchitis.

In Table 4.1 the different classes of compounds present in the extracts are shown. The terpene content is relatively low across the different solvents tested, with the majority portion of the extracts being α-pinene. The terpene content in in the extracts are seen to increase significantly after the addition of a solvent to the cyclohexane. The addition of a solvent to cyclohexane shows a decrease in the amount of aldehydes present in the extracts, while the addition of ethanol showed to detection of ketones in the extracts. In general, alkanes and alkenes are present in all the extracts with an exception to sample 2 extracted with 3:1 cyclohexane – acetone, this could be owed to the alkane content being too small to be detected by the Py-GC/MS analyser. Larger amounts of both alcohols and fatty acid are present in the extracts of pure cyclohexane extractions.

Thus, cyclohexane can be considered a good extracting solvent only when it is used as a mixture with other solvents, in this case together with acetone or ethanol. In terms of the different aged samples used, the extraction solvents used made a greater impact on the composition of the extracts as compared to the ages of the samples. Hence, it can be concluded that all aged sawdust can be beneficiated for the different terpenes, which are all essentially valuable compounds.

Table 4.1 Main compound classes of preliminary Soxhlet extractions[1]

Sample	**Solvent**	**% Extractives**	**% Terpene**	**% α-Pinene**	**% Alkane**	**% Alkene**	**% Ketone**	**% Aldehyde**	**% Alcohol**	**% Fatty Acid**
Sample 1	Cyclohexane	7,59	0,21	0,09	1,32	22,5	1,55	1,63	2,99	7,38
Sample 2	Cyclohexane	7,28	0,04	-	4,98	5,1	5,43	1,39	8,01	22,48
Sample 3	Cyclohexane	6,43	0,01	-	0,61	5,13	8,22	4,12	8,62	8,23
Sample 1	3:1 Cyclohexane-Acetone	8,47	1,23	1,01	0,26	3,69	2,54	0,97	1,85	0,13
Sample 2	3:1 Cyclohexane-Acetone	7,78	0,63	0,48	-	1,7	0,49	-	7,55	3,35
Sample 3	3:1 Cyclohexane-Acetone	7,24	0,34	-	1,59	2,38	1,74	-	4,78	1,74
Sample 1	3:1 Cyclohexane-Ethanol	6,46	2,03	1,75	3,05	6,49	-	-	3,31	1,03
Sample 2	3:1 Cyclohexane-Ethanol	7,84	0,16	0,16	6,14	4,15	-	0,14	0,11	0,21
Sample 4	3:1 Cyclohexane-Ethanol	8,77	3,7	0,03	2,86	5,89	-	-	0,37	0,59
Sample 2	Acetone	5,74	0,23	0,16	3,4	1,92	0,34	2,67	5,5	1,16

1

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[1] Triplicate samples of sawdust were used and the values presented are averages of the runs conducted

4.2 Accelerated solvent extractions

The ASE (Accelerated Solvent Extraction) method was intended to be used to compare results with those obtained from the traditional Soxhlet extraction method, however the equipment was only accessible to conduct studies using pure cyclohexane as the solvent. These results are displayed in Table 4.2 below.

The Box Behnken design (BBD) was used for the ASE experiments to determine the percentage of extractives attainable factoring three independent variables such as extraction time, the number of static cycles and temperature. Each independent variable or factor was evaluated at three levels: temperature was evaluated at 80, 115 and 150 degrees celsius, extraction time was evaluated at 10, 20 and 30 minutes and lastly the number of static cycles were evaluated at 1, 2 and 3 cycles.

Table 4.2 ASE extractions - Pure cyclohexane (Sample 2 – 500-micron)

Temperature (℃)	**Time (min)**	**Cycles**	**% Extractives**	**% α - Pinene**	**% Terpene**
80	10	2	6.42	3.6	4.22
80	20	1	4.53	2.32	2.71
80	20	3	8.76	0.48	0.73
80	30	1	4.17	0.76	0.98
80	30	2	6.66	0.16	0.53
115	10	3	6.69	0.6	0.94
115	20	1	5.45	0.33	0.52
115	20	2	7.21	1.51	2.44
115	30	1	5.32	0.15	0.5
150	10	2	7.39	0.77	1.13
150	20	1	3.70	0	0.4
150	20	3	5.02	0	0.45

** Table produced by design of experiment, rearranged here for logical order

It is evident from the results that significant amounts of α- pinene were detected in the ASE extracts with cyclohexane as the extracting solvent. Two of the extractions carried out at 150°C produced no α-pinene. This indicates that the α-pinene content present in the extracts was too small to be detected. This could also be as a result of the sawdust samples decomposing after exposure to high temperature.

At 80°C, a significant difference is seen in the extractions at 10 mins, 2 cycles; and at 20 mins with 1 cycle; although both these total extraction times are 20 minutes. This is because of the number of cycles used for a complete extraction play a significant role in the extractions. Long extraction times in a single cycle result in decreased amounts of extracts, hence the extraction cycles should be increased and the times reduced.

At 115°C, a reduction in the α-pinene content is noticed as the temperature of the system was now above the solvents boiling points of the solvents. At 150°C, the temperature was too high for the sample to handle and thus all the α-pinene in the extract was decomposed.

All the above extractions were carried out using the same solvent as well as the same age of sawdust (sample 2). From the results obtained for the ASE extraction analyses the most amount of α-pinene found in the sawdust sample is from the run conducted at:

1. 80°C, 10 mins, 2 cycles

The reason for the higher terpene content could be attributed to the time over which Soxhlet extractions are meant to be carried out. This extended time could lead to components being decomposed.

Table 4.3 displays the main classes of compounds identified in the extracts from the ASE extractions. The alkanes content is seen to be the greatest at the 150°C extractions, showing that while decomposing the terpenes in the sample more alkanes are being extracted. The fatty acid content was relatively low throughout the experiment, but higher values were present in the extracts of the 150°C extractions. Most amounts of aldehydes were present in the extracts of the 150°C extractions. This therefore, indicates decomposition of the α-pinene content in the samples at 150°C, and thisallowed for more of the other classes of compounds to be extracted and detected.

Table 4.3 Main classes of compounds of ASE extractions

Temperature (℃)	**Time (min)**	**Cycles**	**% Alkane**	**% Alkene**	**% Terpene**	**% Aldehyde**	**% Fatty Acid**	**% Alcohol**	**% Ketone**
80	10	2	0,32	3,97	4,22	1,75	0,24	2,37	0,08
80	20	1	0,57	6,72	2,71	2,88	0,3	1,7	0,06
80	20	3	1,86	4,51	0,73	0,64	0,16	1,27	0,15
80	30	1	0,21	4,68	0,98	1,94	0,1	1,48	0,14
80	30	2	0,22	4,82	0,53	1,35	0,19	1,18	0,16
115	10	3	4,2	6,39	0,94	0,59	0,44	2,67	-
115	20	1	0,21	3,52	0,52	1,37	0,19	1,21	0,07
115	20	2	0,14	3,29	2,44	0,39	0,21	1,12	-
115	30	1	0,08	4,62	0,5	1,23	0,24	1,29	0,18
150	10	2	1,97	4,93	1,13	2,21	0,19	1	0,49
150	20	1	2,13	6,82	0,4	5,15	0,89	3,75	0,69
150	20	3	4,88	6,52	0,45	2,72	0,95	2,53	0,21

Figures 4.1, 4.2 and 4.3 were obtained from the Statistica^{TM} software that was used for the Box Behnken experimental design of the ASE Extractions. The plots displayed are the interactions between 2 independent variables against the dependent variable, which is the α-pinene percentage. These are Surface and Contour plots relating the variables. Each figure is discussed individually.

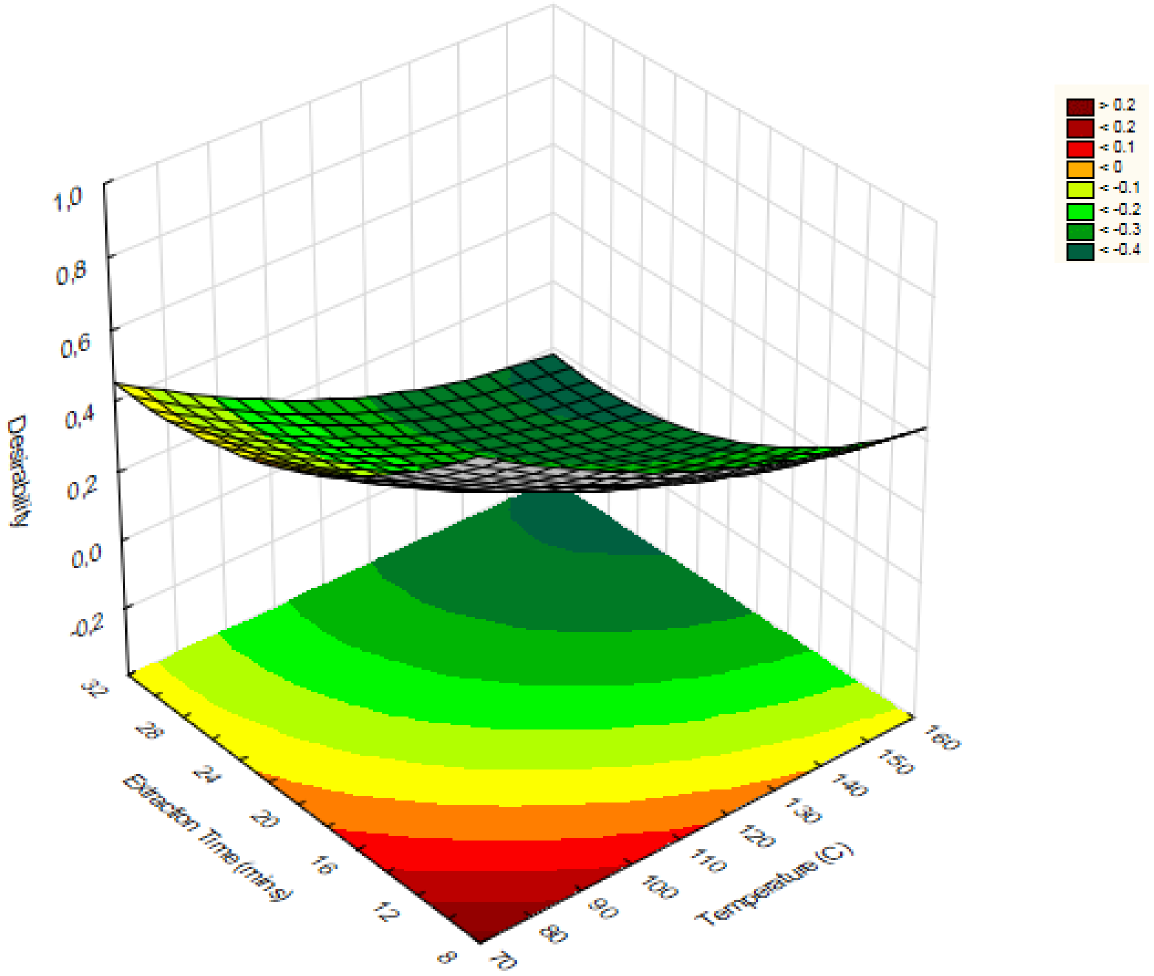

Figure 4.1 Surface and contour plots of extraction temperature and time versus desirability

Figure 4.1 displays the plot of temperature and time against Desirability are shown here. As the temperature is increased from 80 to 150°C , the desirability of the valuable compounds is decreased. An increase in time also decreases the desirability however, a steadier decrease is observed when time is varied. Low temperatures and decreased times are the preferred conditions for maximum α-pinene yield and hence, maximum lipophilic extraction.

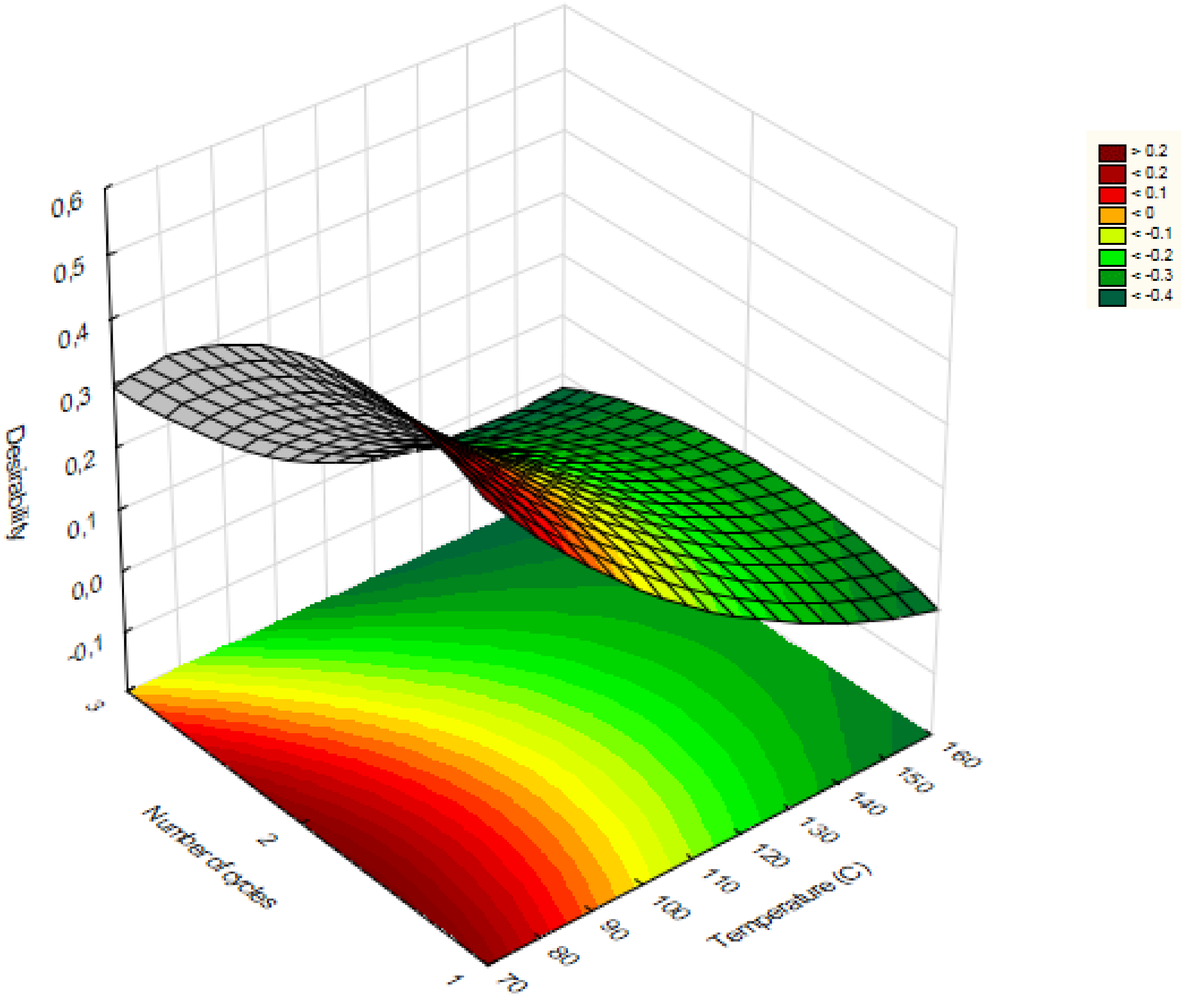

Figure 4.2 Surface and contour plots of extraction temperature and cycles versus desirability

Figure 4.2 depicts the relationship between the temperature of the solvent and the number of cycles against the desirability. The optimum number of cycles occurs at 2 extraction cycles with the temperature ranging between 70 and 115 °C. Less heat and fewer cycles allow for a decrease in volatile compound decomposition and hence, show a greater amount of terpene content with the majority being α-pinene, except for 4 runs in which the other terpene compositions were greater than that of α-pinene. The other terpenes that were present in all extracts were 2-carene and o-cymene, these two terpenes were even shown in the extracts where no α-pinene was observed. Thus, it can be stated that although α-pinene decomposes at increased temperatures, other valuable terpenes can still be extracted. These compounds could also show to be less volatile as these sawdust samples were a relatively old batch.

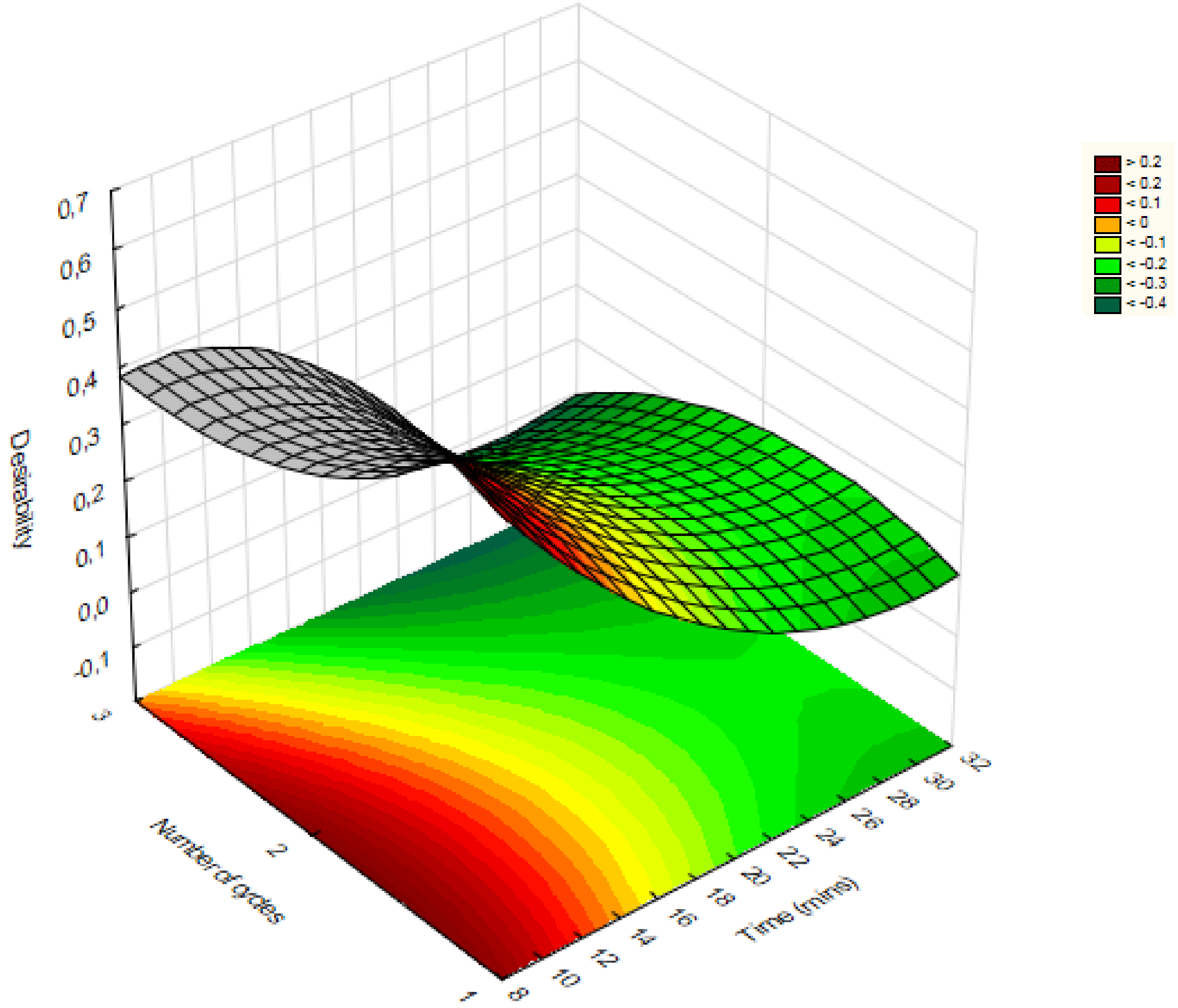

Figure 4.3 Surface and contour plots of extraction cycles and time versus desirability

Figure 4.3 relates the number of cycles and the time of extraction to the desirability. It can be seen from this figure that the optimum conditions for the time and number of cycles is 2 extraction cycles at 10 minutes per cycle. These figures display interactions between the different variables, therefore optimum conditions are assuredly obtainable.

All three of the contour plots displayed above show an elliptical pattern depicting that the interactions between the variables are significant. Temperature and extraction time affect the extraction of desirable components in the same manner; as the temperature is increased the desirability is decreased and the similar pattern is noticed when the extraction time is increased. The number of cycles is least affected by a low temperature as well as a low extraction time.

Therefore, the most desirable results are achieved through conducting extractions at both low temperatures and low extraction times.

4.3 Soxhlet extractions: effect of extraction time, solvent type and particle size

The ASE results, presented in section 4.2, showed that shorter time periods allowed for the presence of terpenes in the extracts. These results had thus led to the conclusion that conducting Soxhlet extractions at shorter extraction times could possibly yield more promising results, as the terpenes could be decomposing when being subjected to the longer extraction times.

The variables studied in these experiments were time, solvent and particle size. The solvents used in these experiments were: 3:1 cyclohexane-ethanol; 3:1 cyclohexane-acetone; and 3:1 acetone-ethanol mixtures, denoted as 1, 2 and 3 respectively, in the table displayed below. The extractions were carried out at 2, 3 and 4 hours and the particle sizes were 375 and 500 microns. As mentioned previously a fresh batch of sawdust was used to conduct this set of extractions. The temperature had to be set to a level that allowed for the solvent to boil to a degree that would be suitable for the extraction cycles to occur at a rate of 6 times per hour.

Table 4.4: Extractions as a function of time and particle size using pure cyclohexane

Time (hrs)	Particle size (microns)	% Extractives	% α-Pinene	% Terpenes
2	375	6,95	0,68	0,68
3	375	8,27	0,55	0,55
4	375	7,06	0,34	0,34
2	500	9,10	0,5	0,5
3	500	8,93	0,27	0,27
4	500	5,72	-	0,13

Table 4.4 displays the values obtained after conducting extractions as a function of time and particle size using cyclohexane as the extracting solvent. This was carried out to determine if the sawdust age affects the extracting capabilities of pure cyclohexane as an extracting solvent. This however, is not evident in the results obtained. Significant amounts of α-pinene are detected in the extracts. Extraction times of two hours are seen to be most suitable for the extraction of maximum amounts of desirable compounds. As the extraction time was increased the terpene content which was composed only of α-pinene decreased. Smaller particle sized sawdust allowed for extraction of more α-pinene.

The experiments were carried out as per the data presented in the first 3 columns of Table 4.5. Also displayed in Table 4.5 is the terpene content present in the extract for each experimental run. The dominant terpene in these extracts were found to be α-pinene, and these percentages are displayed as well.

Table 4.5 Results for Soxhlet extraction as a function of time

Time (hrs)	Particle size (microns)	Solvent	% Extractives	% α-Pinene	% Terpenes
2	375	1	6,83	21,45	22,15
2	375	2	6,98	20,38	20,38
2	375	3	7,74	17,95	17,95
3	375	1	6,74	12,34	12,82
3	375	2	7,12	16,51	17,37
3	375	3	9,07	16,63	16,63
4	375	1	6,80	10,28	10,28
4	375	2	8,47	7,21	8,14
2	500	1	6,86	9,45	9,45
2	500	2	7,81	9,18	9,18
3	500	1	6,76	8,89	8,89
3	500	2	8,44	3,75	3,75
3	500	3	9,13	2,28	2,28
4	500	1	8,49	1,15	1,15
4	500	3	7,31	2,52	2,52

In this set of experiments, the maximum terpene content was extracted at a total extraction time of 2 hours, using the smaller particle size and using 3:1 cyclohexane-acetone as the extracting solvent, followed closely by the extraction with the same conditions except that the extracting solvent was 3:1 cyclohexane-ethanol. At the 3-hour extraction time, a large amount of α-pinene was observed when 3:1 acetone-ethanol was used as the extraction solvent, hence indicating that all three of the extraction solvents had similar extracting capabilities for extracting lipophilic extractives.

4.3.1 Effect of particle size

Two particle sizes were tested, and as can been seen from the overall results, a significant decrease in terpene content occurs when the particle size is increased. An increase in particle size from 350 to 500 micron caused an approximate decrease in terpene content of 50% after an extraction time of 2-hours. A greater decrease is observed when the extraction was carried out for 3-hours. However, for the 3:1 cyclohexane-acetone solvent the terpene content does not differ much for the different particle sizes.

These results were expected as a smaller particle size allows for better interaction between the solvent and the sawdust particles. Therefore, it can be concluded that smaller particle sizes are preferred.

4.3.2 Effect of extraction time

Three extraction times were analysed, 2, 3 and 4 hours. An extraction time of 2-hours yielded a maximum terpene content that ranged between 17 and 21,5 % for the 375-micron particles and ranged between 9 and 9,5 % for the 500-micron particles. These contents dropped to 12 - 16% at 3- hour extractions and to 7-10% at 4-hour extractions using 375-micron particles. to ~2% at 3- and 4-hour extractions using 500-micron particles.

It can therefore be concluded that the terpenes decompose as extractions times are increased and an ideal extraction time would be at 2-hours.

4.3.3 Effect of solvent type

It is apparent from the results achieved that all three solvents display similar extracting capabilities. Solvent 1 is observed to extract the maximum terpenes at 2-hours with both the particle sizes analysed, at 3-hours using 500-micron particles and at 4-hours using 375-micron particles.

Solvent 3 showed a maximum terpene yield at 3-hours using the 375-micron particles and at 4-hours 500-micron particles solvent 3 is seen to extract the maximum terpenes.

Looking at the overall results from the Soxhlet extractions as a function of time, when they are classed by the different extraction solvents used, it is noticed that all 3 solvents have a similar extracting potential. These results indicate that as mixtures with cyclohexane, the solvents extract more valuable compounds as opposed to when cyclohexane is used in pure form as the extracting solvent. The larger particles sizes showed a decreased extraction composition at 3- and 4-hour extraction times, however, only at an extraction time of 2 hours, together with larger particle sizes were significant extractions of α-pinene obtained.

4.4 Seasoning

Various factors affect the composition of extractives present in wood. These variations exist between tree parts i.e. stem, bark, branch etc, types of stem wood and growth conditions amongst others, as well as between differences in age, season, provenance and site. The particle size and extraction time used in to carry out this study were 375-microns and 2-hours respectively.

This study was conducted over a three-month period i.e. week 0 to week 11. Week 0 refers to day 1 of the study, on which the sawdust residue sample was collected from the mill. The sawdust was then added to the silo, which was designed for the seasoning study. A sample was then removed and prepared for extraction using the Soxhlet extraction method and 3:1 cyclohexane – ethanol (v/v) as the extracting solvent. Figure 4.4 depicts the terpene contents present in a sample of seasoned sawdust over a period of 11 weeks. The terpene contents show various peaks as well as declines over the study period.

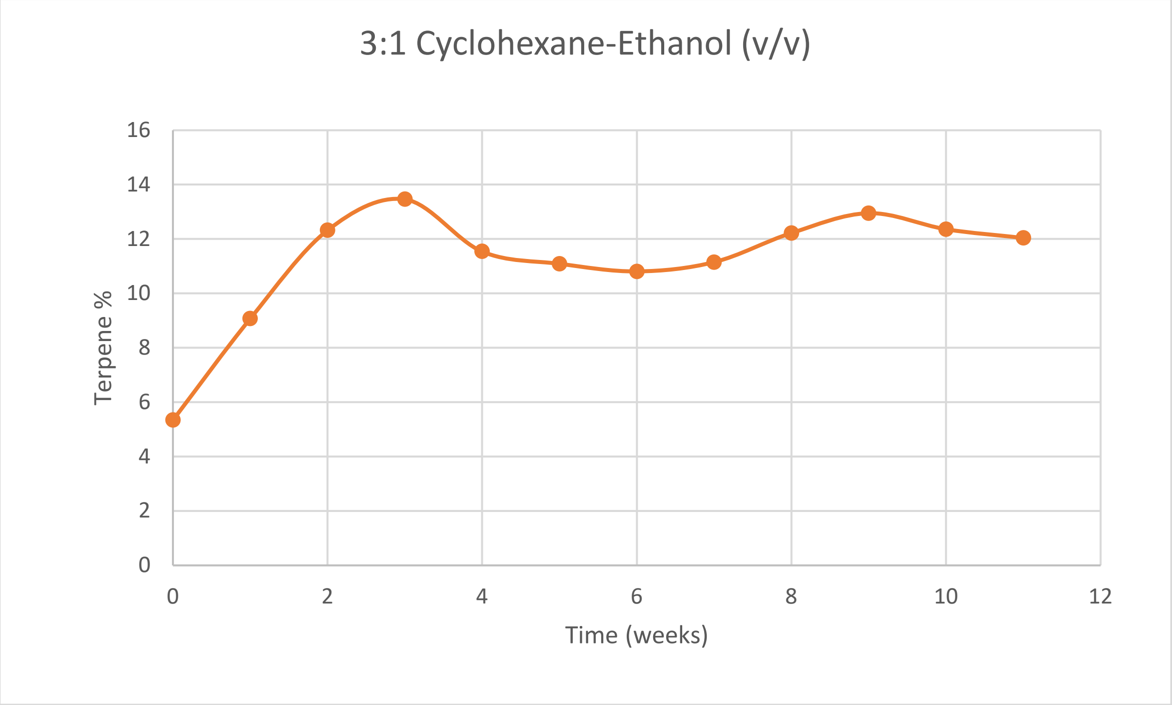

Figure 4.4 Terpene content over 3-month seasoning period

The seasoning analysis results show an initial low terpene content followed by a gradual increase with a peak at the third week and thereafter a slow decrease and increase until the end of the seasoning extractions. These results together with results from the other experimental works of this study, it can be stated that using 2-hours extraction times, the values of the terpene contents are seen to fluctuate between 10 – 14% and at times even exceeding this amount.

The initial low terpene content differs from the terpene content of section 4.3 as different sawdust batches are used even though they both were fresh batches.

It is generally known that when the wood samples are exposed to air, the extractives are evaporated as many of the components of these extractives are volatile. Air-drying sawdust at room temperature does not cause a major decrease in extractive content over periods of time.
However, in this seasoning study a different trend was seen, with extractives contents remaining at high percentages even after 3 months of being exposed to air in a controlled environment.

For the sawdust use in this manner, i.e. for the extraction of lipophilic extractives (with terpenes being the major components) for potential production of valuable products and chemicals, it is, therefore, good to know that there are significant amounts of extractives available in the sawdust even after being stored for long periods of time.

Table 4.6 displays the main classes of compounds identified in the extracts of the seasoning study. Increased contents were observed in majority of the classes with an exception seen in the fatty acids contents, which decreased over the range of experiments performed as the sawdust samples were stored for longer periods.

Various compounds were not detected in the extracts between runs, such as the non-detection of aldehydes between weeks 1 and 4, but detection at week 5. This would be due to the manner in which the seasoning is carried out thereby not sampling from the exact location at every extraction. Some parts of the sawdust could then be exposed to more air while other portions are kept moist beneath the already dry areas. Aldehydes are one example of these compounds, they are volatile and therefore being kept in a seasoning environment with air passages to dry the samples allows for these compounds to escape.

Table 4.6 Main classes of compounds determined in the seasoning study[2]

2

	Seasoning Study						
	% Terpene	**% Aldehyde**	**% Fatty Acid**	**% Ketone**	**% Alkane**	**% Alkene**	**% Alcohol**
Week 0	5,34	0,4	4,1	1,15	5,23	11,94	0
Week 1	9,07	0	3,18	2,17	6,02	11,61	2,81
Week 2	12,52	0	0	6,51	1,7	12,69	0
Week 3	13,46	0	3,75	0,53	3,75	10,38	0,95
Week 4	11,54	7,9	4,22	4,4	7,31	17,72	10,64
Week 5	11,09	16,64	1,65	0	5,86	24,46	0
Week 6	10,81	0	0	0	11,94	41,74	2,4
Week 7	11,15	6,48	2,46	9,78	10,2	30,69	0
Week 8	12,22	1,4	1,63	1,24	10,87	29,15	21,44
Week 9	12,95	0,86	0,93	6,53	28,13	22,12	20,71
Week 10	12,36	2,51	0	9,78	24,95	26,97	18,42
Week 11	12,04	1,65	0,51	10,45	24,68	28,27	19,43

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[2] Triplicate samples of sawdust were used and the values presented are averages of the runs conducted

4.5 Kinetic studies

The effect of time and temperature was evaluated by a kinetic study of the lipophilic extraction process. This was conducted using (3:1) cyclohexane – ethanol (v/v) as the extraction solvent, as this was found to be the solvent with which maximum terpenes were extracted in section 4.3, four temperatures were analysed, and extractions were carried out between 0 – 4 hours. The first sample was removed after 30 minutes for analysis and thereafter hourly analyses were done. After each desired time interval, the extraction was terminated and the round bottom flask, containing the solvent together with the extract was removed. This sample thereafter underwent rotary evaporation to remove the excess solvent and preparation for Py-GC/MS analysis was carried out.

During extraction at temperatures conducted below boiling point, 70 and 75°C, the number of cycles observed was 2 and 4 cycles respectively within an hour. This fewer cycles are due to the solvent not boiling but being heated sufficiently to allow for the solvent to vaporise, although at a much slower rate. At 80°C, the number of cycles were seen to be 6 cycles in an hour and at 85°C, 8 cycles were observed in an hour.

Figure 4.9 shows the concentration of the terpenes present in the extract versus time data for all four of the investigated temperatures. At 70, 75 and 80°C the concentration peak was noticed after two hours of extraction before the concentration began to decrease and eventually stabilise. This trend is more easily noticed in Table 4.7, where the percent of the total terpenes present in the extracts are displayed. Only a small percent of these terpenes was extracted initially and thereafter increased until it reached the 2-hour extraction time after which it began decreasing again. The initial terpene content being relatively small would be due to the short time period that the sample was in contact with the solvent.

The total terpene content is seen to increase as the temperature is increased but decreases at 85°C. The solvents used in this study had boiling points that were ~ 80°C. Thus, the trend implies that once the temperature exceeds that of the solvents boiling points, the extractives begin to decompose. This could also be due the solvents rapidly boiling at 85°C and hence, not spending enough time in contact with the sample. Although, during the study carried out at 85°C, the maximum terpene yield occurred after an hour of extraction, the percent extracted was a relatively low amount when compared to the lower temperatures. At 70, 75, and 80°C between 72 and 86 percent of the total terpenes in the extracts were removed after 2 hours of extraction and at 85°C, only 63 percent of extractives are removed after 1 hour.

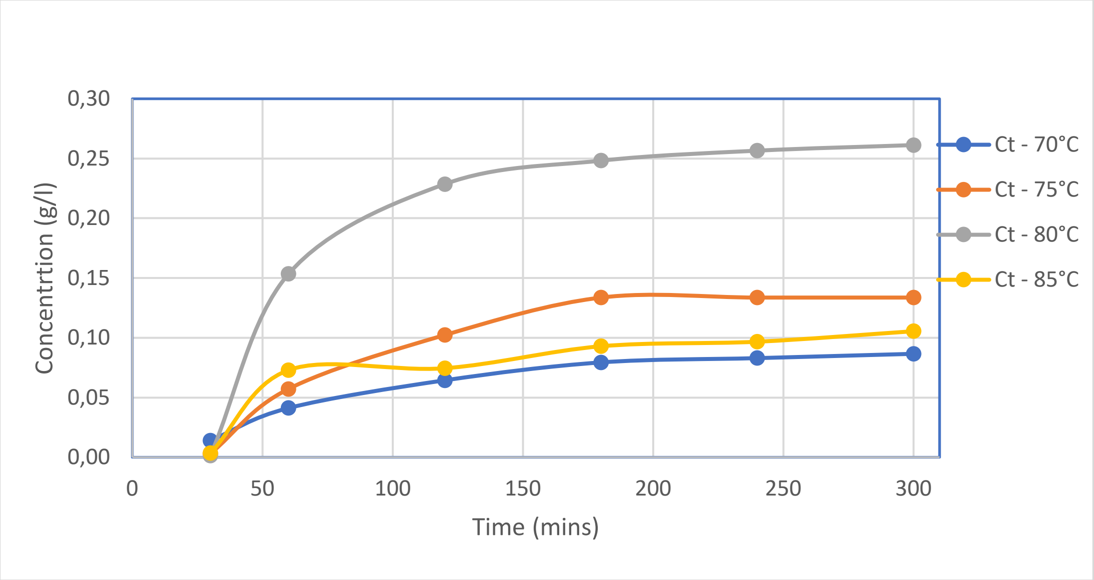

Figure 4.5 Cumulative concentration of extracted terpenes at different temperatures

The highest total terpene concentration is seen at 80°C. This is the expected results as it is closest to the boiling point of the solvents. Higher temperatures decompose some of the terpenes in the extracts thus resulting in lower total terpene concentration.

Table 4.7 Terpene contents extracted during kinetic study

	Terpene contents (%)			
Time (mins)	70°C	75°C	80°C	85°C
30	1,32	0,18	0,09	0,25
60	3,27	4,4	4,86	5,6
120	3,74	5,01	5,31	1,4
180	2,35	2,03	1,05	1,12
240	0,49	0	0,34	0,5
300	0,36	0	0,29	0,4
Total Terpenes	**11,53**	**11,62**	**11,94**	**9,27**

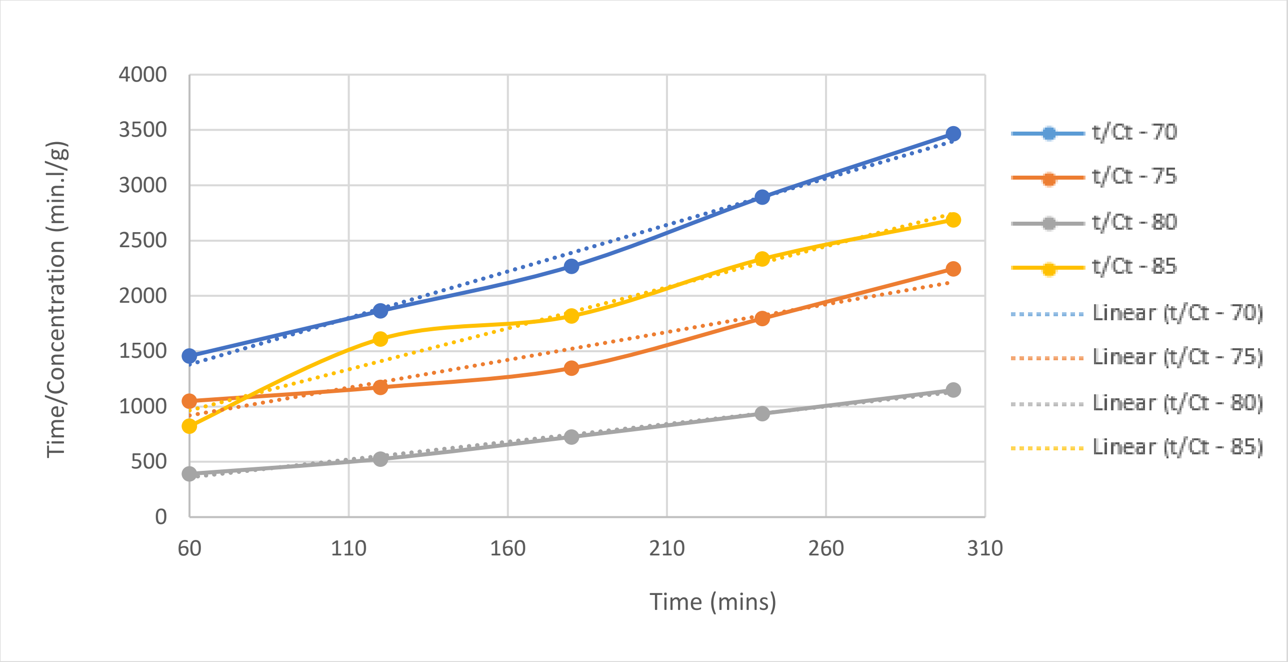

Figure 4.6 Second order extraction kinetics of terpenes from pine wood sawdust

Extractions from plant biomass are most often described using first- and second-order kinetic models. Therefore, these two models were tested on the results obtained to determine the kinetic model that would best represent these results. In a first-order extraction the integrated model equation is $\ln[A] = -kt + \ln[A_0]$, where A represents the concentration at any time, t. A_0 is the initial concentration and k is the rate constant. To determine if the extraction fitted a first-order model, a plot was generated of ln[A] versus time. This plot is expected to yield a straight-line with a negative slope. However, in this study, a curve was produced when the natural logarithm equation was applied to the results as shown in figure 4.7 and hence, the system being first-order was ruled out and the second-order was tested.

The time/concentration values were computed and were used to create the plot of Figure 4.6. The linear fit of each of these curves were then determined. The linearised form thus indicates that the second-order kinetic model fits the data well. The straight-line graphs that were generated represent the function, $\frac{t}{C_t} = \frac{1}{kC_s^2} + \frac{t}{C_s}$, where $\frac{t}{C_t}$ represented the y-axis and *t* represented the x-axis. From the equations of the straight lines, the slopes were used to determine the extraction capacity, C_s, and the y-intercepts were used to determine the second order extraction rate constant, *k*. The values determined from the straight lines are presented in Table 4.8. The equations 4.3 - 4.6 were then generated using, equation 4.2 as the standard equation, and substituting the C_s and *k* values presented in Table 4.10.

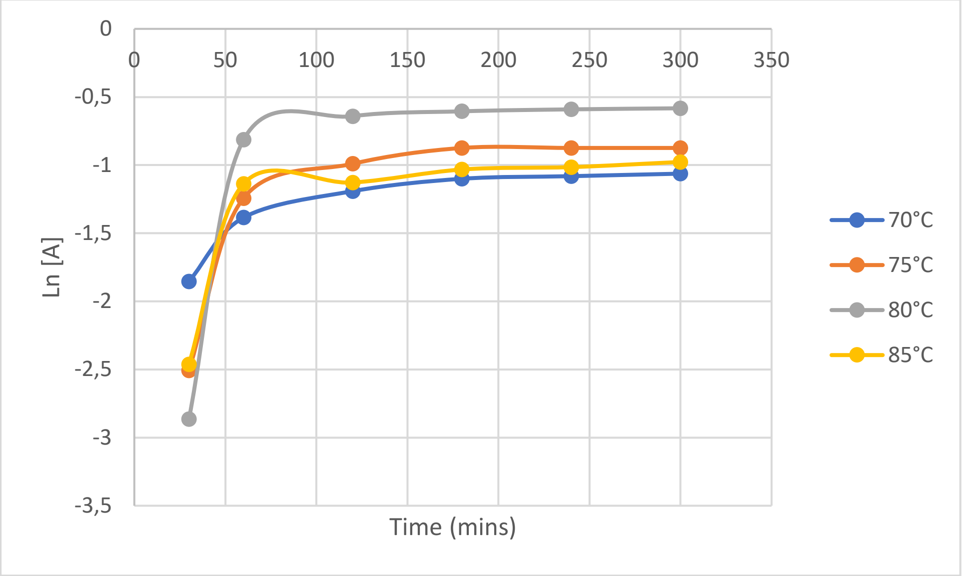

Figure 4.7 First order extraction kinetics of terpenes from pine wood sawdust

Table 4.8 The slope and intercept of linear fit graphs at each temperature

Temperature (°C)	Slope	y-intercept
70	8.4118	874.92
75	5.0237	617.58
80	3.208	167.52
85	7.4224	518.51

Table 4.9 Extraction rate constant and saturated concentration values

Temperature (°C)	Cs $\left(g/_l\right)$	k $\left(l/_{g.min}\right)$	h $\left(g/_{l.min}\right)$
70	0.1189	0.0809	1.1437×10^{-3}
75	0.1991	0.0409	1.6213×10^{-3}
80	0.3117	0.0614	5.9654×10^{-3}
85	0.1347	0.1063	1.9287×10^{-3}

$$C_t = \frac{kC_s{}^2 t}{1+kC_s t} \qquad (4.2)$$

Equation 4.2 displayed above is used to represent the concentration of the terpenes in the extracts at any time, t, and at a specified extraction temperature.

This is the kinetics model equation that was developed and represents the concentrations at any time at 70°C.

$$C_t = \frac{1.1437 \times 10^{-3} t}{1 + 9.619 \times 10^{-3} t} \tag{4.3}$$

This is the kinetics model equation that was developed and represents the concentrations at any time at 75°C.

$$C_t = \frac{1.6213 \times 10^{-3} t}{1 + 8.143 \times 10^{-3} t} \tag{4.4}$$

This is the kinetics model equation that was developed and represents the concentrations at any time at 80°C.

$$C_t = \frac{5.9654 \times 10^{-3} t}{1 + 0.019 t} \tag{4.5}$$

This is the kinetics model equation that was developed and represents the concentrations at any time at 85°C.

$$C_t = \frac{1.9287 \times 10^{-3} t}{1 + 0.014 t} \tag{4.6}$$

The values for concentration were determined from the kinetic models developed and these values were seen to correlate well to the actual concentrations achieved. The only exceptions were noticed after the first time-interval.

Table 4.10 Comparison of concentration values from actual experiment and kinetics models

Time (mins)	70°C		75°C		80°C		85°C	
	k	0,0809	k	0,0409	k	0,0614	k	0,1063
	C_s	0,1189	C_s	0,1991	C_s	0,3117	C_s	0,1347
	C_t	**C_t from kinetics model**	**C_t**	**C_t from kinetics model**	**C_t**	**C_t from kinetics model**	**C_t**	**C_t from kinetics model**
30	0,014	0,027	0,003	0,039	0,001	0,114	0,004	0,041
60	0,041	0,044	0,057	0,065	0,153	0,167	0,073	0,062
120	0,064	0,064	0,102	0,098	0,229	0,217	0,075	0,085
180	0,079	0,075	0,134	0,118	0,248	0,242	0,099	0,097
240	0,083	0,083	0,134	0,132	0,257	0,256	0,103	0,104
300	0,087	0,088	0,134	0,141	0,261	0,266	0,112	0,109

Displayed in Appendix E are pyrograms of the kinetic study conducted, only the pyrograms obtained where the maximum terpene contents are observed were displayed.

From the kinetic study it is observed that the temperature of the solvents is required to be close to the boiling point in order to achieve a maximum terpene yield. Temperatures below the boiling point, do not allow for the solvent for to actually boil, but it does provide sufficient heat for the solvent to begin vaporising and condensing in the Soxhlet tube however at a slower rate.

4.6 Optimised conditions for extraction of old sawdust samples

The optimised results of section 4.3 were determined using the results and data obtained from the Statistica Software and are presented as follows: -
Extraction time: 2 hours
Extraction Solvent: 3:1 Cyclohexane-Ethanol
Particle Size: 375 microns

These optimised conditions were carried out on the older sawdust samples that were investigated in section 4.1 to determine if the shortened times also affect the composition of the extracts from sawdust residues that are up to 2 years old. The results are displayed in Table 4.11, with a maximum terpene content of 14,62% and a minimum of 3,24%. Although the terpene content is seen to be the lowest when the oldest sawdust is tested, it can be seen that these values are much greater that those obtained from a 6-hour extraction.

Pyrograms of the optimised results are presented in Appendix E, these contain the pyrograms of all four samples that were tested using optimised conditions.

Table 4.11 Optimised condition on old sawdust samples

Sawdust Type	**Solvent**	**% Extractives**	**% α - Pinene**	**% Terpene**
Sample 1	3:1 Cyclohexane – Ethanol	7,56	13,58	14,62
Sample 2	3:1 Cyclohexane – Ethanol	8,27	8,67	10,89
Sample 3	3:1 Cyclohexane – Ethanol	8,23	3,39	6,83
Sample 4	3:1 Cyclohexane – Ethanol	8,96	3,24	3,24

The operating conditions of the study were temperature, total time of extraction, different solvents used, the size of the sawdust particles. Where the effect of temperature on the extractions was determined, the operating temperatures were varied below and above their boiling points.

The total time of the extraction determines whether sufficient time is allowed complete extractions. Long extraction times as well as high temperature result in decomposition of the components extracted.

The particle sizes used were within a range specified; smaller particle sizes are more suitable for extractions due to the increased interactions between the solvents and particles.

These conditions were operated at a 95% confidence level.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The aim of this study, to investigate technologies to beneficiate wood biomass via extractions has been achieved. Extraction experiments began with preliminary Soxhlet extractions, conducted over an extraction period of 6-hours, which have been found to decompose majority of the extractives available in the sawdust, mainly due to the constant heat applied to the extracts over a long period of time. Successful extractions of lipophilic extractives were thereafter, achieved during experiments conducted in all other sections. Up to 4-hour extraction times are possible to obtain a significant quantity of extractives, with 2-hour extraction times providing the highest quantity of terpene contents in the extracts. Solvent mixtures with cyclohexane are more effective in extracting lipophilic extractives than pure cyclohexane, even when pure cyclohexane is used on fresh sawdust samples, only a slight difference is noticed between the different aged samples. From Chapter 4, section 4.3; the optimum conditions for extraction of lipophilic extractives containing a more valuable composition of compounds was found to be;

- Extraction time of 2-hours
- Extracting solvent was 3:1 Cyclohexane-Ethanol (v/v)
- And a 375-micron particle size

From the seasoning study, it can be stated that all aged sawdust residues would be viable for lipophilic extraction as the terpene content over the 3-month study is seen to only fluctuate between 10 and 14 % rather than totally evaporate. The conclusion of all aged sawdust samples being viable is also verified when the optimum conditions were applied to the samples of the preliminary study (1-2- year old samples). The kinetics model was verified being second order for the solid-liquid extraction which was shown by the linearised plots generated. As the temperature was increased, the terpene contents were seen to increase as well, but after exceeding the boiling points (~80℃) of the solvents the terpene contents decreased. Indicating the decomposition of the extractives at high temperatures. Applying the optimum conditions to the samples of Section 4.1, increases the extractives contents as the terpene contents are seen to increase. An increase of approximately 8 times the value of Section 4.1 is noticed. It is also noted that the amount of extractives in the sawdust does not determine the composition of the extracts, hence, the compositions were more closely studied to investigate the effects of the different variables.

Therefore, with the intention of maximising the extraction of lipophilic extractives from pine wood sawdust, and its potential conversion to a South African blend pine oil, extraction should be carried out at the optimum conditions.

5.2 Recommendations for future studies

- To use the ASE method of extraction to perform experiments with fresh sawdust and different solvents. Use the obtained results to conduct a comparison between the Soxhlet extractions as a function of time.
- Study the effect of the seasoning over a longer period, 5 months.
- Conduct an extraction study in which sawdust samples are obtained from trees of various ages and determine its effect on the quantity of extractives obtained.

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APPENDIX A: SAMPLE CALCULATIONS

A.1 Kinetic Study

$$\frac{t}{C_t} = \frac{1}{kC_s^2} + \frac{t}{C_s}$$

The plot of this should be linear and would therefore have a slope and an intercept on the y-axis.

The slope is equivalent to $\frac{1}{C_s}$ and the y-intercept is equivalent to $\frac{1}{kC_s^2}$.

$$\frac{1}{C_s} = 8.4118$$

From this **C_s = 0.1189**.

$$\frac{1}{kC_s^2} = 874.92$$

From this, **k = 0.0809.**

$$C_t = \frac{kC_s^2 t}{1 + kC_s t}$$

$$C_t = \frac{0.0809 \times 0.1189^2 \times t}{1 + (0.0809 \times 0.1189)t}$$

$$C_t = \frac{1.1437 \times 10^{-3} t}{1 + 9.619 \times 10^{-3} t}$$

A.2 Compound classes from Py-GC/MS results

To determine the percent of each compound class, a list of classes is determined. In this study the compounds were grouped into the following classes: -

Alkanes
Alkenes
Terpenes
Aldehydes
Ketones
Alcohols
And Fatty acids

Table A.1 is a list of compounds contained in an extract. The terpene content is then determined by adding the values highlighted and α-pinene is seen as the major terpene, where as the other terpenes contained in the extract are, 3-Carene and o-Cymene.

2,98 % = Total terpene content
2,24 % = α-pinene content
0,74 % = other terpenes

Similarly, all other compounds are grouped into the different classes.

Table A.1: Compounds identified from Py-GC/MS analysis

Ret. Time	Area %	Compound Name	Ret. Time	Area %	Compound Name
1,983	1,22	Acetaldehyde	8,865	0,32	1-Nonene
2,036	1	1-Methylcyclopropanemethanol	9,184	0,07	Cyclooctene
2,193	1,25	CH3C(O)O(CH2)3CH=CH2	10,494	0,21	Benzene
2,385	0,16	4-Penten-1-ol	11,702	0,1	trans,trans-nona-2,4-dienol
2,523	0,7	1-Hexene	13,321	0,06	2-Hepten-4-one, 6-methyl-
2,775	0,18	Ammonium acetate	14,259	0,17	1-Decene
2,895	0,35	1,3,5-Hexatriene, (Z)-	15,067	0,05	Octanal
3,159	0,4	1,3-Cyclohexadiene	15,519	0,06	Cyclohexane
3,351	0,5	1-Heptene	15,76	0,11	3-Carene
3,696	0,08	3-Hexen-2-one	15,939	0,09	Cyclohexane
4,141	0,12	3-Hepten-1-ol	16,161	0,13	o-Cymene
4,432	0,06	Cyclobutane	16,343	0,15	o-Cymene
4,605	0,06	1,3,5-Hexatriene	16,605	2,24	.alpha.-Pinene
4,737	0,33	Toluene	20,747	0,13	3-Tetradecene
5,24	0,29	1-Octene	21,725	0,14	Nonanal
5,516	0,36	Hexanal	22,729	0,1	1,4-Cyclohexadiene-1-carboxylic acid
6,263	0,33	Bicyclo[3.1.0]hexane, 1,5-dimethyl-	23,076	0,12	5-Undecyne
7,498	0,09	1,4-Cyclohexadiene	24,171	0,23	Benzene
7,796	0,1	E,Z-3-Ethylidenecyclohexene	27,601	0,12	1-Dodecene
8,002	0,09	o-Xylene	29,936	0,08	6-Dodecyne
8,21	0,09	Octa-2,4,6-triene			

APPENDIX B: MSDS OF CHEMICALS USED

Table B.1 Material Safety Data Sheets of solvents for the extraction of lipophilic extractives

Chemical Name	Chemical Formula	Identification	Eye Contact	Skin Contact	Inhalation	Ingestion	Flammability	Disposal
Cyclohexane (Science Lab.com., 2013b)	C_6H_{12}	110-82-7	Irritant. Remove contact lenses. Flush eyes with running water. Keep eyelids open. Seek medical attention.	Irritant, permeator. Irritant. Wash skin with soap and water while removing contaminated clothes. Get medical attention. Wash clothes thoroughly before reuse	Remove to fresh air. Give artificial respiration and oxygen if breathing is difficult. Get medical attention.	Do not induce vomiting. Loosen tight clothing. If individual is not breathing, perform mouth-to-mouth resuscitation. Get medical attention	Flammable. Auto ignition point: 245°C (473°F), Flash point: -18°C (-0.4°F), Flammable limit %, lower: 1.3%, upper: 8.4%.	Consult federal, state and local waste regulations to determine appropriate disposal conditions.
Acetone (Science Lab.com., 2013a)	C_3H_6O	67-64-1	Irritant. Remove contact lenses. Flush eyes with running water. Keep eyelids open. Seek medical attention.	Irritant, permeator. Irritant. Wash skin with soap and water while removing contaminated clothes. Get medical attention. Wash clothes thoroughly before reuse	Remove to fresh air. Give artificial respiration and oxygen if breathing is difficult. Get medical attention.	Do not induce vomiting. Loosen tight clothing. If individual is not breathing, perform mouth-to-mouth resuscitation. Get medical attention	Flammable. Auto ignition point: 465°C (473°F), Flash point: -20°C (-4°F), Flammable limit %, lower: 2.6%, upper: 12.8%.	Consult federal, state and local waste regulations to determine appropriate disposal conditions.

Ethanol (Science Lab.com., 2013c)	C_2H_5OH	64-17-5	Irritant. Check for and remove contact lenses. Immediately flush eyes with running water for about 15 minutes. Keep eyelids open. Get medical attention.	Irritant. Immediately flush skin with plenty of water. Cover the irritated skin with an emollient. Remove contaminated clothing and shoes. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention.	Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. Give oxygen if breathing is difficult. Get medical attention.	Do not induce vomiting. Loosen tight clothing. . Never give anything by mouth to an unconscious person. Get medical attention.	Flammable. Auto ignition point: 363°C (685.4°F), Flash point: Closed cup 12.78°C (55°F), open cup 17.78°C, (64°F) Flammable limit %, lower: 3.3%, upper: 19%.	

APPENDIX C: ABSTRACTS ACCEPTED FOR “UKZN RESEARCH DAYS” EVENT

Beneficiation of sawdust: chemical fractionation of lipophilic extractives

Zaakerah Badat[1], Bruce Sithole[1,2], Viren Chunilall[2] and Annegret Stark[3]

[1]Discipline of chemical Engineering, University of KwaZulu-Natal, Durban, South Africa
[2]Forestry and Forest Products Research Centre, Natural Resources and the Environment, Council for Scientific and Industrial Research, Durban, South Africa
[3]SMRI Sugarcane Biorefinery Research Chair, University of KwaZulu-Natal, Durban, South Africa

- *Corresponding author: E-mail address;* zkrh.badat@gmail.com *Tel: +2776 0699 241*

Abstract

As one of the lignocellulosic biomasses, sawdust is a by-product of the mechanical milling of timber (wood) into various usable sizes [1]. Only a portion of the sawdust is being utilised by mills, in furnaces and to run the kilns. Another small portion is used by dairy farmers as animal bedding and supplied to plants for the manufacture of wood pellets [2], while the majority of it is being dumped.
The question that thus arises is, can high value products be generated efficiently and cost effectively; from sawdust? One potential product is pine oil. Pine oil is an essential oil obtained from pine trees and its common uses include: as an ingredient in cleaners, disinfectants, solvents fragrances, medicines and aromatherapy [3]. It is traditionally obtained as tall oil, a by-product of the kraft pulping process. Can we obtain it by direct extraction from a waste material, pine sawdust? In this study, we assess the effectiveness of various solvents to extract lipophilic extractives from pine sawdust.

The conventional Soxhlet Extraction method is being used to carry out the extractions. Cyclohexane, ethanol, and acetone are the selected extracting solvents. These solvents were chosen as they are selective for lipophilic extractives, hence giving little to no extraction of lignans [4]. Analysis of the extracts are conducted using Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS). The characteristics will later be studied, and the optimum conditions will be investigated. Modelling studies will then be conducted to evaluate the feasibility of the developed beneficiation processes.

The focus of this study is to beneficiate pine sawdust by extraction of a valuable material, pine oil, that can be used in a large variety of industrial and domestic applications. Success in this process will result in beneficiation of saw dust waste into a high value material.

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[2] Canfield, C. 2008. Sawdust: Once a nuisance, now valuable. Available: *https://www.seattletimes.com/business/sawdust-once-a-nuisance-now-valuable/*.

[3] Kelkar, M. V., Geils, W. B., Becker, R. D., Overby, T. S. & Neary, G. D. 2006. How to recover more value from small pine trees: Essential oils and resins. Biomass and Energy, 30, *316-320.*

[4] Sjostrom, E. & Alen, R. 1999. Analytical methods in wood chemistry, pulping and papermaking, Germany, Springer.

Abstract 1 was accepted for the SAIChE Research Day which was held at UKZN Howard College Campus on the 15th August 2018. A poster was presented. This focused on the results achieved following the Soxhlet extractions as a function of time.

Abstract 2 was accepted for the SACI Postgraduate Colloquium that was held on the 2nd February 2018 at DUT, Hotel School, Ritson Campus. This presentation focused on the preliminary results achieved from the Soxhlet extractions as well as the results obtained from the ASE extractions.

Beneficiation of sawdust: chemical fractionation of lipophilic extractives

Zaakerah Badat[a], Bruce Sithole[a,b], Viren Chunilall[b] and Annegret Stark[c]

[a]Discipline of chemical Engineering, University of KwaZulu-Natal, Durban, South Africa, E-mail: zkrh.badat@gmail.com

[b]Biorefinery Industry Development Facility, Council for Scientific and Industrial Research, Durban, South Africa

[c]SMRI Sugarcane Biorefinery Research Chair, University of KwaZulu-Natal, Durban, South Africa

Key words: Pine sawdust, Accelerated Solvent Extraction (ASE), Soxhlet extraction, Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS)

Sawdust is an agricultural waste that is produced as a by-product at sawmills and during the conversion to useful products. Sawdust wastes produced from these stages are comparatively uncontaminated and can therefore be retrieved and reused. The large quantities of sawdust being produced each year, make it an impossible task to completely reprocess this material.

Currently only a portion of the sawdust waste is being used in furnaces, as animal bedding or for the manufacture of wood pellets. Thus, still leaving behind large amounts that are unused. The question that thus arises is, can high value products be generated efficiently and cost effectively, from sawdust? One potential product is pine oil. Pine oil is an essential oil obtained from pine trees and its common uses include: as an ingredient in cleaners, disinfectants, solvents fragrances, medicines and aromatherapy.

The conventional Soxhlet Extraction method was used, as a function of time, to carry out the extractions. Three solvent mixtures were selected as the extracting solvents, these were, 3:1 (v/v) Cyclohexane-Ethanol, 3:1 (v/v) Cyclohexane-Acetone and 3:1 (v/v) Acetone-Ethanol. Analysis of the extracts were conducted using Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS).
Maximum terpene content was observed when a total extraction time of 2 hours was used, together with smaller particle sizes. The three solvents used in this study were shown to have similar extracting potentials, with results indicating that a mixture of 3:1 (v/v) Acetone-Ethanol extracted a slightly greater amount of terpene as compared to the other two solvents.

The ultimate aim of this study was to beneficiate sawdust via solvent extraction methods. Further work on this project include modelling studies to evaluate the feasibility of the developed beneficiation processes. Success in this process will result in beneficiation of sawdust waste into high value materials.

APPENDIX D: PYROGRAMS FROM PY-GC/MS

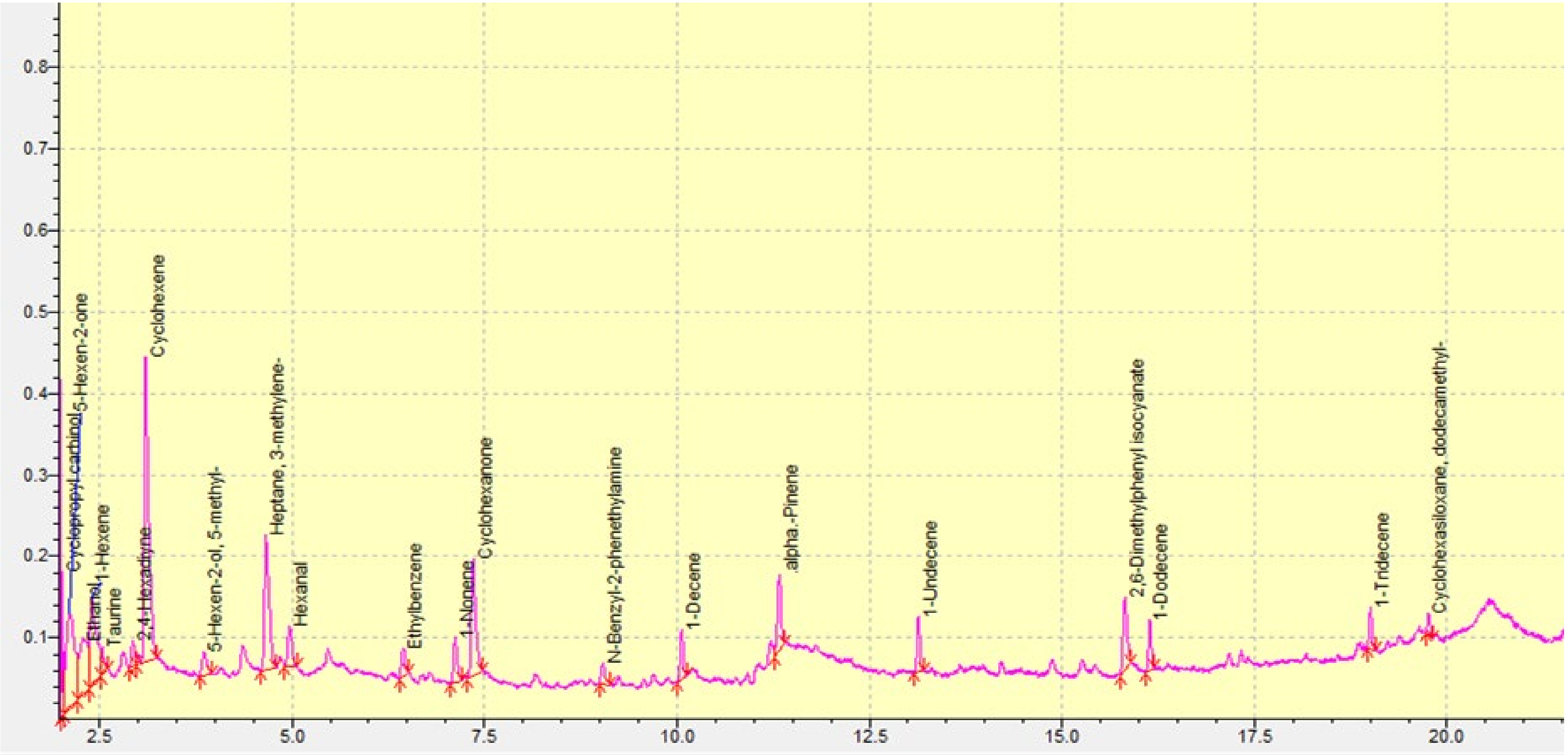

Figure D.1 Pyrogram for kinetic studies performed at 70°C

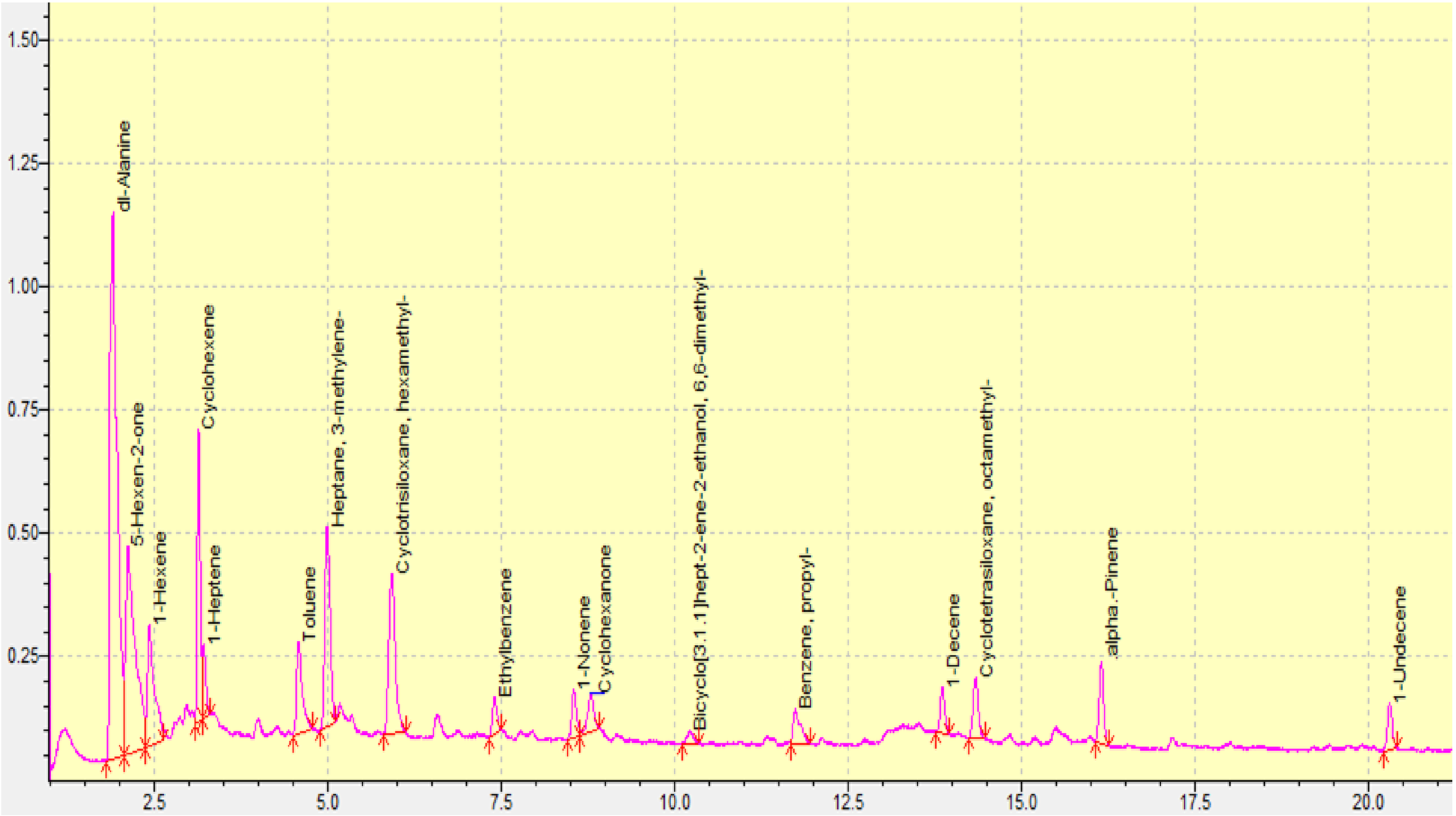

Figure D.2 Pyrogram for kinetic studies conducted at 75°C

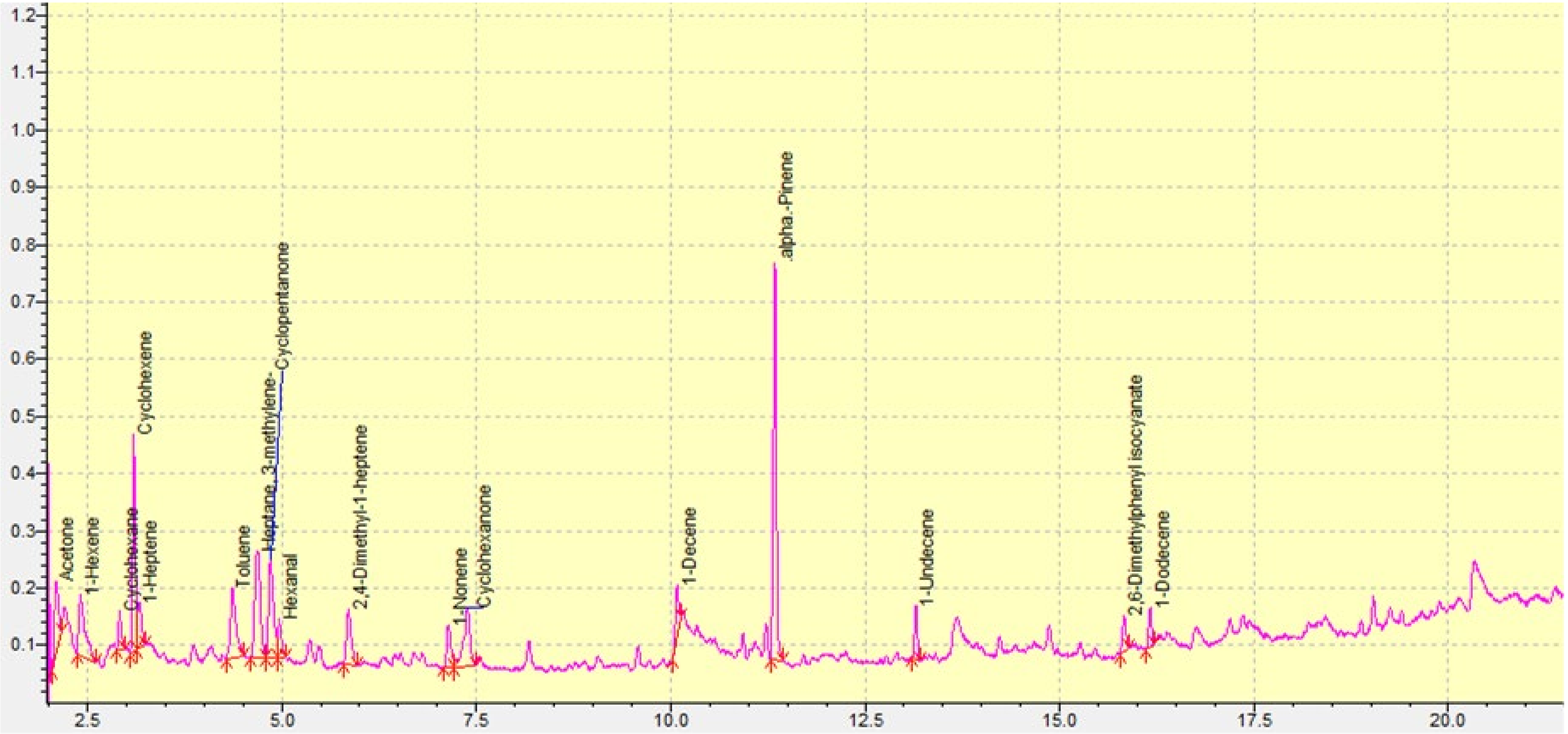

Figure D.3 Pyrogram for kinetic studies conducted at 80°C

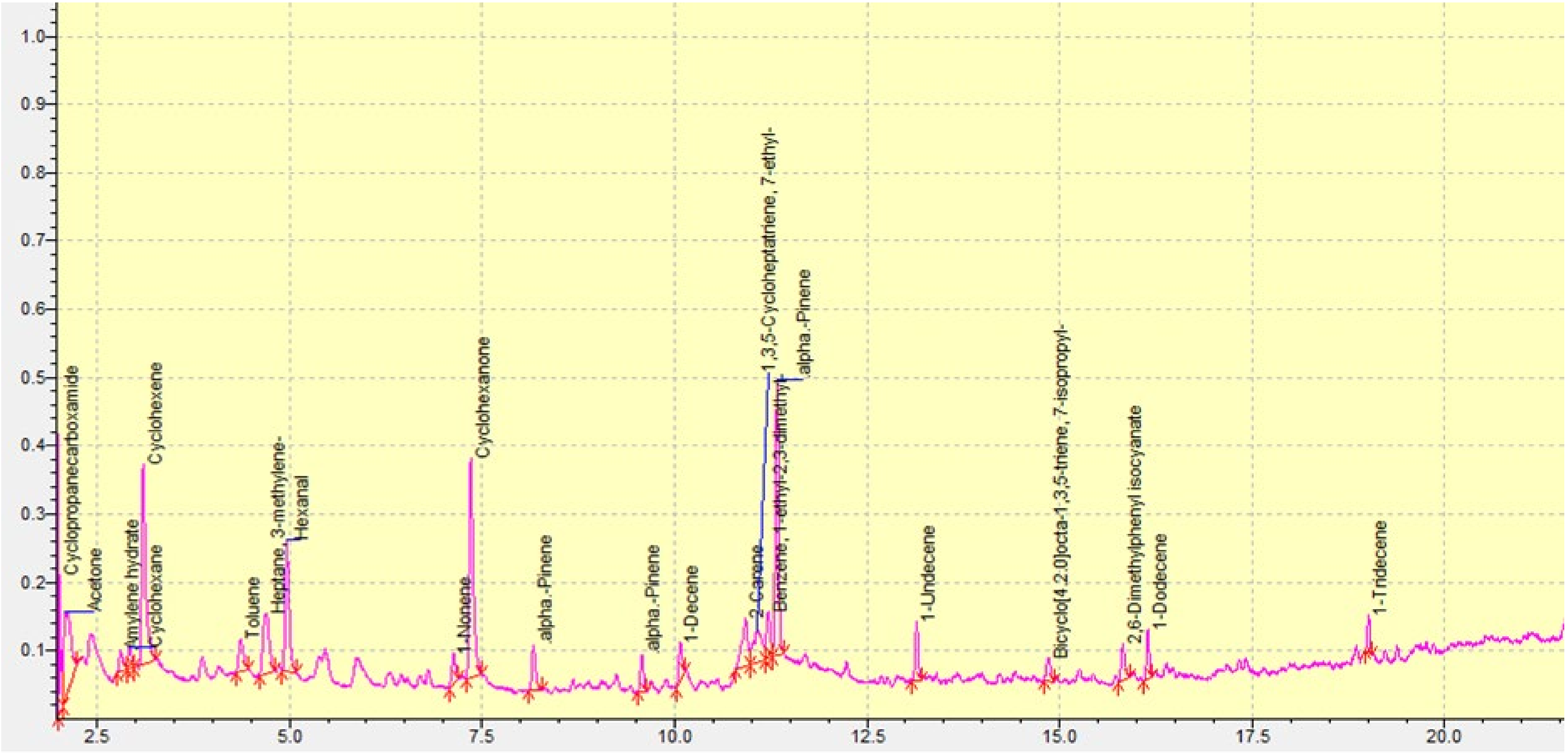

Figure D.4 Pyrogram for kinetic studies conducted at 85°C

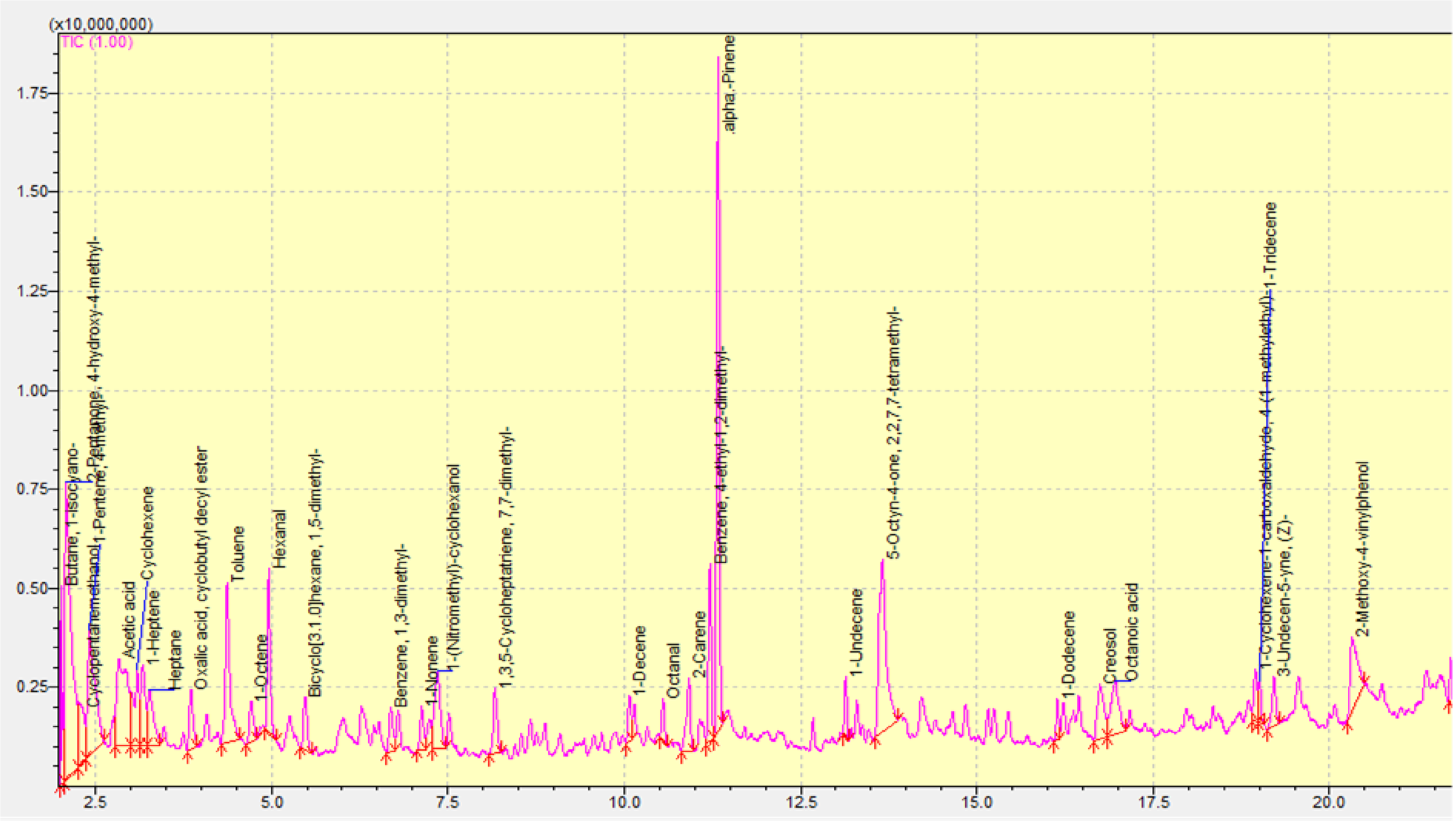

Figure D.5 Pyrogram for sample 1 conducted at optimised conditions

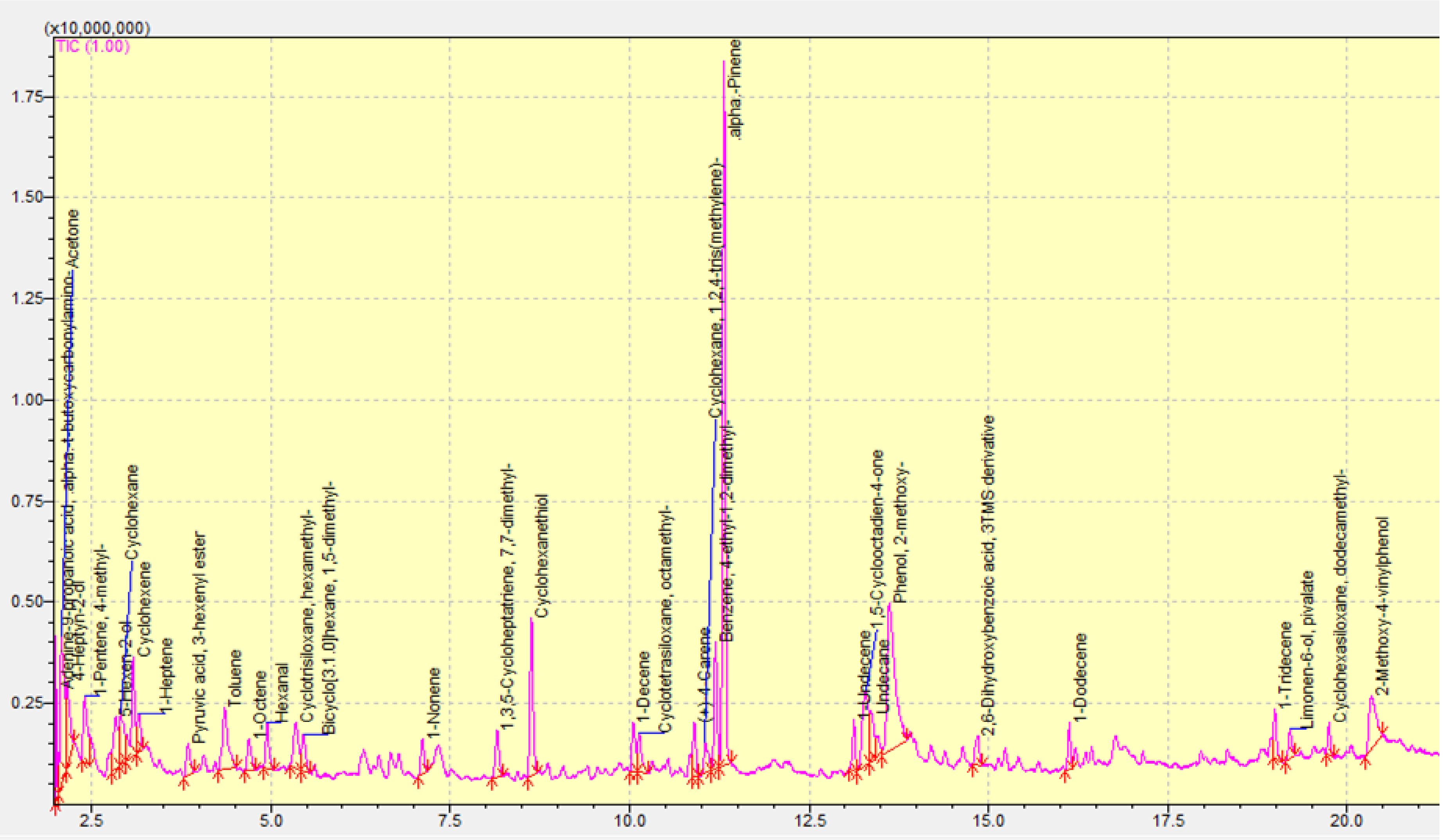

Figure D.6 Pyrogram for sample 2 conducted at optimised conditions

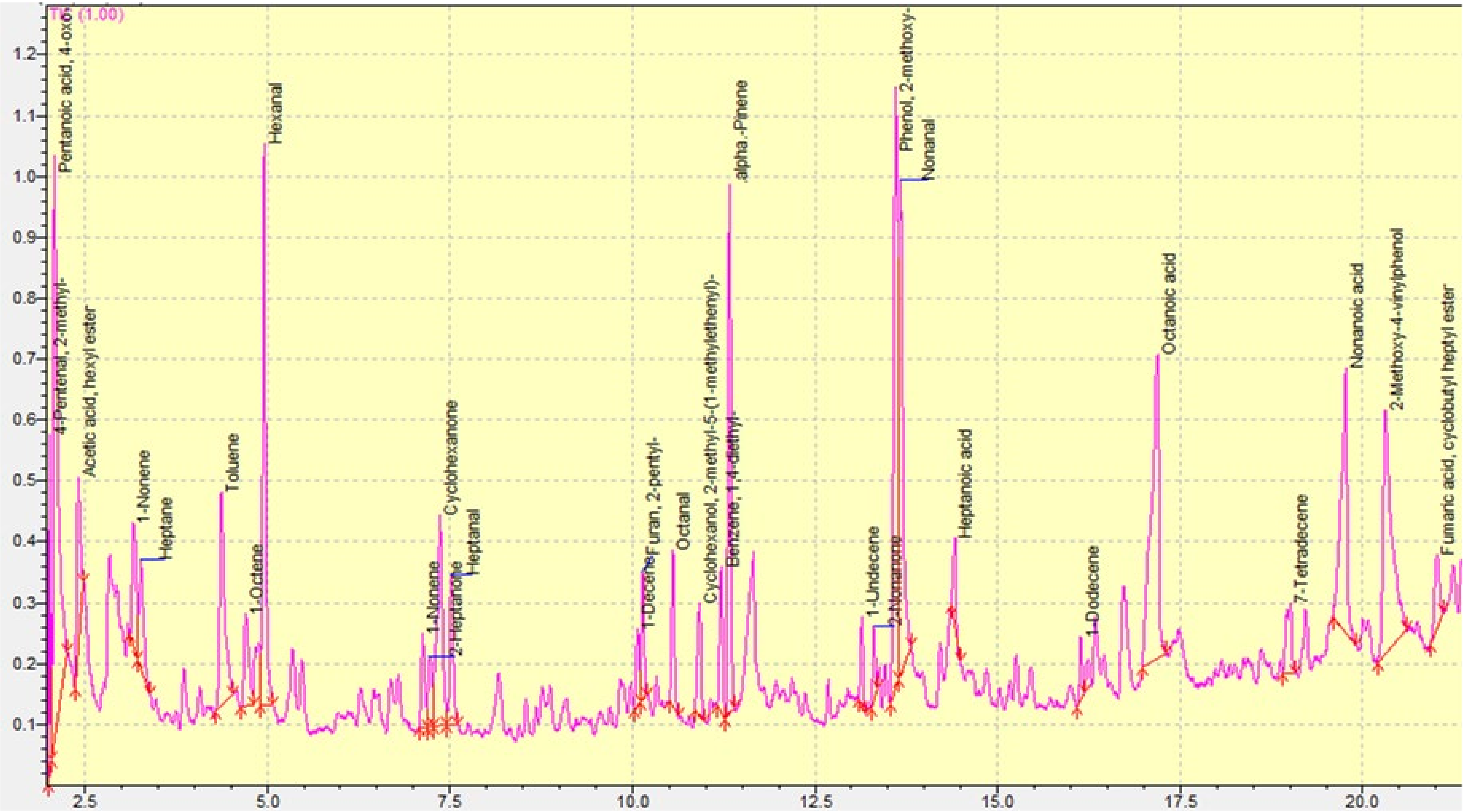

Figure D.7 Pyrogram for sample 3 conducted at optimised conditions

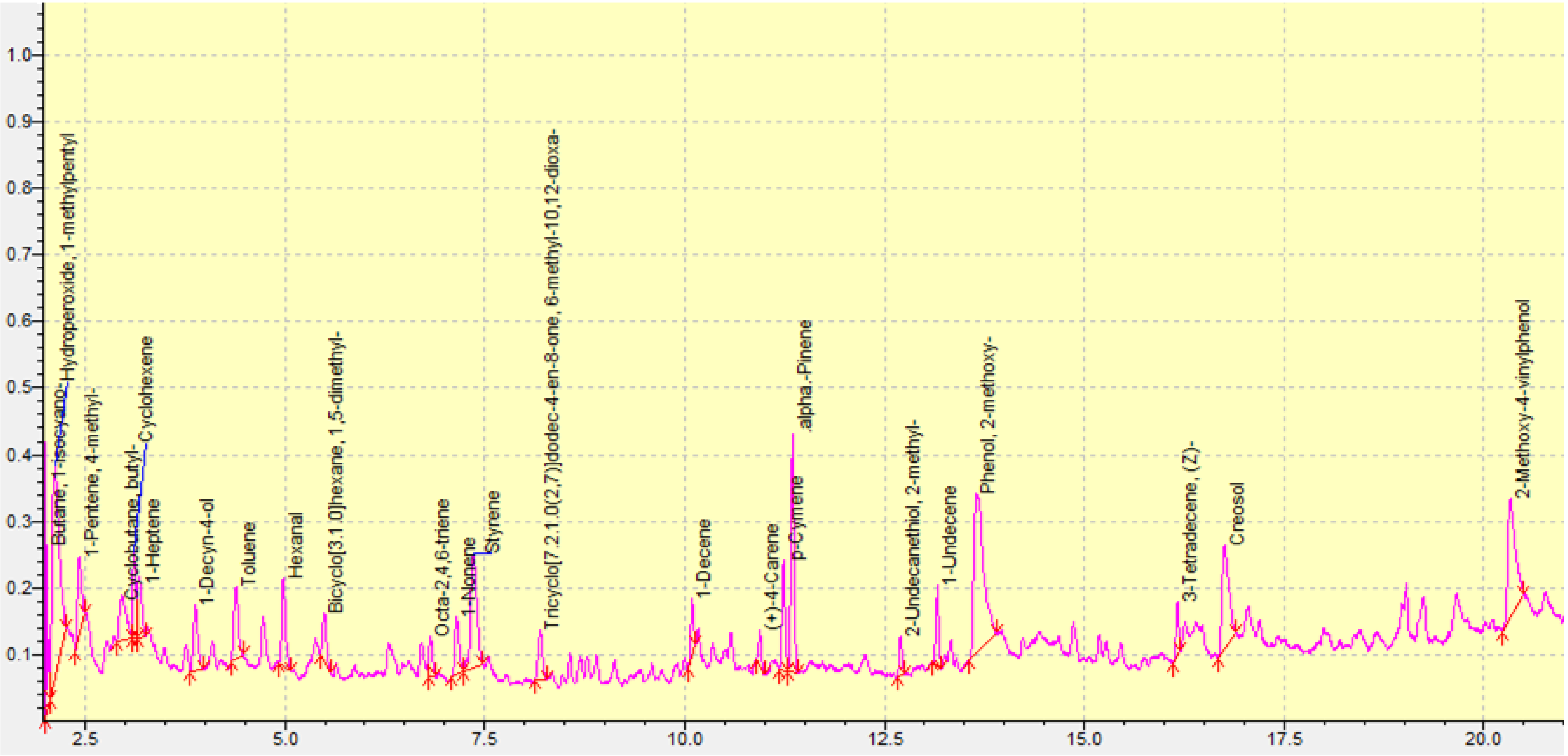

Figure D.8 Pyrogram for sample 4 conducted at optimised conditions