ABSTRACT

WANG, YINQIAO. Loblolly Pine Residues Utilization. (Under the direction of Dr. Kelley and Dr. Park.)

As one of the main tree species in the south, loblolly pine accounts for more than half of the total wood consumption in the United States. However, the residues left after the harvest are not studied and utilized adequately. As a sustainable resource, loblolly pine residues have various utilizing possibilities. Extraction is one of the technology options which has broadly industrial usage. In addition, the choice of the extraction techniques was critical to isolate the aimed components. This study compared and evaluated two extraction techniques. Both the Soxhlet extraction and the steam distillation were conducted to obtain the extractives from different fractions of loblolly pine residues. A minor fraction of the residue is composed of non-structural extractives that can be converted into value-added products. The composition and amount of the extractives were determined and analyzed. The tree age, tree fractions, and the extraction solvent types as the three factors that may affect the extractive properties have been compared via statistical analysis. Based on quantitative and qualitative measures of the extractive contents, principal component analysis models were built to determine the relationship between chemical components and the genetic features of the residues. A feasible application of the extractives is proposed in the study. As the main constituents of the essential oil extracted by steam distillation, α-pinene and β-pinene were used for the production of the terpin hydrate. Terpin hydrate as a commercial pine chemical was synthesized and characterized. The effects of the main synthesizing factors, including acid concentration, temperature, and Tween mixture amount, were investigated and optimized. Economic impacts were evaluated by simulating the entire process on an industrial scale. Based on the internal return rate (IRR) of 17.7%, the pathway is profitable and considerable for further study.

Loblolly Pine Residues Utilization

by
Yinqiao Wang

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Forest Biomaterials

Raleigh, North Carolina

2021

APPROVED BY:

Dr. Steve Kelley
Co-chair of Advisory Committee

Dr. Sunkyu Park
Co-chair of Advisory Committee

Dr. David C. Tilotta

BIOGRAPHY

Yinqiao Wang got her Bachelor of Engineering degree at Nanjing Forestry University. After graduating, she entered the master's program in Forest Biomaterials department at NC State University to better understand the engineering and chemical fundamentals that will serve as the foundation of the new bioeconomy. With a dual master's degree in Statistics, she plans to apply her interdisciplinary skills to develop her career in the bioproducts relative field.

ACKNOWLEDGMENTS

I want to thank my advisors, Dr. Kelley and Dr. Park, for their support, encouragement, and guidance during my entire master's studying period. Only words cannot express how grateful I am. They always patiently guide me to explore my potential, inspire me to achieve my goals, and assist me to challenge. Without them, this study would not be finished.

I also want to thank my committee member, Dr. Tilotta, for your valuable suggestions and your time in joining my committee. I cannot accomplish this study without the unconditional love and support from my dear parents. Also, thank you to all the great friends and colleagues I met in the Forest Biomaterials department.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1 Introduction

Sustainability is a popular concept that attempts to integrate social, environmental, and economic benefits and costs while addressing practical problems while balancing both near-term and longer-term impacts (Hussain et al., 2014, UNSD, 2018, as cited in Mensah, 2019). Commonly, environmental sustainability focuses on climate change, water and air quality impacts, biodiversity, and ecological integrity while also considering economic and developing society (Mensah, 2019).

The utilization of forest harvesting residues can impact environmental sustainability. The accumulated harvesting residues may influence the other forest resources, preserve the water quality, and impact the wildlife habitat (Osborne, 2013). Harvesting the woody biomass can provide for durable wood products, provide sustainable bioenergy as an alternative to non-renewable fossil fuels and bring economic benefits for loggers and landowners. Removal of the woody residues helps decrease the labor and cost for subsequent land management (Megalos et al., 2019). In addition to the advantages listed above, removing forest residues can bring added environmental benefits include reducing the risk of insect, disease, and wildfire loss (Megalos et al., 2019). Removing forest residues requires a market for these residues. One potential market is as a feedstock for the production of value-added products.

This study investigated the potential for using different fractions of loblolly pine residues to produce value-added extractives that could be used for the production of fine chemicals in the downstream process. This work evaluated the mass and chemical composition using various techniques, e.g., Soxhlet extraction, steam distillation, gas chromatography-mass spectrometry (GC-MS), and gas chromatography with flame ionization detection (GC-FID).

CHAPTER 2 Background

2.1 Loblolly pine residues

The southern United States is a region from Texas to Virginia with high forest densities and diversity. It accounts for 55% of the national forest land while generating about 64% of the total wood harvest (Smith et al., 2001, as cited in Loxley, 2018). Generally, trees can be classified into two main categories as softwood and hardwood, based on their differing physiological characteristics (Yang et al., 2012). Softwoods such as *Pinus spp* are commonly planted in managed plantations and are major sources of timber, pulp chips, bioenergy, and bioproducts.

Pine is one of the most critical softwood species, which occupies about 34 percent of the southern US forest land (Wear & Greis, 2012). The most common southern pines include the loblolly pine (*Pinus taeda L.*), longleaf pine (*Pinus palustris Mill.*), shortleaf pine (*Pinus echinata Mill.*), and slash pine (*Pinus elliottii Engelm.*). Among the pine species, loblolly pine is the major plantation-grown species with an area of 14.4 million hectares (as cited in Restrepo et al., 2019; South & Harper, 2016). A strong timber market and the emergence of new markets for bioenergy and bioproducts could increase the pine planted area between 24% and 36% by 2060 (Wear & Greis, 2012). The merchantable stemwood of loblolly pine provides timber production while its bark and unmerchantable crowns, branches, and needles are commonly not collected and could be used as a feedstock for bioenergy or bioproducts.

Besides the economic gains from the residues, the utilization of the biomass residues can provide extra environmental benefits. To limit the emissions of greenhouse gas from fossil fuels, biomass is considered a renewable and more sustainable alternative. Biomass is the most prominent form of renewable energy, with more than 85% coming from the forest industry. The usage of woody biomass for bioenergy is predicted to rise dramatically to at least 170 million green tons by 2050 (Wear & Greis, 2012). The forestry-derived biomass is a mixed resource whose properties depend on the species location, climate, and harvesting time and method (Acquah, 2010; as cited in Acquah et al., 2015). Depending on the harvesting method, forest residues can be divided into three main classes: primary products used for energy purposes such as fuelwood, charcoal; forest residues, which are the materials produced during harvest; and mill residues, which are created during biomass processing (van Stralen et al., 2016). The quantity of the latter two is considerable, with about 13% of the total potential biomass in the United States and an approximate portion of 22% of the total potential biomass amount in the southern region (as cited in Huang et al., 2011; Koch, 1972).

Figure 2.1 Example of loblolly pine limbs, fine branches, and needles classified as forest residues from commercial harvesting (Source: Megalos et al., 2019).

The treetops, branches, and needles are all included in forest residues from the harvesting of pine forests (van Stralen et al., 2016). The tree bark is considered a mill residue since it is produced along with stemwood processing and is commonly removed at the sawmill or pulp chipping operation (van Stralen et al., 2016). These residues are all sources of forest biomass that could potentially be utilized to produce renewable bioenergy.

Compared with other energy sources, the relatively high cost of collecting and transporting forest waste residues has limited its use. Obtaining higher value byproducts is one way to increase the economic viability of collecting forestry residues.

Figure 2.2 Loblolly pine bark mill residues collected at a sawmill (Source: Jackson, 2008).

A review of the chemical composition and characteristics of the forest and milled loblolly pine residues and an analysis of their potential as feedstocks for the production of bioenergy and bioproducts is provided below.

The chemical composition and characteristics of a loblolly pine tree vary significantly among different parts. A complete pine tree can be classified as the above-ground biomass and the below-ground root section. The above-ground biomass part contains the stem and crown. For commercial purposes, the crown is generally classified as the portion that can no longer be economically processed into dimension lumber, usually below a 4 or 6-inch diameter, to the top of the tree (Loxley, 2018). The crown is composed of branches and needles.

The form, weight, and distribution of loblolly pine needles is depended on their positions relative to the crown. However, the weight of needle biomass after reaching a certain age is comparatively constant. The tree age and the planting site are the two main factors that influence branch biomass's weight. Understanding the typical mass of the above-ground tree derived from foliage and branches has a practical impact on residue utilization.

The stem section contains the stemwood, and the outside bark. The bark is defined as the tissues circumambient and exterior of the vascular cambium. Generally, it comprises about 13-21% of a log on a dry weight basis (Feng et al., 2013). Bark is commonly removed at sawmill or pulp mill and is combusted for the production of low-grade heat or steam. Due to natural processes and also the harvesting methods, barks have a higher ash content than wood. This ash can cause fouling and harm the combustion systems (Feng et al., 2013). Thus, the economic benefit created by the combustion of bark is minimum. As a tree matures, the total mass of the bark increases, but the weight percentage of bark compared to the whole tree decreases due to the increasing wood percentage.

Like all trees, loblolly pine is comprised of three high molecular weight components: lignin, cellulose, and hemicelluloses. There are also low molecular weight substances commonly labeled as extractives that are non-structural and include a wide array of different compounds.

Extractives can be removed with polar or non-polar solvents. The specific solvent, extraction process, extraction time, and temperature all influence the mass and composition of the recovered extractives. Although extractives only represent a minor fraction of the total tree weight, they have a broad application range and serve as the source for a number of different value-added chemicals. The extractives from different tree species have significant differences. The composition of the extractives within a tree varies due to the different morphological sites and functions (Sjöström & Alen, 2010). Based on the type of extraction solvent and the solubility of the resulting extractive, several major classes can be summarized as aliphatic and alicyclic compounds, polysaccharides, phenolic compounds, and other compounds (Yang et al., 2012).

In this work, the three most valuable and high-demand extractives can be classified as volatile components, resin acid, and fatty acid.

Oleoresins are a collection of resin acids, monoterpenes, and other terpenoids found in the resin canals of the softwoods. Turpentine is a specific subgroup of low boiling monoterpene isomers. In loblolly pine resin, volatile pine oil, also called essential oil, can be isolated as natural products. The essential oil was separated for key components by the turpentine industry with high concentration and accounted for 25% of the total resin weight (Sharma et al., 2016). For loblolly pine, the most common terpene hydrocarbons in the essential oil are α-pinene, β-pinene, which are unsaturated monoterpenes (chemical formula: $C_{10}H_{16}$) (Saeidnia, 2014; Wilbon et al., 2012). For example, essential oils extracted from loblolly pine needles by hydro distillation contained 37 % to 43% of α-pinene and 12% to 15% of β-pinene (Adams et al., 2014). Due to the relatively large concentrations and ease of isolation, α- and β-pinene were used as the starting materials for many industrial applications such as fragrances and flavors (Lakshmikumaran & Sridharan, 2016). The presence of reactive double bonds allows the α- and β-pinene to be easily converted to polyterpenes via polymerization. Polyterpenes have been broadly used in disinfectants, specialty chemicals, pharmaceuticals, solvents (Lakshmikumaran & Sridharan, 2016).

Rosin consists of the less volatile diterpene components of resin. Over 90% of the rosin is the resin acid (Abdel-Raouf & Abdul-Raheim, 2018). There are three categories of rosin generated by the loblolly pine trees referred to in the manufacturing process and extraction methods: gum rosin, wood rosin, and tall oil rosin(Abdel-Raouf & Abdul-Raheim, 2018). Gum rosin is directly collected from the living pine tree wounds. The wood rosin is the rosin extractive from the tree stumps. The tall oil rosin is the resinous material obtained as a by-product of the pulping Kraft process. These three rosin sources can be further divided into two main chemical compound groups: resin acids and fatty acids. Resin acids from pine rosin have tricyclic carboxylic skeletons containing primarily abietic- pimaric- isopimaric- acid and several isomers for specific applications (Abdel-Raouf & Abdul-Raheim, 2018). The resin acids and their derivatives are widely used in many industrial fields such as pharmaceutics, pulp and paper industry, coating applications, printing ink formulations, and adhesives, etc. (Abdel-Raouf & Abdul-Raheim, 2018). Due to the rigid, multi-ring structures and their potential for condensation polymerization, resin acids have attracted attention as an alternative to petroleum-feedstock for polymer synthesis (Liu et al., 2009).

The second category of compounds common to rosins is fatty acids. Fatty acids are long-chain monocarboxylic unsaturated acids. The primary industrial source of fatty acid for the loblolly pine tree is the fractionation recovered from the tall oil, which isomers form containing oleic, linoleic, linolenic, and palmitic acids. The palmitic acid, a short-chain fatty acid with 16 carbons, is mostly recovered from this portion. Fatty acids from the loblolly pine tree have similar features to the fatty acids extracted from vegetable plants. However, the fatty acids from pine trees are broadly used in non-food applications, such as paints and coatings, soaps, specialty inks, mining chemicals, lubricants, metalworking fluids, and adhesives (Lakshmikumaran &

Sridharan, 2016). A thorough understanding of the distribution of these components is critical for better utilizing the fatty and resin acid in the extractives.

2.2 Extraction techniques

Natural products extraction has a long history that can be traced back to thousands of years ago. With the various chemical compounds from extractives, the plant tissue as a leading source of extractives has been widely applied in the pharmaceutical and nutraceutical industry (Pilz & Bart, 2011). Therefore, extraction is a feasible approach to increase the economic potentials of woody biomass residues.

Due to the complexity of the extractives, it is impractical to use one extraction technique to isolate all the extractives present in pine trees. The preferred extraction protocol is determined according to the chemical composition of the targeted extractives. At the same time, choosing a suitable extraction technique and solvent is vital for any commercially viable process. The targeted structures will have different functional groups, chirality, and concentration, which allows them to use as the active ingredients for combinatorial medicines or foods (Zhang et al., 2018). This section will review common extraction techniques and compare the pros and cons of each approach.

The conventionally explored extraction methods typically consist of solid-liquid extraction using organic solvents, which include maceration, percolation, and reflux extraction (Zhang et al., 2018). Maceration is preferably used with volatile, thermolabile, or bioactive materials with a long extraction time and relatively low efficiency. It relies on the evaporation of the extractive, which is collected by condensation. The main benefit of maceration is its simplicity and use for temperature-sensitive compounds. It has been reported that the yield of extractives achieved by the maceration is similar to the yields from heat-assisted, microwave-assisted extraction, and ultrasonic-assisted extraction (Albuquerque et al., 2017; as cited in Zhang et al., 2018). Due to the low operating temperature, maceration is a competitive method for industrial-scale applications.

Percolation is another common technique that is broadly used for ground particles. The particles are placed on the filter, and the solvents are slowly poured onto the materials. The solvent liquid is allowed to strain through the porous filter bed. Although the extraction efficiency may be increased by replacing the saturated solvent with fresh solvent, the application of the percolation is limited by the feature of the solids. Although the use of fresh solvent makes the final separation of the targeted extractive and the solvent more complex and expensive. Some fine powder and plants may soak the solvent and swell (Pilz & Bart, 2011). Thus, extra efforts are required to determine suitable particle size, ideal implementation procedure, and high maintenance cost.

Reflux extraction is a process that repeatedly boils and condenses the extraction solvent. By trapping the solvent vapors, the solvent volume remained the same throughout the whole extraction time, and the added heat typically increased the solubility of the solvent. Compared to maceration and percolation, reflux extraction has the advantages of being faster and of using less solvent, and it allows for the use of a wide range of solvents. The disadvantages of refluxing include degradation of thermally-labile materials and higher operating costs (Zhang et al., 2018).

Soxhlet extraction is a practical and effective extraction that takes advantage of the benefits of both percolation and reflux. The solvent in the bottom flask was heated and condensed into the middle glass tube. The gradually accumulated solvent percolated the solids in the extracted thimble. The solvent is periodically siphoned off in a second tube and transferred back into the bottom flask. The extractives were carried to the flask with the solvent. This extraction is continuous with fresh solvent boiled out of the bottom flask. It is often used as a benchmark to understand to benefit new, alternative extraction techniques. In order to obtain the desired compounds, solvent selection is one of the core factors to consider. Meanwhile, solvent selectivity, solubility, cost, and safety also influence the choice of solvent (Zhang et al., 2018). The benefits of Soxhlet extraction include simple process, low cost, and high efficiency. At the same time, the significant drawback of this method is thermal degradation probability and usually consuming a moderate volume of organic solvent compared to the extraction techniques such as steam distillation, which leads to environmental damages.

More environmentally friendly extraction techniques such as hydro distillation and steam distillation are widely applied in the industry. Hydro distillation and steam distillation are similar extraction techniques targeting volatile compounds collected as essential oil. The three main physicochemical processes involved in the two techniques are hydro diffusion, hydrolysis, and decomposition by heat (Azmir et al., 2013). The solid materials were filled in the bottom flask with sufficient water added. By boiling the water, the volatile components were transported by steam as a mixture vapor phase. This vapor phase was condensed as a liquid mixture flowing into a separator. The essential oil was separated from the liquid mixture. The remaining water-rich phase with water-soluble phenolics is the hydrosol phase which can be further utilized, such as a mild antiseptic (Mohammad Azmin et al., 2016; Oreopoulou et al., 2019). Instead of mixture solids and water in the bottom flask, the bottom flask only contained water which was used to generate steam. The plant material was packed into a compartment set above the water flask, which allowed the steam to pass through. The good point of hydro distillation is the relatively high essential oil yield compared to the steam distillation method. However, the downside of hydro distillation is that a prolonged time or additional solvent is required to separate water from the oil product (Mohammad Azmin et al., 2016). Both hydro distillation and steam distillation are inexpensive and environmentally friendly without using organic solvents. Despite several advantages, both methods have drawbacks included the potential risk of thermal degradation and energy consumption.

2.3 Production of terpin hydrate for α-pinene

The essential oils recovered from pine trees contain an abundant amount of α-pinene and β-pinene. Due to their ease of isolation and relatively low-costs, α- and β-pinene are used as the starting materials to synthesize many critical chemical compounds and polymers for the flavor, perfumes, fragrance, and pharmaceutical industry (Wilbon et al., 2012). A multitude of chemical reactions is performed with α-pinene and β-pinene, among which hydration is one of the most practicable and valuable reactions. α-pinene can be used to produce a mixture of monocyclic and bicyclic alcohols with strong proton acid as the catalyst, such as sulfuric acid, nitric acid, and phosphoric acid (Comelli et al., 2013; Yi et al., 2015).

Terpin hydrate is one example of an industrial product produced from α-pinene and β-pinene. Terpin hydrate has been widely used as the starting materials to produce medicines, pesticides, fragrances, and fine chemicals. Mixtures of isomeric cis- and trans-N,N'-diacyl-p-menthane-l,8-diamines have been formed through the Ritter reaction between terpin hydrate and various nitriles (Kovals'skaya et al., 1989; as cited in Yi et al., 2015). Handrick et al. (1982) reported that condensation of anhydrous terpin hydrate and 3'-acetoxyolivetol in the presence of fused zinc chloride in dichloromethane produced delta-9-tetrahydrocannabinol (delta-9-THC) as a synthon. The synthon helped synthesize other pharmaceutical compounds, while its metabolites have a similar pharmacological effect and higher activity than delta-9-THC (Handrick et al., 1982; as cited in Yi et al., 2015). Eucalyptol applied in the medical industry could be prepared by terpin hydrate dehydration under sulfuric acid aid (Mitchell & Sasser, 1987). In the fine chemicals industry, terpin hydrate as the raw materials was converted into menthane diamine, which is broadly used as the curing agent, copolymer, antibacterial precursor, and reaction additives, via a convenient, low toxicity two-step reaction (Yi et al., 2015).

Other than the pharmaceutical application mentioned above, terpin hydrate is an essential intermediate used in a two-step process to produce α-terpineol synthesized process. The two-steps hydration of α-terpineol has the drawbacks of long reaction time, high energy consumption and environmental concerns, and equipment corrosion caused by large volume strong acid usage. A one-step hydration alternative can be achieved by directly converting α-pinene into α-terpineol in the one-pot reaction. However, the one-step pathway has a lower yield and less 'pleasant' odor than α-terpineol made by the two steps hydration.

The two steps hydration reaction mechanism is electrophilic addition reaction (Finar, 1973; Li et al., 2009). The α-pinene molecule opens the ring structure and forms the first carbocation under the attack of a proton (Comelli et al., 2013). Undergoing the rearrangement, the bicyclic skeleton of the first carbocation opens to generate the second carbocation, a tertiary carbocation. One molecule of water then adds to the double-bond, followed by the addition of a second water molecule, which adds to the four-carbon ring structure (Stinson, 1984). In the presence of additional water, the hydrate form is generated.

Zhao et al. (2000) studied the optimization conditions of the terpin hydrate synthesis process. The commercial turpentine, which contained 92% of α- and β-pinene, was used as the raw material. Peregal solution was added as the emulsifier to mix the acid and turpentine. The highest yield achieved through their work is reported to be 79%, although the raw terpin hydrate product was not dried after the vacuum filtration. The optimal temperature range is reported to be 28 °C to 30 °C, the reaction times range between 16 to 24 hours, and the turpentine and 30% acid mass ratio is 1:1.5.

Wang et al. (2003) enhanced the process by using ultrasound to increase the mass transfer between the immiscible phases. The yield of the vacuum-dried terpin hydrate product achieved by this method averaged 76%, with a shorter reaction time of 17 hours. The reaction temperature range was fixed at 26 °C to 28 °C, and the turpentine and 35% acid mass ratio was 1:1.72.

Li et al. (2009) combined sulfuric acid and phenyl acrylic acid, which has a strong ability to attack electrons. This method increased the activity of the hydrogen ions and aided the hydration reaction. Within the temperature range of 15 °C to 20 °C, the yield of terpin hydrate was 73%. The mass ratio of turpentine and 27% acid (1 mg phenyl acrylic acid not considered) is 1:1.50. The Tween 20 and Tween 80 were used as the emulsifier with a mass ratio of 1:1.

Chen (2011) explored adding an emulsifier additive to decrease the diameter of the emulsion and thus increase the mass transfer. By adding the tetrapropyl ammonium bromide, the macro emulsions were decomposed as micellar emulsions with a diameter of 0.1 μm. The highest yield of terpin hydrate was 84% achieved after 24 hours reaction with a reaction temperature of 24 °C, and turpentine and 35% acid mass ratio 1:2.

Despite the fact that the yield of terpin hydrate has been increased by the aid of ultrasound, organic carboxylic acid, or the emulsifier additive, these approaches are expensive and may cause difficulties for later purification operations. Therefore, this work evaluated several alternative synthetic approaches to terpin hydrate synthesis.

CHAPTER 3 Experimental

3.1 Soxhlet extraction

3.1.1 Moisture content

The moisture content was measured for the test samples according to the NREL/TP-510-42619 (2005). Approximate 1 g of the sample was weighed and dried in a drying oven at 105°C to a constant mass. The test was repeated three times for each sample type. Moisture content was calculated on an oven-dried basis as the equation (3.1) below:

$$\text{Moisture content (\% d.b.)} = \frac{\text{Weight}_{\text{Sample before drying}} - \text{Weight}_{\text{Sample after drying}}}{\text{Weight}_{\text{Sample after drying}}} \times 100\% \quad (3.1)$$

3.1.2 Extraction procedure

Eight samples were harvested from two different loblolly pine tree families. Tree A family is 23 years old, and tree D family is 13 years old. Four fractions, including the whole chips, needles, branches, and bark, were prepared separately from tree A and tree D. All the samples were air-dried to constant weight, then milled and passed through a 2 mm sieve.

For the Soxhlet extraction, the suitable solvent is a critical factor. The ethanol solvent was used according to the NREL's procedure, as quantification for the water-soluble portion is not the goal of this study (Sluiter et al., 2008). In comparison, the methyl isobutyl ketone (MIBK) was used because it is a commercially viable extraction solvent that could achieve high extraction efficiency for the materials containing high aliphatic and aromatic extractive (Cheremisinoff, 2003). The ethanol, ACS spectrophotometric grade, with a purity of 95%, and the MIBK, ACS reagent grade with purity over 98%, were purchased from Sigma Aldrich and used as received.

Soxhlet extractions were conducted triplicate according to SCAN-CM 67:03 (2003) and NREL/TP-510-42619 (2005). Equal to 3g of oven-dried ground samples were used for the successive extraction with MIBK (bp 117°C) or ethanol (bp 79°C) for a minimum of 24 extraction cycles. The extracted thimbles were dried in a drying oven at 105°C to constant mass. The extraction was evaluated by the weight loss percent of the thimbles and the extract production percent with the following equations (3.2 and 3.3) on an oven-dry basis:

$$\text{Weight loss (\% d. b.)} = \frac{\text{Weight}_{\text{Sample before extraction}} - \text{Weight}_{\text{Sample after extraction}}}{\text{Weight}_{\text{Sample before extraction}}} \times 100\% \tag{3.2}$$

$$\text{Extractives (\% d. b.)} = \frac{\text{Weight}_{\text{flask plus extractives}} - \text{Weight}_{\text{extractives}}}{\text{Weight}_{\text{Sample used}}} \times 100\% \tag{3.3}$$

Even though the weight loss percent is a more common parameter for the extraction process, the extractive percent is preferable because of the direct relation with our study. The following discussion is focused on the extractive percent. The detailed results for weight loss percent were listed in the appendix.

3.1.3 Quantification by GC-FID analysis

Trimethylsilyl derivatives of the extractives were analyzed by GC-FID following the procedure, which is modified (Arshadi & Gref, 2005; Eriksson et al., 2018; ÖRså & Holmbom, 1994). The heptadecanoic acid with purity greater than 98%, and BSTFA and the TMCS with purity over 99% were purchased from Sigma Aldrich and used as received. A weighed sample of the extractives was dissolved in 10 mL dichloromethane solvent (ACS grade, purchased from Fisher Scientific). One mL of the extractive, with the heptadecanoic acid as the internal standard was transferred to a vial. The solvent was evaporated with nitrogen flow, after which 80 µl N, O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA), and 40 µl trimethylsilyl chloride (TMCS) were added into the dried sample. The sample was reacted in the 70°C oven for 45 minutes. After that, the excess BSTFA and TMCS were evaporated by nitrogen before being dissolved with 1 mL dichloromethane. The dichloromethane with purity over 99% was purchased from Fisher Scientific and used as received. Agilent GC 6890N gas chromatograph equipped with a flame ionization detector and an HP-5 MS capillary column (30 m x 250 μm x 0.25 μm) cross-linked with 5 % phenyl methyl siloxane were used to evaluate the extractives. 1 μl sample was injected with the split ratio of 1:10. The GC was temperature programmed with the initial temperature of 100°C, increasing at a rate of 10 °C/min to 220°C then at 1 °C/min to 235°C and at 10 °C/min up to 260°C (5.5 min hold). The FID detector was operated using hydrogen at the flow rate of 40 mL/min, air at 300 mL/min, and helium as the makeup flow at 20 mL/min. Based on the oven-dried samples, the concentration of individual compounds was determined by comparing the peak area of the added internal standard.

3.1.4 Characterization by GC-MS analysis

Agilent 7820 GC gas chromatograph equipped with a mass spectrometer operating in the electron-impact mode (EI 70eV) was used to characterize the extractives. The HP-5 MS capillary column (30 m x 250 μm x 0.25 μm) cross-linked with 5 % phenyl methyl siloxane was installed for the instrument. The column was temperature programmed according to the similar procedure described in the previous section. The NIST Mass Spectral Search Program and the relative references were used to identify the compounds.

3.1.5 Statistical analysis

Exploratory data analysis was performed using R version 3.6.1. In order to satisfy the normality and equal variance assumptions, the logarithmic transform was applied to the dataset before conducting the universal two-way analysis of variance (ANOVA). The statistically significant factors were determined by the p-value, with a threshold alpha of 0.05.

Principal component analysis (PCA) is a simple, non-parametric method for high-dimensional data analysis which has been applied in diverse disciplines (Wold et al., 1987). The objective of this type of analysis is to use the first meaningful few components to account for most of the information in the original data set. The data set from this study contains 48 objective samples and multiple interrelated chemical component variables. It is very difficult and time-consuming to identify all the structures of the chemical compounds through the spectrum's results. Therefore, PCA was conducted on the experimental data set using the software R under version 3.6.1. The data was normalized before performing PCA.

3.2 Steam distillation

3.2.1 Moisture content

The three different loblolly pine fractions, needles, branches, and barks, were collected from tree C and D families in 3 days to 1 week after harvest. All the trees were nine years old. The samples were all oven-dried according to NREL/TP-510-42619 (2005).

3.2.2 Steam distillation

Based on the methods from Charles and Simon (1990) and Biorenewables Education Laboratory (2011), about 250 g of fresh residues were cut into about 1cm wide small pieces and placed in the biomass flask. The boiling beaker was filled with about 1.25 L of deionized water and a few boiling stones. Steam was generated by heating and passed through the chamber with fresh residue materials. The steam distillation was carried out for 90 minutes after the first drop of the vapor condensed into the separated funnel. The upper layer liquid in the funnel was collected as the essential oil. The condenser and the separate funnel were washed using the ethyl ether to collect residual essential oils. The residue moisture in essential oil obtained from the needle fraction was removed by being treated with sodium sulfate, while the remained moisture in other fractions was estimated as 10%. The ethyl ether was removed by the rotary evaporator. The recovered essential oil was weighed and stored for later usage. The laboratory-grade ethyl ether and sodium sulfate were purchased from Fisher Scientific and used as received. The weight loss of the different fractions was determined by the equation (3.4) on a dry basis, while the yield of the essential oil was calculated by the equation on a dry basis (3.5).

$$\text{Weight loss (\% d. b.)} = \frac{\text{Weight}_{\text{Sample before distillation}} - \text{Weight}_{\text{Sample after distillation}}}{\text{Weight}_{\text{Sample before distillation}}} \times 100\% \tag{3.4}$$

$$\text{Essential oil yield percent (\% d. b.)} = \frac{\text{Weight}_{\text{Essential oil}}}{\text{Weight}_{\text{Sample before distillation}}} \times 100\% \tag{3.5}$$

Figure 3.1 Steam distillation apparatus filled with the needle residues.

3.2.3 GC-FID analysis

The recovered essential oil sample was analyzed using GC-FID according to ASTM D6387 (2019). The α-pinene with purity over 98% and β-pinene with purity over 99% were purchased from Sigma Aldrich and used to prepare the standard solution. The n-Decane, with purity greater than 99%, was purchased from Fisher Scientific and used as the internal standard. Weighed about 50 mg of the essential oil sample, added about 15 mg n-Decane, then dissolved in 1mL hexane, purchased from Fisher Scientific with purity over 99%.

The quantification tests were performed on the Agilent GC 6890N. The gas chromatograph was equipped with a flame ionization detector and an HP-5 MS capillary column (30 m x 250 μm x 0.25 μm) cross-linked with 5 % phenyl methyl siloxane. The main operating conditions are listed as follows. The initial column temperature is 50°C (5 min hold) and ramping at the rate of 4 °C/min to 240°C. After holding for 10 mins, the column temperature increased at 1 °C/min to 250°C. The FID detector was equipped with hydrogen flow at the rate of 40 mL/min, air flow at the rate of 300 mL/min, and helium makeup flow at the rate of 20 mL/min. The detector turned on at the temperature 250°C. The sample was injected using split mode with a split ratio of 10:1. The amount of α-pinene and β-pinene in the essential oil was calculated using the standard curve derived from the response of the known α-pinene and β-pinene samples and the n-Decane internal standard.

3.3 Terpin hydrate synthesis

3.3.1 Experimental setup

The α-pinene and sulfuric acid (purity over 98%) were purchased from Sigma Aldrich. The Tween 20 and Tween 80 were obtained from Fisher Scientific and used as the emulsifier with mass ratio 1:1. The terpin hydrate synthesis reaction was conducted following the procedure (Prakoso et al., 2017; Li et al., 2009): 50 mL of α-pinene and 0.5 mL, 10% Tween mixture solution was added into a 250 mL Erlenmeyer flask under the ice water bath. Then, 50 mL sulfuric solution was slowly dropped into the flask. Due to the α-pinene and sulfuric acid forming heterogeneous liquid, the Tween mixture worked as the emulsifier in the system. The emulsification could produce smaller droplets with larger contacting surface areas that benefited the hydration reaction (Lee et al., 2013). The mixture was stirred at the speed of 800 r/min for 24 hours at room temperature.

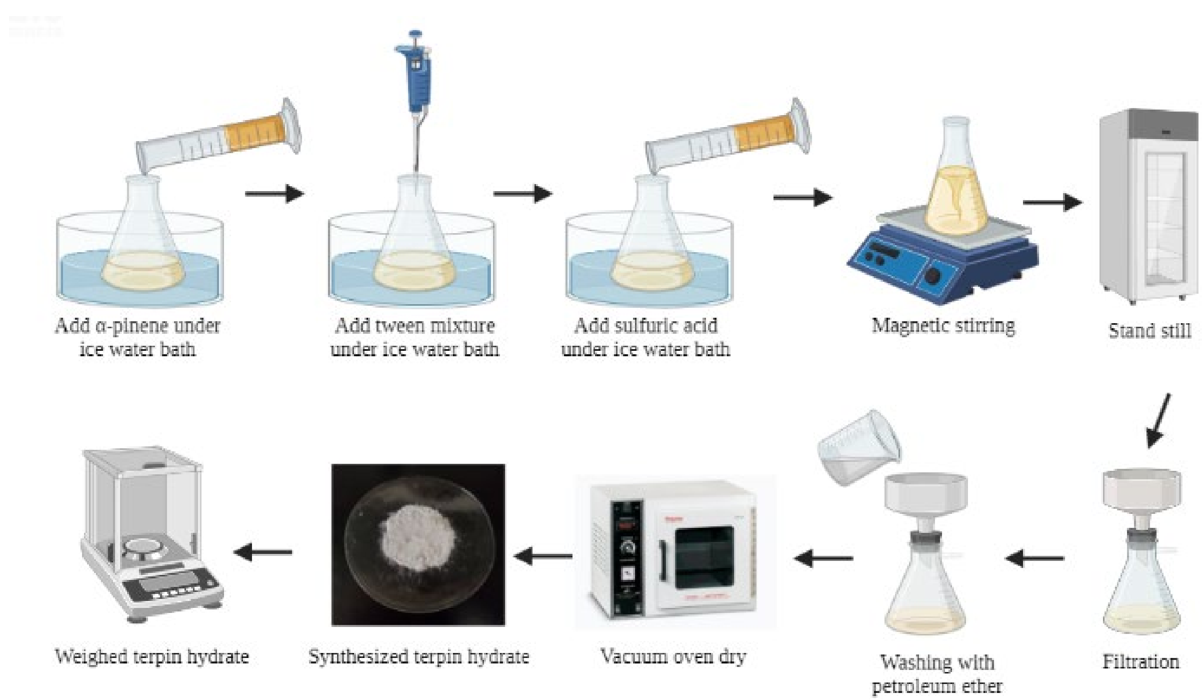

Figure 3.2 Terpin hydrate synthesis process illustration (Created with BioRender.com).

When the reaction finished, the mixture was cooled in the refrigerator, which allowed to crystallization of the terpin hydrate product. The product crystals and the solution were separated using the Buchner funnel. Petroleum ether (ACS grade, purchased from Fisher Scientific) was used to wash the terpin hydrate product. When no droplet was formed through the vacuum filtration, the product was transferred into a vacuum drying oven until constant weight. The weight of the synthesized product was recorded to determine the yield according to the following equation (3.6):

$$\text{Yield, \%} = \frac{\text{Weight}_{\text{Vacuum dried terpin hydrate}}}{\text{Weight}_{\text{Terpin hydrate received in theory}}} \times 100\% \tag{3.6}$$

The separated solution was transferred into a conical tube and centrifuged at the speed of 3000 r/min for 10 mins to achieve two separate layers. The upper layer consisted of the unreacted α-pinene and other organic by-products, while the lower layer contained the aqueous acid fraction. The upper layer was recycled, weighed, and reused for a second reaction intended to maximize the overall yield. The second reaction was conducted following the same procedure. The amount of the sulfuric acid and the Tween was based on the weight of the recycled organic solution.

3.3.2 Experimental design

To maximum the terpin hydrate yield, the main reaction conditions were explored. Optimization for the synthesis process is aimed to obtain the optimum outcome while saving time, energy, and resources input (Das & Dewanjee, 2018). A full factorial design was carried out for the three experimental conditions with two levels for each condition. All the possible combinations of the conditions were run to investigate the main effects and interactions on the yield. The three experimental conditions were temperature, acid concentration, and Tween mixture amount, resulting in a total of 8 experimental conditions. Based on the initial results, an additional level of the Tween mixture was included, resulting in a mixed-level factorial experimental design. The balanced and orthogonal properties of the mixed-level factorial design resulted in the information of each level being evenly distributed, and the significance of the factors was able to be evaluated (Guo et al., 2007).

Three main experimental conditions were chosen to study, including acid concentration, temperature, and the amount of Tween mixture. As the catalyst for the ring-open and rearrangement of the α-pinene to produce terpin hydrate, the concentration of sulfuric acid is critical for the hydration reaction. Hydration was also influenced by temperature. The isomerization would be the primary reaction when the temperature was relatively high, while the reaction efficiency would be limited under a too low temperature. In addition, the amount of emulsifier added was another factor that worthy of studying. With the properties of the heterogeneous mixture, the hydration reaction would be hard to happen to achieve a high terpin hydrate yield. However, too much of the emulsifier would make the terin hydrate difficult to separate, leading to a low yield. Other experimental conditions were fixed in this study.

3.3.3 Characterization of the terpin hydrate

Based on the procedure developed by Kurlansik et al. (1967), the dodecyl alcohol (purchased from Fisher Scientific, purity over 99%) was used as the internal standard. About 25 mg internal standard and about 50 mg terpin hydrate were weighed and recorded accurately. The absolute alcohol (from Fisher Scientific purity over 99%) was added to dissolve and mix the internal standard and the terpin hydrate. Terpin hydrate reference was prepared using the commercial terpin hydrate (from Fisher Scientific, purity from 98% to 100%) following the same process. The terpin hydrate recrystallization was carried out by the absolute alcohol (from Fisher Scientific, purity over 99%) (Zhao et al., 2000).

Alternatively, the samples were also subjected to an additional treatment following the method from the research from Kubiak (1968). The internal standard solution was prepared by weighing 5.5 mg of the 3-tert-butylphenol (from Fisher Scientific, purity over 98%) and dissolving by 1 mL chloroform (from Fisher Scientific, purity over 99%). Commercial terpin hydrate was dried at 60°C in the vacuum oven for 3 hours to prepare the anhydrous terpin reference. The residual moisture was determined using a TA Instruments, TA Q500 thermogravimetric analyzer (TGA). The anhydrous terpin sample was heated from 30°C to 300°C, at 5°C/min.

About 31.6 mg was accurately weighed and dissolved in 35 mL chloroform with 5 mL internal standard solution. About 34 mg terpin hydrate sample was measured and dissolved in 16 mL saturated sodium chloride solution (from Fisher Scientific, purity of over 99%). Added 35 mL chloroform, the heterogeneous solution was shaken at 140 r/min until the terpin hydrate solids were completely dissolved. Then, the sample was centrifuged for 10 mins at 3000 r/min. The upper aqueous layer and the lower organic layer were used for GC tests.

The quantification tests were conducted using the same instrument and procedure described in the previous essential oil GC-FID test section. The terpin hydrate quantity was calculated using the standard curve based on the purchased terpin hydrate and the synthesized terpin hydrate. The terpin hydrate recovery rate based on this procedure was calculated according to equation 3.7.

$$\text{Recovery rate, } \% = \frac{\text{Weight of terpin hydrate}_{\text{Calculated by the chromatographic result}}}{\text{Weight of terpin hydrate}_{\text{Used for the experiment (50 mg)}}} \times 100\% \tag{3.7}$$

Due to the chemical properties of the terpin hydrate, degradation could happen to the terpin hydrate with acid in the samples (Kubiak, 1968). The dehydration and rearrangement of terpin hydrate result in a mixture of monoterpene and terpineol compounds. The decomposed compounds characterization was performed on Agilent 7820 GC gas chromatograph equipped with a mass spectrometer operating in the electron-impact mode (EI 70eV). An HP-1 MS capillary column (30 m x 250 μm x 0.25 μm) with 100% dimethylpolysiloxane was installed. The column was temperature programmed according to the similar procedure described in the previous section. The NIST Mass Spectral Search Program and the relative references were used to identify the compounds.

3.3.4 Statistical analysis

One-way analysis of variance (ANOVA) model was built on the experimental data of terpin hydrate synthesis under R version 3.6.1. The normality and equal variance assumptions for the model were checked. The statistically significant factors were identified based on the p-value at the alpha level of 0.05.

3.3.5 Basic technical-economic analysis

To evaluate the economic potential of converting the pine residues into a value-added product, the experimental data from steam distillation and terpin hydrate synthesis were used.

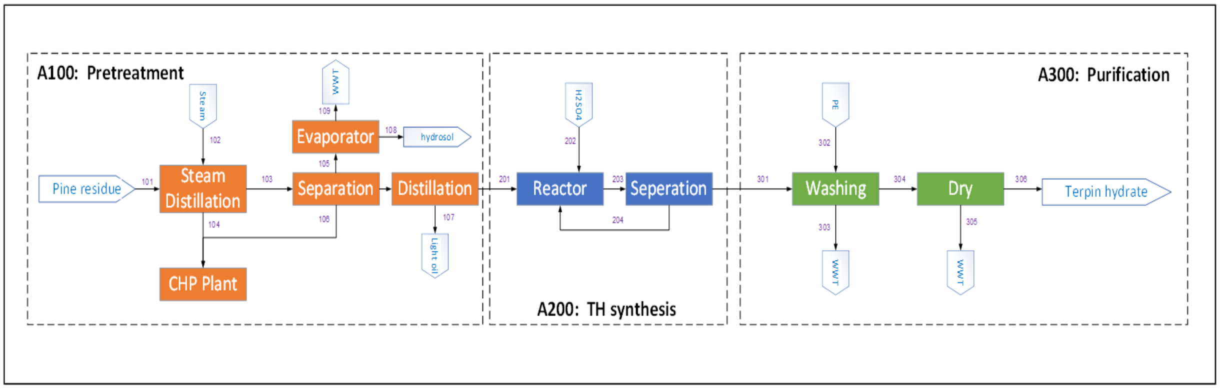

Figure 3.3 Process flow charts of loblolly pine residue utilization.

Figure 3.3 shows the industrial process model, which was built on three sections. The first section (A100) is the pretreatment of the feedstock materials. The essential oil is extracted from the residues by steam distillation. After that, the received fraction is separated into wasted materials, essential oil, and an aqueous stream. The hydrosol, which contains the water-soluble phenolic constituents, is concentrated by evaporation. The essential oil stream is further distilled to separate the α-pinene and β-pinene. The unused fraction can be sold as by-products. The extracted residues are sent to combined heat and power plant (CHP) to combust for energy. The second section (A200) is the terpin hydrate synthesis process. The production of terpin hydrate follows the same steps designed for the experiment. The acid-catalyzed hydration happens in the reactor. Then, the effluent mixed with the product, impurities, and the wasted acid are separated. The raw terpin hydrate obtained from filtration is washed and dried in the lase purification section (A300). Assumptions made for the economic analysis are listed in table 3.1. The capital investments, variable operating costs, and fixed costs are estimated according to current market values (https://www.alibaba.com/) and other studies (Pokharel et al., 2019; US DoE, 2011). The main process parameters are listed in table 3.2. A discount cash flow rate of return spreadsheet was used to calculate the internal rate of return (IRR).

Table 3.1 General assumptions for the economic analysis model

General Assumptions	
Project year	2021
Plant capacity	500 dry metric tons of pine residue/day (1000 green metric tons of pine residue/day)
Plant life	30 years
Federal tax rate	21%
Financing	40% equity
Loan terms (years)	10
Loan interest	8%
Project contingency	30%
Construction period (years)	3
Plant Operating days/year	350

Table 3.2 Main process parameters for the economic analysis model

Process Parameters		
Steam Distillation & Separation	Feed flowrate	500 dry mt/d
	Steam used	1000 mt/d
	α-pinene mass fraction in light oil	50%
	α-pinene recovery from separation	0.95
	Organic chemicals concentration in hydrosol	10%
Terpin Hydrate Synthesis	Terpin hydrate yield	70%
	Terpin hydrate selectivity	90%
	Solid mass fraction after separation	40%
	Sulfuric acid usage (mass% to α-pinene)	171%
	Tween mixture usage (mass% to α-pinene)	0.13%
Purification	Washing removed efficiency	90%
	Terpin hydrate loss in the wash (mass%)	5.00%
	Petroleum ether usage (mass% to terpin hydrate)	97%
	Terpin hydrate decomposition in the drying process	5%

CHAPTER 4 Results and Discussion

Using the method described in Chapter 3, a series of experiments were conducted to better understand 1) the variations in the chemical composition of extractives recovered from different biomass fractions, 2) the effects of different extraction methods, and 3) the potential for converting specific extractives to higher-value chemicals.

4.1 Effect of pine feedstock on extractives

4.1.1 Soxhlet extraction

To better understand the effectiveness of the pine feedstocks on the composition of the extractive exhaustive Soxhlet extraction was performed. Two solvents, ethanol and methyl isobutyl ketone (MIBK) were used for these extractions.

The solvent extractable for the fractions from Tree A and Tree D were compared in Figure 4.1. Overall, the extractables from the older tree, tree A, are higher than those from the younger tree D. As expected, there are no significant differences in the yield of extractives recovered from the needle and branch fractions from tree A and tree D. Tree A branch has a similar weight loss percent using different solvents, while the tree D needle has no significant difference in extractives' percent. Generally, extraction using ethanol as the solvent resulted in a higher weight loss and extractives' percent. It is reasonable because ethanol's relative polarity (compared to water polarity) is 0.654 higher than MIBK 0.412 (Snyder et al., 2010; Welton & Reichardt, 2010). Although the more polar solvent leads to a lower yield of phenolic substances, the total yield is higher with more potent antioxidant properties of the chemical compounds (Nawaz et al., 2020).

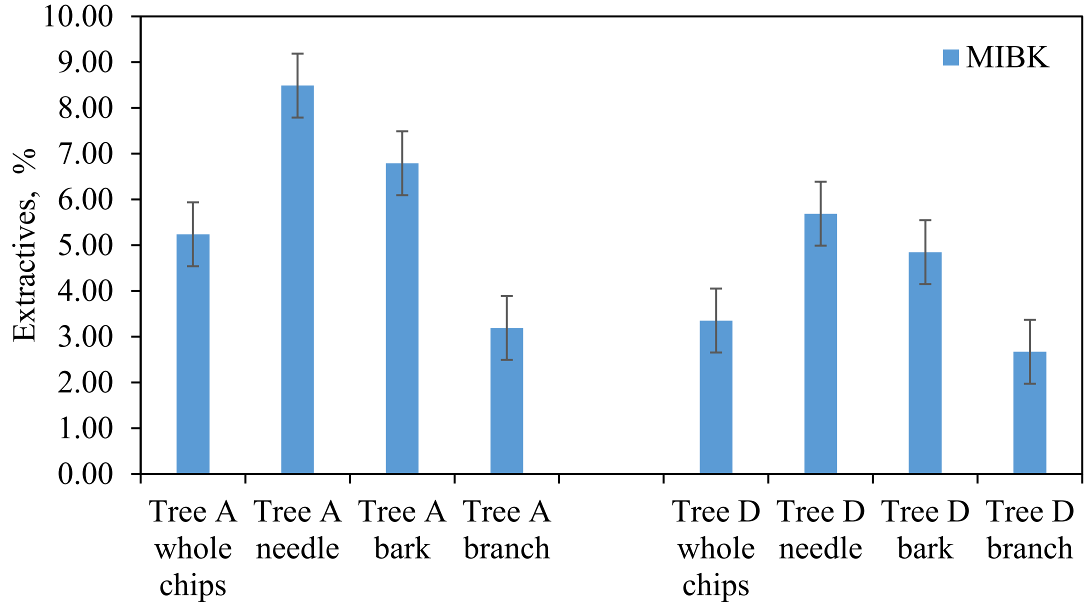

Figure 4.1 Extractive percent comparison between different fractions and trees [MIBK].

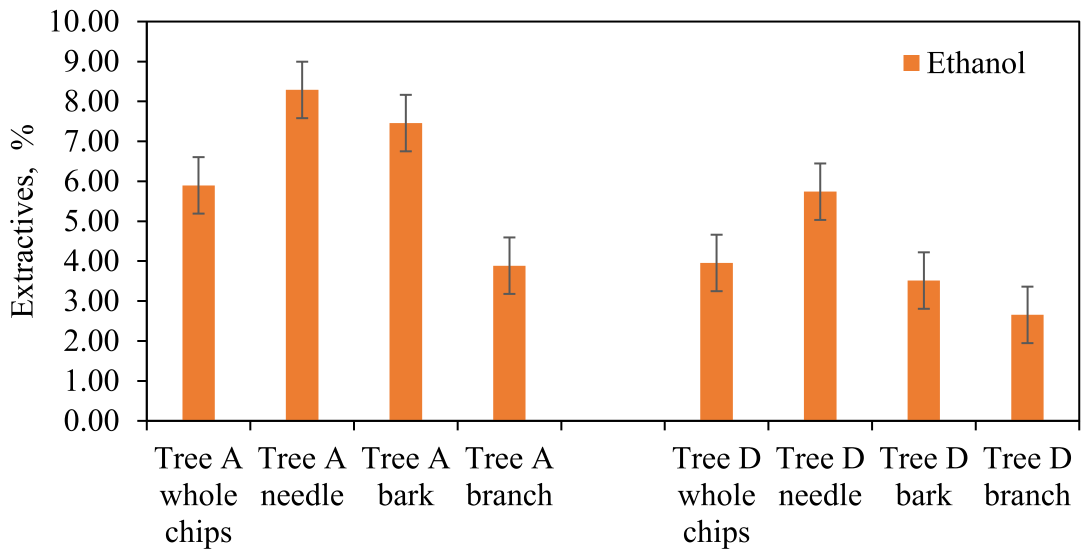

Figure 4.2. Extractive percent comparison between different fractions and trees [Ethanol].

Table 4.1 Analysis of variance for the extractive percent

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Response Extractives					
fractions	**1**	**0.3270**	**0.3270**	**9.9267**	**0.0031**
ages	**1**	**0.6646**	**0.6646**	**20.1747**	**0.0001**
solvent	1	0.0039	0.0039	0.1199	0.7310
fractions:ages	1	0.0078	0.0078	0.2358	0.6299
fractions:solvent	1	0.0029	0.0029	0.0871	0.7694
ages:solvent	1	0.0155	0.0155	0.4718	0.4961
fractions:ages:solvent	1	0.0124	0.0124	0.3777	0.5423
Residuals	40	1.3176	0.0329		

Based on the results from a Universal Two-way ANOVA analysis (Table 4.1), the two-way or three-way interactions were not significant according to the p-values larger than 0.05, among the three factors: fractions, ages, and solvent. The extractive percent were statistically significant differences between the four studied fractions and the two tree ages. Because the p-values for fractions is 0.0031 and for age is 0.0001, which were both smaller than 0.05. The result did not provide enough evidence to make a distinction between the two types of solvents (p-value = 0.7310 > 0.05). Hence, the fraction and age of the starting materials were the most important factors related to the amount of extractive received for the Soxhlet extraction. The interactions between different factors were not necessary to be considered.

A detailed comparison of the overall mean response was completed for the eight different samples (Fig. 4.3). While the ANOVA concluded that there was little statistical difference between the solvents, ethanol tends to give a higher extractive percent for whole chips and branches, which is a meaningful feature for further experiment. Using a suitable solvent for the recovery of different fractions has laboratory and commercial reasons. These results provide valuable guidance for choosing the optimal solvent to achieve the maximum recovery of extractives and also allow for consideration of the important commercial properties of the solvents.

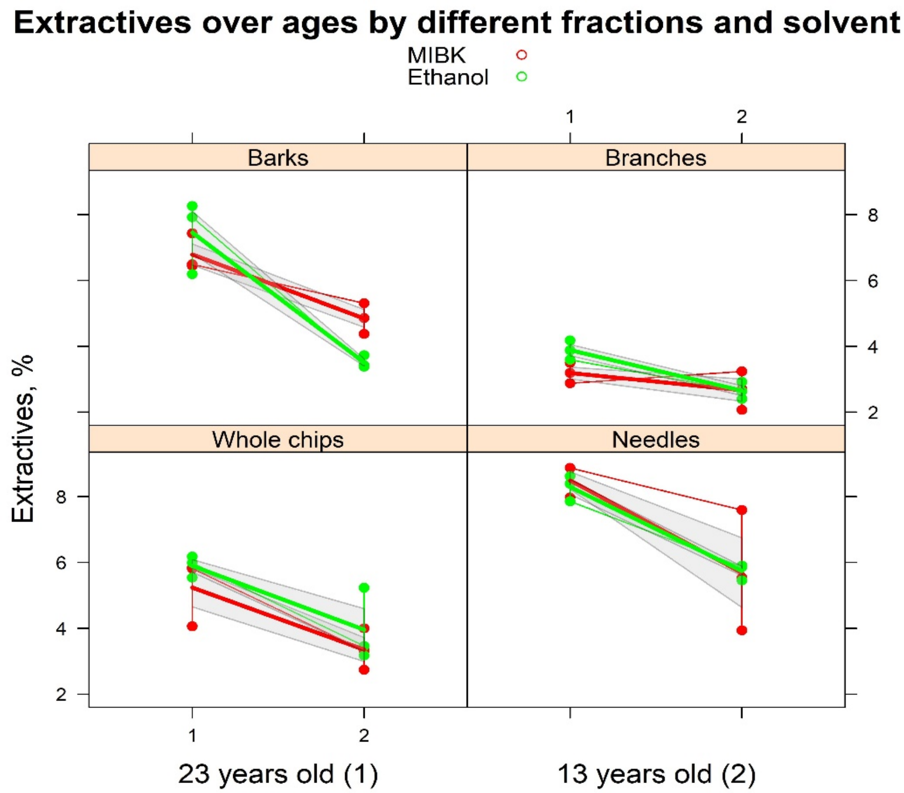

Figure 4.3 Overall mean response for the extractive percent. Grey zone indicates the 95% confidence interval for the group means.

4.1.2 Principal component analysis of extractive composition

The chemical composition of the extractives was evaluated with gas chromatography. To better understand trends in the data, principal component analysis (PCA) was carried out on the chromatograms. The normalized intensity of the chromatograms. This led to a data set that contained 48 individual experiment samples and over 30 thousand intensity values at different retention times. The PCA score plots indicate the percentage of variance explained by each principal component in the model, as well as the similarities and differences among the different pine residue fractions. The PCA loading plots are used to identify the main chemical compounds, which can represent the most important chemical compounds for the different fractions. Figure 4.4 shows that the combination of the first and second principal components explains about 65% of the total variability in the data set. The score plot in figure 4.4 shows that the samples from different fractions cluster together on PC1 and PC2. This indicates that the largest variation in the chemical composition is due to the anatomical fraction of the trees. The score plot also shows that the samples from the whole chips and branches tend to dominate PC 1, while the needle and bark samples are associated with PC2. This is reasonable since the whole chips and branches are both dominated by the wood fraction. The needle and bark fractions both have secondary metabolites. These trends are present for both trees and for both extraction solvents.

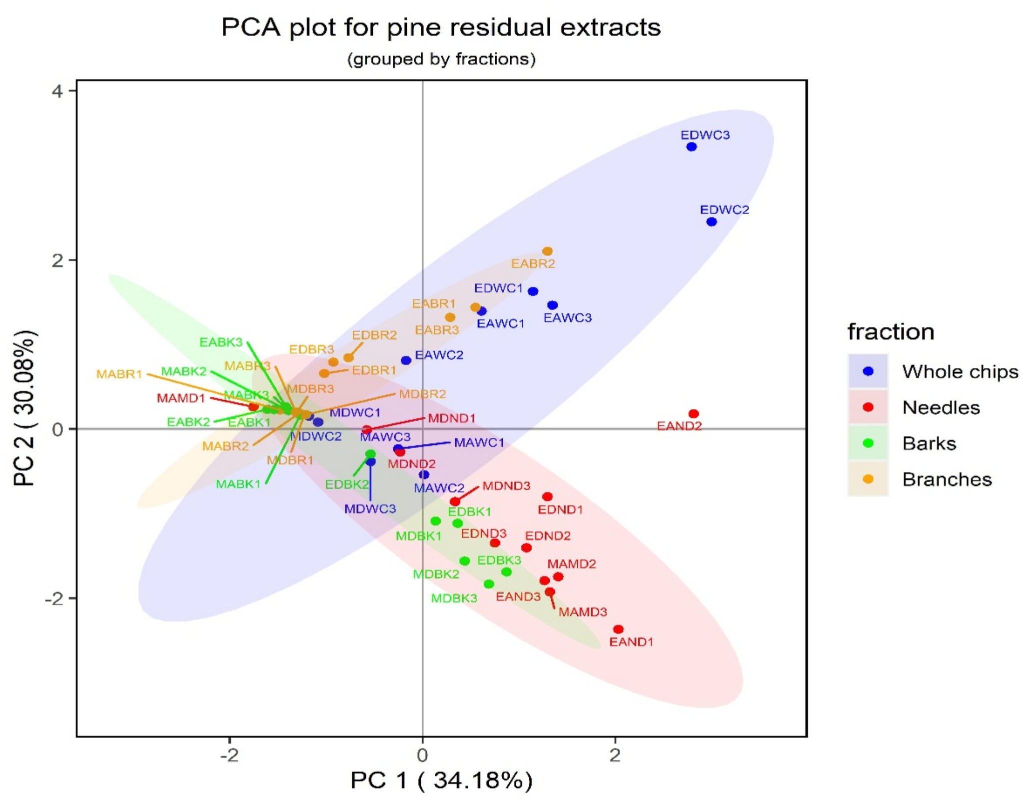

Figure 4.4 PC 1 & 2 score plot for the pine extractives. Colors indicate different fractions, and ellipses represent 95% confident intervals.

The same general conclusions can be drawn from the score plot (Figure 4.5) for PC3 and PC4, which explain 13% of the chemical variation contained in the GC traces. As expected, there is a greater overlap between all the samples since the PC3 and PC4 have less chemical detail. There is still a similar trend with overlap between the whole chips and branches, and also between needles and barks. The other variables include the ages of the tree and the different types of the extraction solvent, were also evaluated in the model. Without any significant findings, the results were not discussed here.

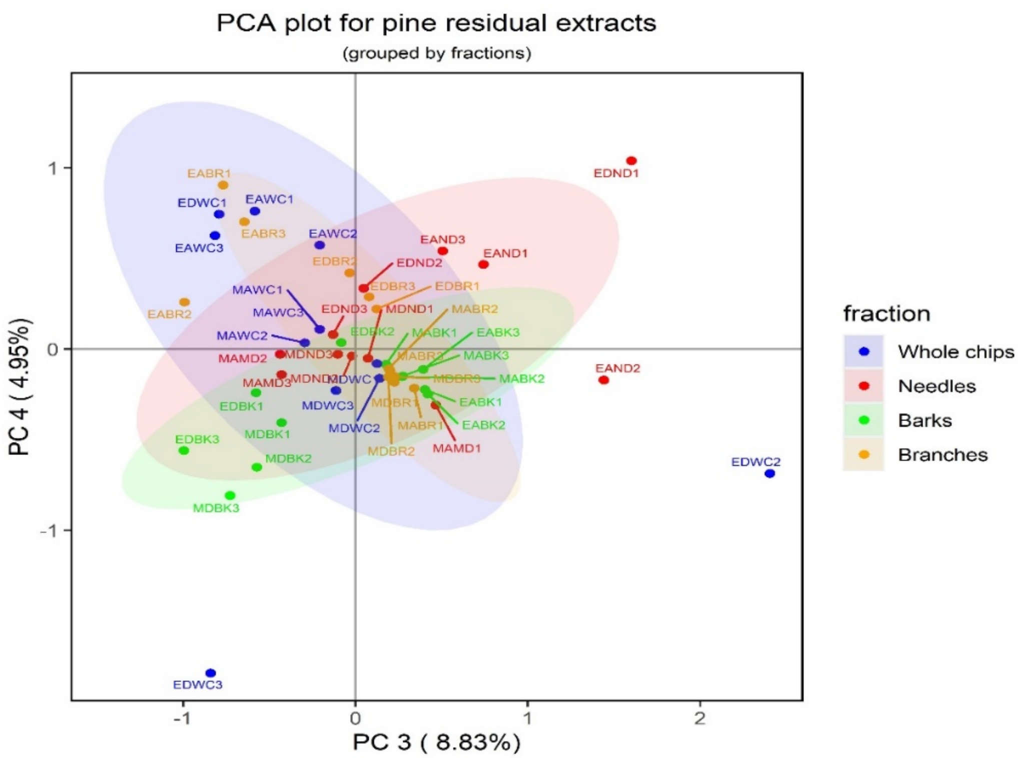

Figure 4.5 PC 3 & 4 score plot for the pine extracts. Colors indicate different fractions, and ellipses represent 95% confident intervals.

The predominant compounds that separate the samples along PC1 and PC2 were identified by evaluating the loading plots, which contain the retention times and peak information. The first twenty potential compounds with the largest peak area were identified.

The peak area and the corresponding chemical compounds were determined. Hence, a second PCA analysis was conducted using only the peak area from these 20 chemicals compounds and 47 experimental sets; sample EAND2: Tree A 23 years old needle fraction set 2 using the ethanol as solvent was removed as an outlier. Figure 4.6 shows the PC score plot for PC 1 and PC 2 from the second PCA model. The samples from the bark and branch fractions tend to negatively associate with PC 1, while the needle and whole chips fractions tend to have a positive association with PC 1. Although PC 2 cannot separate bark and branch fractions, a positive PC 2 value indicates the sample is more like the whole chips fraction, and a negative value relates to the needle fraction.

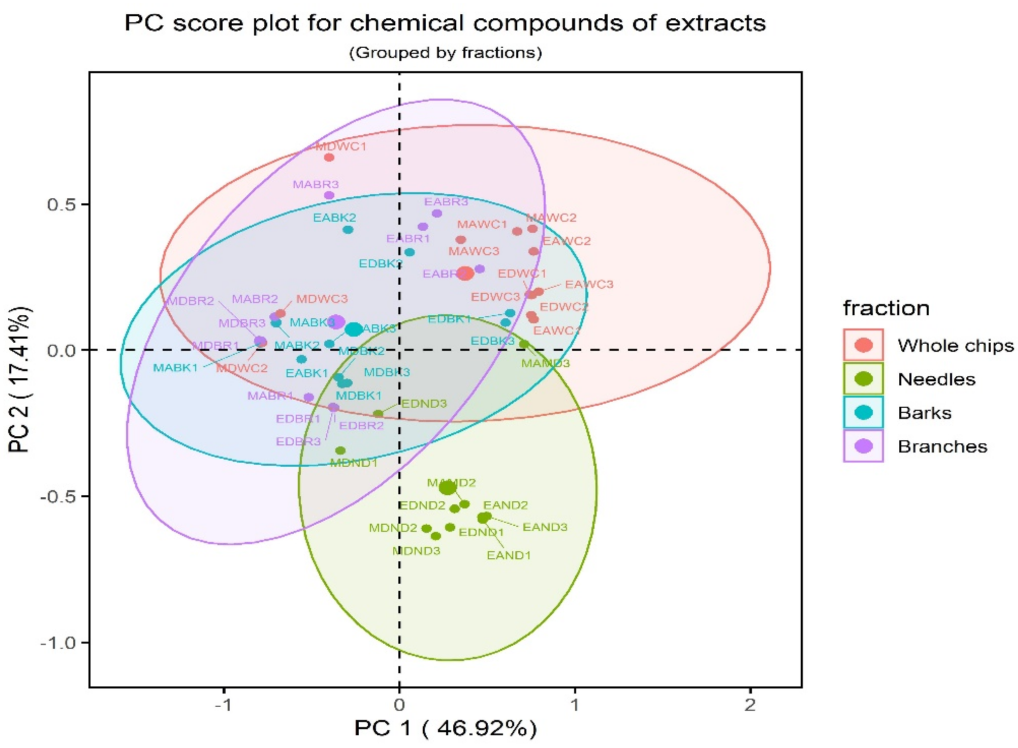

Figure 4.6 PC 1 & 2 score plot for the pine extracts. Colors indicate different fractions, and ellipses represent 95% confident intervals.

4.2 Use of steam distillation as a low-cost extraction system

Steam distillation is a simple process for the recovery of essential oils. While solvent extractions can provide a high yield of extractives, the complexity of solvent extraction, recovery and recycling all add significant capital and operating costs. Thus, a series of steam distillation extractions were conducted to evaluate the efficacy of this simple, low-cost approach.

Figure 4.7 shows the results for steam distillation for the needle, bark, and branch fractions. The wood fraction was omitted since the high cost of this feedstock would preclude its use in a commercial process. The bark fractions showed the highest yield of essential oil and the followed was the branch fraction, and the essential oil yield of the needle fraction was the lowest. Because the essential oil from needle fractions was treated to remove the moisture, this process may cause some unexpected loss of the oil.

In contrast to Soxhlet extraction, which provided many non-volatile diterpenes and triterpenes, the essential oil recovered from steam distillation was composed of a variety of monoterpene. The α-pinene and β-pinene were dominant components (Figure 4.8). Based on GC-FID analysis, α-pinene accounted for 41.2 wt.% and β-pinene for 7.5 wt.% of the essential oil isolated from the needle fraction.

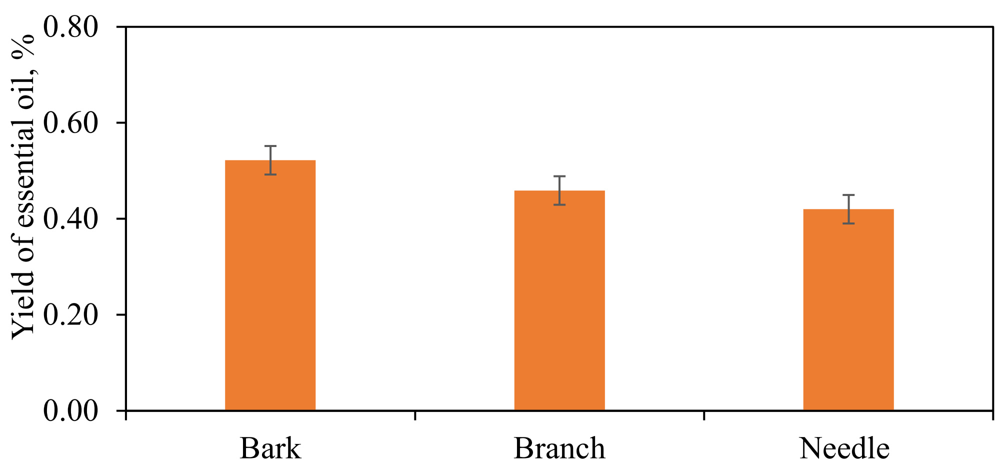

Figure 4.7 Yield of essential oil for different fractions.

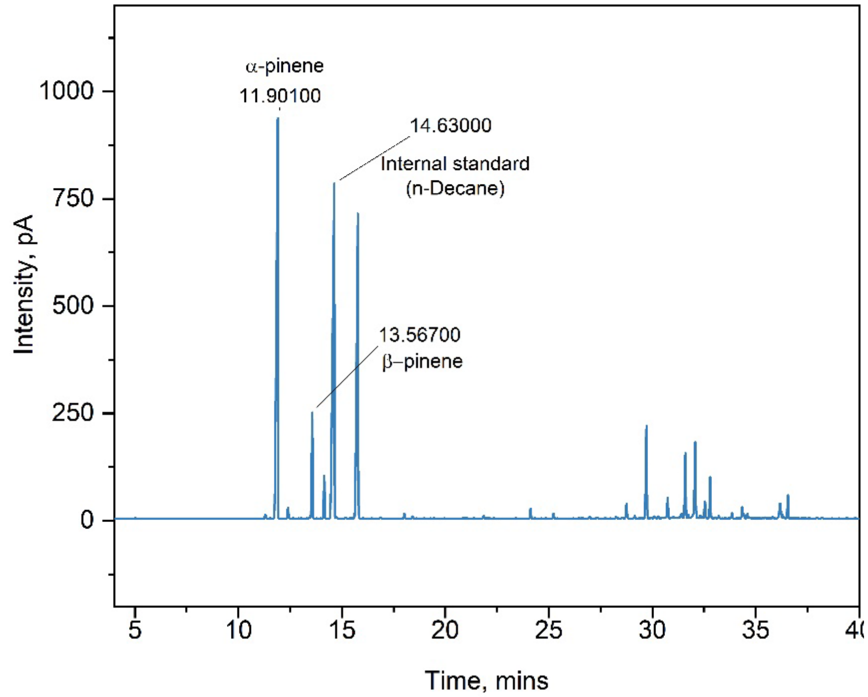

Figure 4.8 GC-FID chromatogram of steam extracts from the loblolly pine needles.

4.3 Conversion of Pinenes to Terpin Hydrate

4.3.1 Production and Analysis of Terpin Hydrate

As discussed in section 4.2, α-pinene and β-pinene were the two main constituents in the essential oil. Due to the similarity of the chemical structure and property, both α- and β-pinene can be used to produce the terpin hydrate, which can be produced via ring-opening and rearrangement followed by the addition of three molecules of water. This reaction has a series of intermediate products, as shown in figure 4.9. The synthesis of terpin hydrate is also accompanied by other side reactions, which produce a mixture of chemical components such as terpinolene and terpineol.

Because both the hydration and isomerization reactions are catalyzed by strong acid (Comelli et al., 2013), the monoterpene components can be converted into terpineol by directly adding one water molecule. This is the mechanism behind one-step α-terpinol hydration. If the dehydration reaction happens to the terpin hydrate molecule, it can be decomposed into terpineol with two water molecules lost. At the same time, the terpin can be produced by terpineol, which double bond breaks and one molecule of water is added. The relationship between terpin and terpin hydrate is quite complex, owing to their eutectic form (di Martino et al., 1999). Under the proper conditions, the two compounds can convert between each other back and forth. The numbers with red circles in figure 4.9 correspond to the chemical compound peaks in figure 4.11.

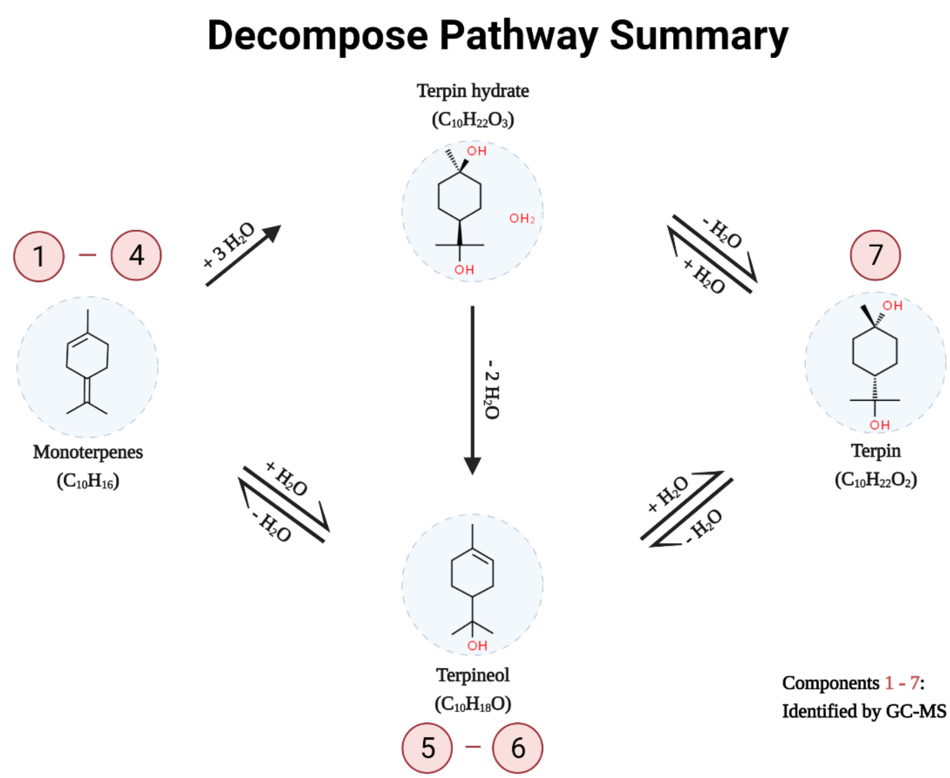

Figure 4.9 Chemical reactions during the decomposition (Created with BioRender.com.; Chemical Structure Search: http://www.chemspider.com; Hassan et al., 1985).

In order to determine the yield and purity of the desired terpin hydrate needs to be accurately identified and quantified. The terpin hydrate and intermediate products were identified and quantified by GC-MS and GC-FID, respectively. However, since the terpin hydrate was quite sensitive to temperature and residue acid, a suitable pretreatment was critical to successfully quality and quantity the terpin hydrate product. Figure 4.10 shows that the chromatogram of the starting pinene, the terpin hydrate known, and the crude reaction product. One of the standard methods to avoid the influence of degradation is recrystallization. The terpin hydrate product was recrystallized by absolute alcohol several times before preparing the samples for the GC test. However, based on this method, it is not reasonable to claim the terpin hydrate was synthesized since other reactions might happen during the process.

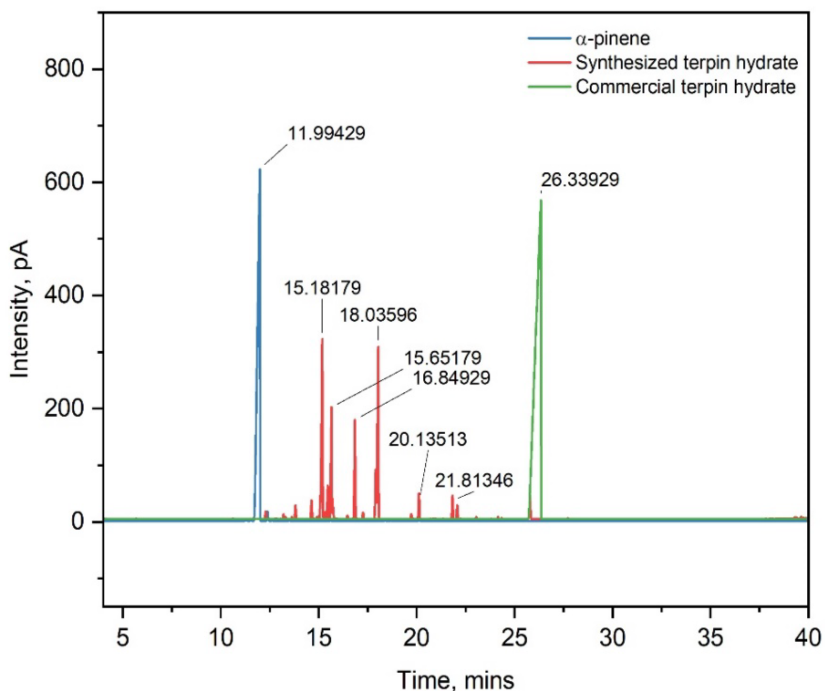

Figure 4.10 GC-FID chromatogram of the commercial α-pinene, the synthesized terpin hydrate, and commercial terpin hydrate.

Figure 4.11 shows that the commercial terpin hydrate peak was almost fully decomposed with trace acid and resulted in a similar chromatogram to the crude terpin hydrate product. This result verified that even traces of residual acid could degrade the terpin hydrate. The products from the decomposition of terpin hydrate were identified as 1: Terpinolene, 2: D-limonene, 3: γ-Terpinene, 4: (+)-4-Carene, 5: β-Terpinol, 6: α-Terpinol, and 7: Terpin. The potential chemical reactions that happened during the decomposition process have been illustrated in figure 4.9. These decomposition products would cause the terpin hydrate yield to be underestimated.

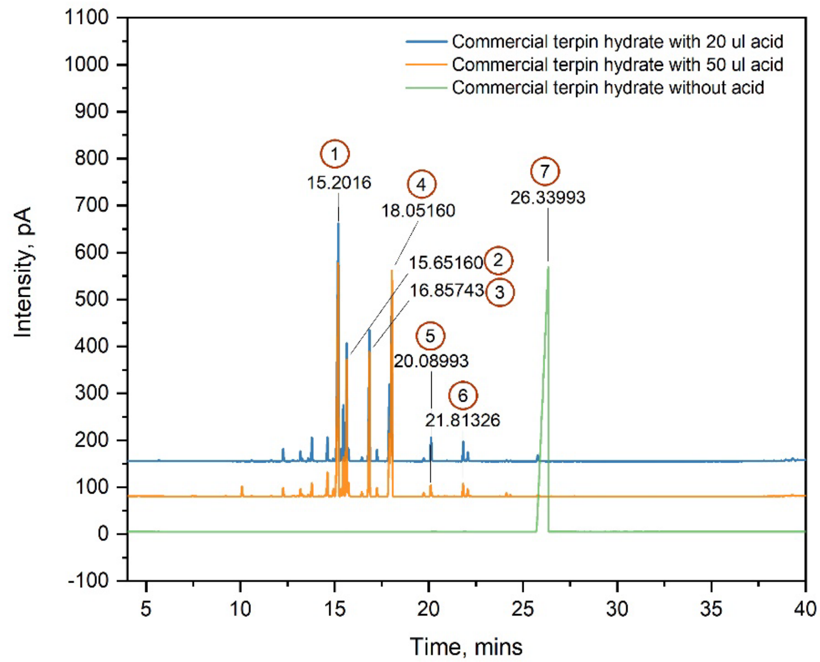

Figure 4.11 Effects of residue acid on the stability of terpin hydrate.

Hence, an alternative pretreatment procedure was needed to remove the acid prior to the final product recovery. Neutralizing the crude reaction product was the simplest potential solution. To evaluate this option, the crude terpin hydrate was washed with 3% sodium hydrate solution instead of petroleum ether. As shown in figure 4.12, terpin hydrate was found in the crude mixture, but numerous degradation products remained.

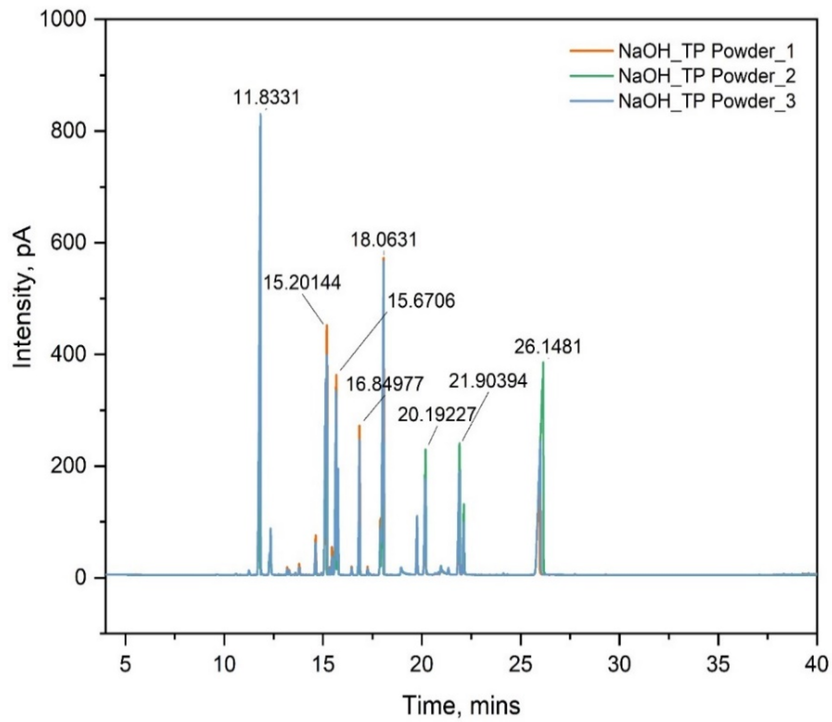

Figure 4.12 GC-FID chromatogram of the terpin hydrate washed by sodium hydrate solution.

An alternative for removing the residue acid was developed. A two-phase system consisting of saturated sodium chloride and chloroform was used to extract the desired terpin hydrate product from the intermediate products in the crude reaction mixture. The shaking and centrifugation were used to assist in the separation of the layers.

Terpin hydrate would lose one molecule of water before showing the exact peak. Anhydrous terpin was made as a reference and the residual moisture was verified by TGA. The synthesized terpin hydrate showed one prominent peak at retention time around 25.8 min, comparing figures 4.13 and 4.14. There was no peak of degraded compounds for the synthesized terpin hydrate. Thus, this procedure could be used for the terpin hydrate qualification and quantification. The recovery rate for the commercial terpin hydrate was 89.6% on average, which was higher than the synthesized terpin hydrate with 48.7%.

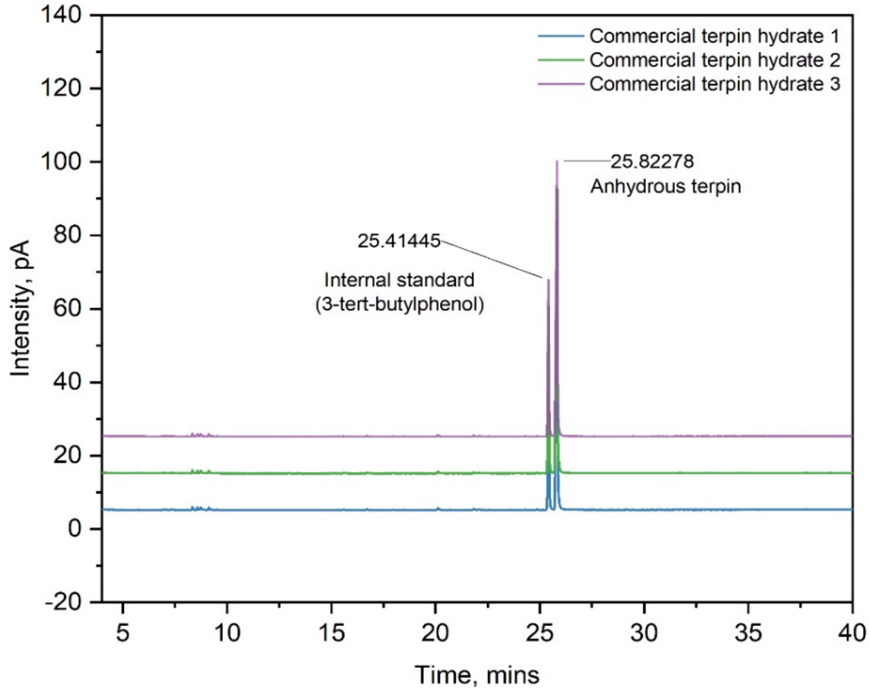

Figure 4.13 GC-FID chromatogram of the commercial terpin hydrate product.

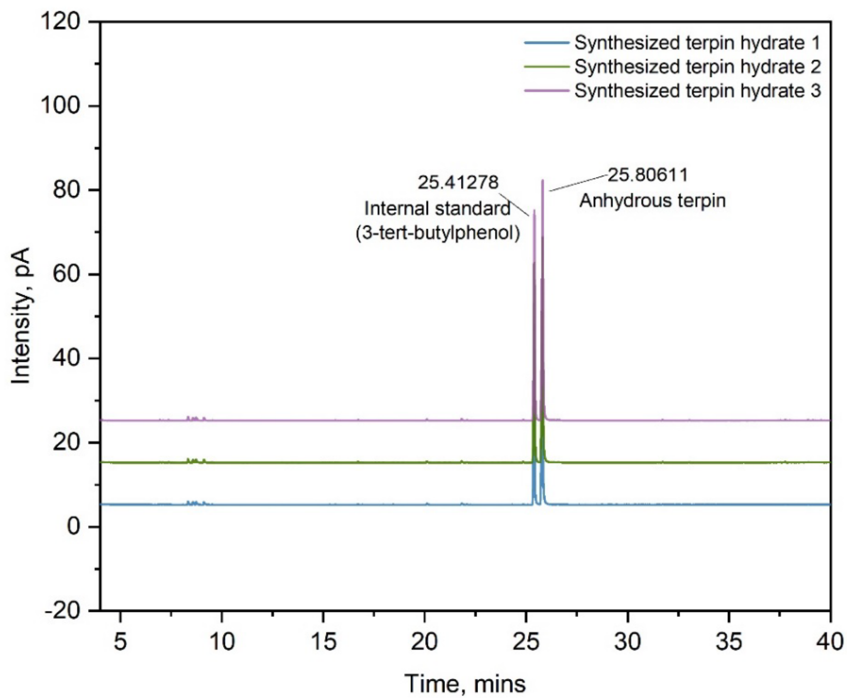

Figure 4.14 GC-FID chromatogram of the pretreated terpin hydrate product.

4.3.2 Optimization of Terpin Hydrate Synthesis Process

With a reliable process for recovery and quantification of the terpin hydrate product, the effects of the reactions' conditions could be evaluated. Based on the literature three variables were evaluated 1) the reaction temperature, 2) acid concentration and 3) the amount of Tween surfactant. Figure 4.15 shows the mean and variance in the yield for these different experimental conditions. The acid concentration was the most significant variable associated with the yield of terpin hydrate. Increasing the temperature could improve the yield of terpin hydrate while adding more Tween mixture had negative effects on the terpin hydrate yield.

Based on the average yields between the 30 wt.% and the 35 wt.% sulfuric acid, the higher acid concentration gave higher yield and lower variance. A higher yield is obtained at 30°C relatives to 24°C. The higher temperature increased the reaction rate and may have also increased the effectiveness of the Tween surfactant. The samples run at 24 °C showed the largest variance. A higher yield was achieved with lower levels of Tween. The emulsifier was intended to help two layers mixture as a homogeneous solution. This character is good for the reaction, while it would be problematic for terpin hydrate separation. After standing still in the refrigerator for hours, the crystallization of the terpin hydrate made it easily separated as crystals. The emulsifier's effect could either retard or accelerate the crystallization (Rizzo et al., 2015). The research from Fuller et al. (2018) reported that heterogeneous interfacial nucleation needs similarity of chemical structure between the emulsifier and the chemical compounds in the dispersed phase. However, the terpin hydrate product does not have a structure with alkane chains. Therefore, the short-chain saturate Tween 20 molecule should have a similar performance as the long-chain unsaturated Tween 80, which is not able to promote the crystallization of the terpin hydrate (Fuller et al., 2018). The suspension would take out part of the terpin hydrate during the layer separation operation, decreasing the yield of aimed product. Under this terpin hydrate reaction system, the too emulsifier would be a harmful factor reducing the yield of the terpin hydrate.

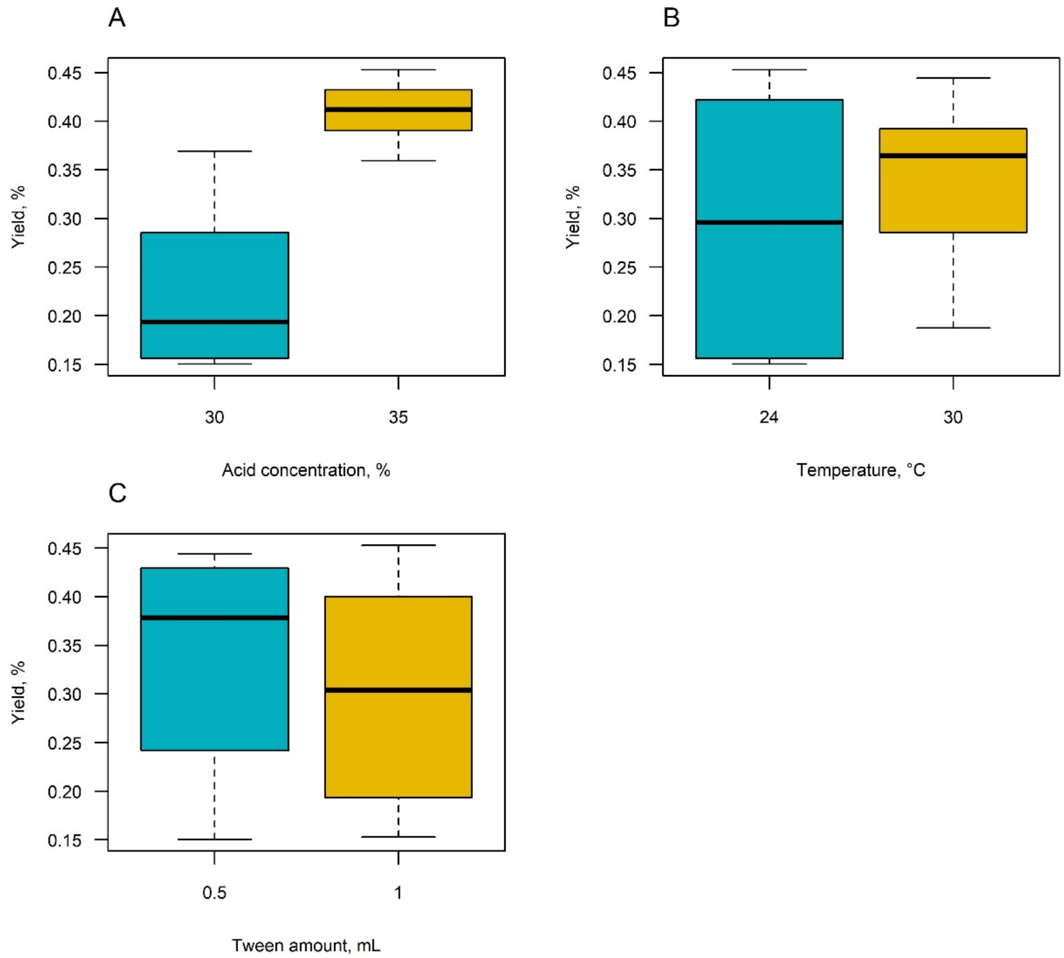

Figure 4.15 Mean yield of terpin hydrate under different experimental conditions (acid amounts, temperatures, and Tween mixture amounts).

The interaction between the experimental factors is shown in figure 4.16. Figure 4.16 A shows the interaction between the acid concentration and the reaction temperature. This shows that for both temperatures increasing the acid concentration increased in the yield. And increasing the temperature had a greater impact on the terpin hydrate yield when a low acid concentration is used. By contrast, when high acid concentration is used, increasing the reaction temperature decreases the yield of terpin hydrate slightly. Hence, to achieve the highest yield of terpin hydrate, a high acid concentration and a low temperature should be used. Figure 4.16 B shows little interaction between acid concentration and Tween mixture usage since the two lines are almost parallel. Figure 4.16 C shows the interaction between temperature and the tween mixture amount. Under a low temperature, the terpin hydrate yield increases with the increasing of the Tween mixture usage. However, if the reaction is conducted under a high temperature, increasing the Tween mixture amount reduces the yield of terpin hydrate.

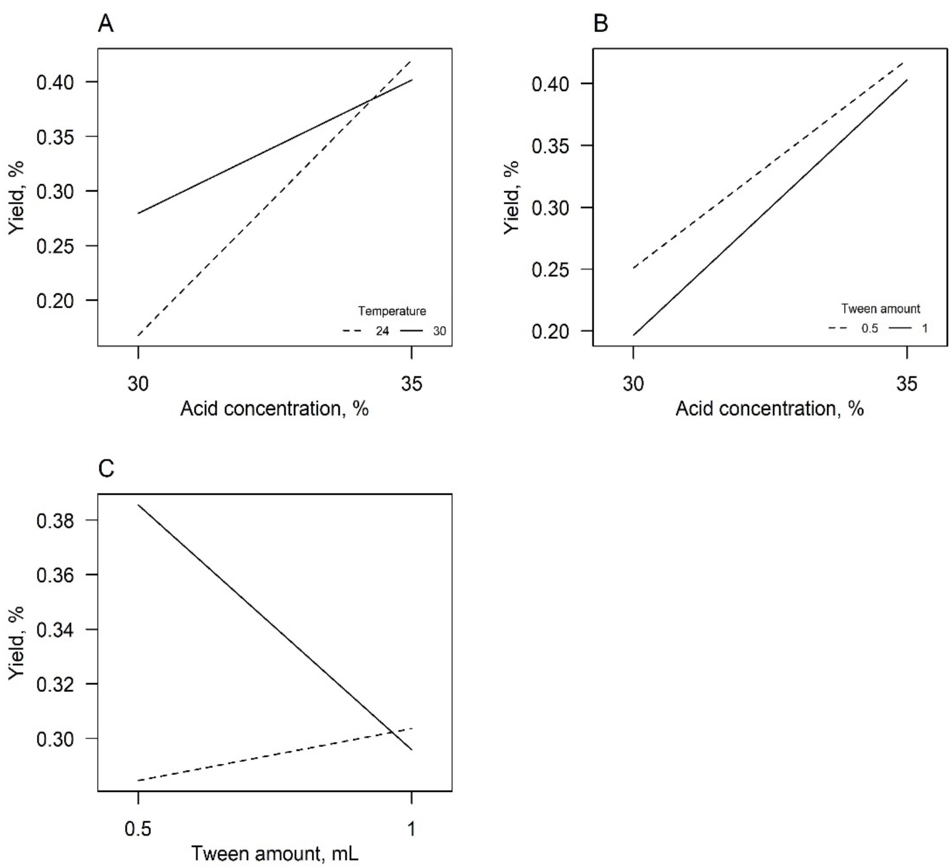

Figure 4.16 Plots of the interactions between different experimental conditions.

Further investigation of the acid concentration effect was conducted by fixing the other experimental conditions and varying only the acid concentration. Figure 4.17 shows the yield of terpin hydrate under different acid concentrations. Although the terpin hydrate yield increased when acid concentration increased from 30% to 35%, the yield dropped dramatically when acid concentration increased from 35% to 45%. The acid-catalyzed hydration and the isomerization of α-pinene happened simultaneously (Comelli et al., 2013). It appears that the isomerization became the prominent reaction under a high acid concentration. Therefore, the low to medium acid concentrations are more suitable for the hydration reaction.

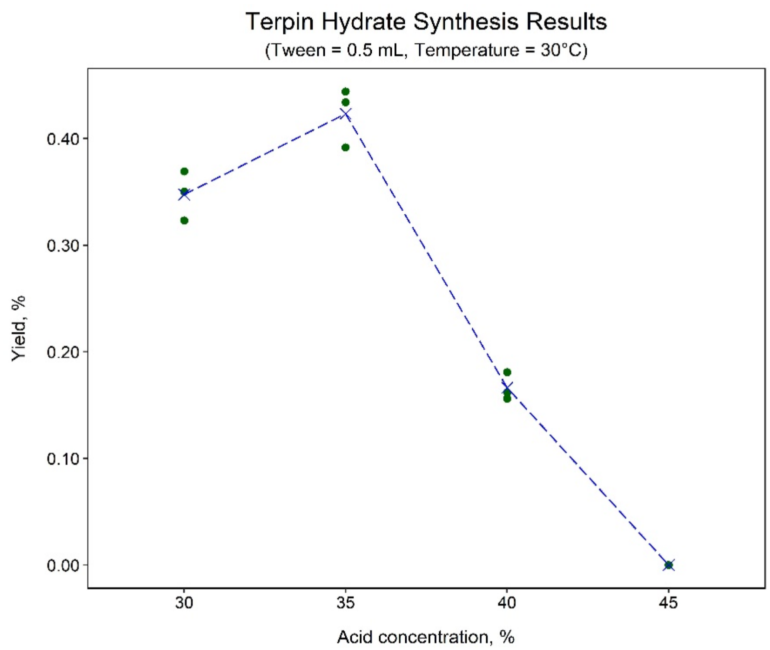

Figure 4.17 Acid concentration effects for the yield of terpin hydrate.

An intermediate level of Tween was also explored. The result is shown in figure 4.18. When using 0.75 mL Tween mixture and 35% acid concentration, the reaction achieved a relatively high yield at 30°C. However, the highest yield remained at 24°C with 1 mL Tween mixture, 35% sulfuric acid. Table 4.2 shows the ANOVA test results. The main effects for acid concentration, temperature, and the Tween mixture amount, together with interactions for acid concentration and temperature, as well as for the temperature and Tween mixture, are all statistically significant according to the p-values, based on the p-value (p-value = 0.1146 > 0.05) of the high order three-way interactions, there is no evidence for the interaction among acid, Tween amount, and temperature. Meanwhile, the interaction of acid concentration and Tween amount shows a large p-value (p-value = 0.3987 > 0.05) which indicates the interaction between acid and Tween mixture amount is not statistically significant. Thus, the three-way interaction and the interaction term of acid and Tween mixture amount were removed from the model, refitting the model. The result of the reduced model is summarized in table 4.3. With p-values smaller than 0.05, the reduced model contained all the experimental conditions that influence the terpin hydrate yield.

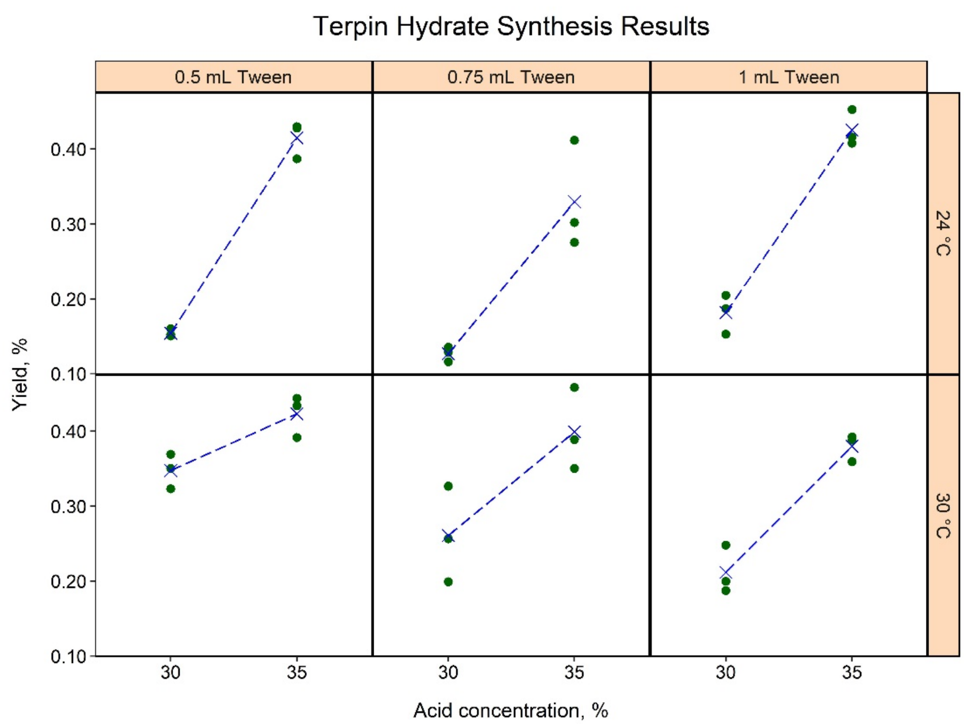

Figure 4.18 Plots of the yield under different experimental conditions.

Table 4.2 Analysis of variance for terpin hydrate yield [Full model]

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Acid	**1**	**0.2972**	**0.2972**	**209.7479**	**0.0000**
Temp	**1**	**0.0380**	**0.0380**	**26.8032**	**0.0000**
Tween	**2**	**0.0192**	**0.0096**	**6.7796**	**0.0046**
Acid:Temp	**1**	**0.0264**	**0.0264**	**18.6492**	**0.0002**
Acid:Tween	2	0.0027	0.0014	0.9556	0.3987
Temp:Tween	**2**	**0.0238**	**0.0119**	**8.3899**	**0.0017**
Acid:Temp:Tween	2	0.0067	0.0033	2.3559	0.1164
Residuals	24	0.0340	0.0014	NA	NA

Table 4.3 Analysis of variance for terpin hydrate yield [Reduced model]

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Acid	**1**	**0.2972**	**0.2972**	**191.7820**	**0.0000**
Temp	**1**	**0.0380**	**0.0380**	**24.5074**	**0.0000**
Tween	**2**	**0.0192**	**0.0096**	**6.1989**	**0.0059**
Acid:Temp	**1**	**0.0264**	**0.0264**	**17.0518**	**0.0003**
Temp:Tween	**2**	**0.0238**	**0.0119**	**7.6713**	**0.0022**
Residuals	28	0.0434	0.0015	NA	NA

4.3.3 Basic economic analysis

In order to demonstrate the economic potential of this process, a techno-economic model with detailed mass and energy balances as well as excel-based discounted cash flow rate of return (DCFROR) calculations is developed. Table 4.4 shows the breakdown analysis of the total capital investment. Since the production of the essential oil requires a large amount of steam, a high cost comes from the energy cost for generating the steam, while the equipment installation for the steam distillation and separation accounts for the highest cost of the installed cost. In comparison, the terpin hydrate production only increases a small amount for both purchased cost and the installed cost because of no heating demand of the terpin hydrate production.

Table 4.4 Breakdown analysis of the total capital investment

Total Capital Investment		
Process Area	Purchased Cost, $	Installed Cost, $
Feedstock Handling	482,445	713,840
Steam Distillation & Separation	6,772,482	13,007,996
Terpin Hydrate Synthesis	101,821	238,703
Terpin Hydrate Purification	183,392	296,194
Utility & CHP plant	3,791,743	5,721,806

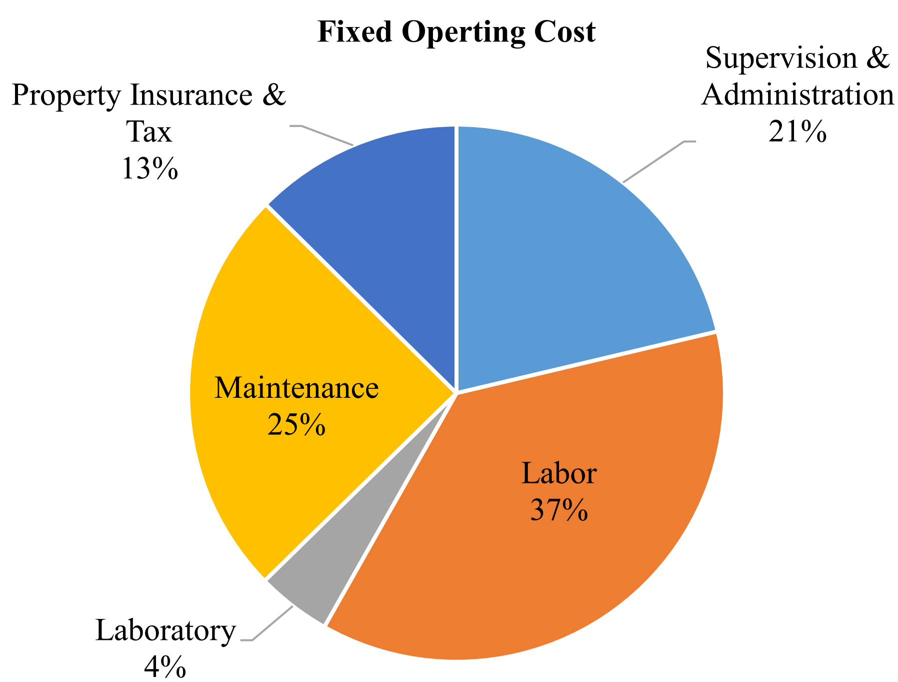

Figure 4.19 Breakdown analysis of the fixed operating cost.

Figure 4.19 shows the breakdown analysis of the fixed operating cost for the process. The costs of the raw materials can be found in table 4.5. Most of the raw materials cost is the pine residue and steam, owing to the yield of the essential oil production being quite limited.

However, the electricity released from the combustion of the used pine residues generates profit. Meanwhile, the valuable by-products, including hydrosol and the other monoterpenes, reduced the overall operating cost. The high value of the terpin hydrate product brings in a significant increase in profit. Based on the 17.7% internal return rate (IRR), converting pine residues into terpin hydrate should be a desirable and considerable pathway.

Table 4.5 Operating cost, sales credit, and IRR

Material cost, $/year	
Pine residue	4,015,958
Steam	7,344,488
Sulfuric acid	78,616
Tween mixture	734
Petroleum ether	54,238
Fixed operating cost, $/year	
Total Fixed operating Cost	3,456,411
Product sales profit, $/year	
Electricity	6,315,990
Terpin hydrate	7,036,187
Other light oil	1,288,000
Hydrosol	9,625,000
Internal rate of return (IRR) for the entire plant	**17.65%**

CHAPTER 5 Conclusion

Loblolly pine residues, needles, bark, branches, and whole wood chips were used as the feedstock for the production of value-added chemicals. Different extraction methods were evaluated, including Soxhlet extraction, with two solvents and steam distillation. The effects of the age of the original tree and the sources of the residue were evaluated. Statistical analysis was conducted to determine the factors that had an impact on the yield of extracted materials. The fraction of the pine tree had the greatest impact on the yield. In addition, a principal component analysis model was built on the extraction data set. The different residue fractions and the chemical compounds in the extractives were investigated by examining the PC score plot and the PC loading plot.

Three fractions of the residues, needles, bark, and branches were used evaluated with steam distillation. The yield of volatile essential oil was determined. GC-FID was used for the characterization of the main monoterpene components. Since the two major components, α-pinene and β-pinene, account for almost 50% of the total weight of the essential oil, further utilization was started with these two compounds.

Terpin hydrate, which is widely used in fine chemicals manufacturing, was synthesized from α-pinene. An analytical procedure to isolate and quantify the terpin hydrate was developed. Synthesis variables of acid concentration, reaction temperature, and the amount of Tween surfactant were optimized based on a factorial experimental design. The main effects of the three experimental conditions all statistically significantly influenced the yield of terpin hydrate. Apart from the main effects, there were interactions between acid concentration and temperature, the temperature, and tween mixture amount, which were examined through the ANOVA model. The economic analysis was performed for this residue utilization process. The financial benefits of the simulated industrial process were evaluated by the internal return rate, which was 17.7%.

This study has some limitations explained below. The Soxhlet extraction using organic solvent was conducted on the air-dried samples, while the steam distillation aimed at the volatile monoterpenes, which used fresh residues. This difference made the results of the two techniques incomparable. Additional studies may use fresh residues for different extraction techniques. The identification of the chemical components relied on the NIST Mass Spectral Search Program, which made uncertainty due to the low probability of the matching and the complexity of the extractives. Due to the experimental scale, the amount of essential oil obtained from steam distillation is quite limited. Thus, the heavy oil that contains the sesquiterpene, diterpene, and triterpene compounds could not be separated. The following work may design to run on a larger scale. With enough amount, the terpin hydrate synthesis would be able to directly conduct on the essential oil instead of using the pure chemical. Meanwhile, the water used in the steam distillation experiment was directly disposed of based on the assumption being made. However, the additional analysis of the water-soluble phenolic components could help to better understand and utilize the hydrosol. Although terpin hydrate synthesis is a mature process in the industry, there is still a lot unknown about the reaction mechanism. A deep understanding of the reaction mechanism could help increase the conversion percent of the raw materials in the hydration process while aiding the selectivity of the aimed chemicals. Apart from the above, the study and analysis of the terpin hydrate crystallization, recrystallization mechanism, and the emulsifier behavior in the reaction system could be meaningful for improving the yield final terpin hydrate product. For economic impacts, there are several uncertainties have been considered in this study. The sensitivity analysis may be conducted to further evaluate the economic impacts of the industrial process.

REFERENCES

Abdel-Raouf, M. E., & Abdul-Raheim, A. M. (2018). Rosin: Chemistry, derivatives, and applications: A review. *BAOJ Chemistry*, *4*(1).

Acquah, G. E. (2010, December). *Characterization of Forest Harvest Residue from the Great Lakes-St Lawrence Forests of South-eastern Ontario*. University of Toronto. http://hdl.handle.net/1807/25399

Acquah, G. E., Via, B. K., Fasina, O. O., & Eckhardt, L. G. (2015). Non-destructive prediction of the properties of forest biomass for chemical and bioenergy applications using near infrared spectroscopy. *Journal of Near Infrared Spectroscopy*, *23*(2), 93–102. https://doi.org/10.1255/jnirs.1153

Adams, J., Gibson, K. E., Martin, E. M., Almeida, G., Ricke, S. C., Frederick, N., & Carrier, D. J. (2014). Characterization and variation of essential oil from *Pinus taeda* and antimicrobial effects against antibiotic-resistant and -susceptible *Staphylococcus aureus*. *Forest Products Journal*, *64*(5–6), 161–165. https://doi.org/10.13073/fpj-d-14-00018

Albuquerque, B. R., Prieto, M., Barreiro, M. F., Rodrigues, A., Curran, T. P., Barros, L., & Ferreira, I. C. (2017). Catechin-based extract optimization obtained from *Arbutus unedo L.* fruits using maceration/microwave/ultrasound extraction techniques. *Industrial Crops and Products*, *95*, 404–415. https://doi.org/10.1016/j.indcrop.2016.10.050

American Society for Testing and Materials. (2019). *Standard Test Methods for Composition of Turpentine and Related Terpene Products by Capillary Gas Chromatography* (ASTM D6387-19). ASTM International, West Conshohocken, PA. https://doi.org/10.1520/D6387-19

Arshadi, M., & Gref, R. (2005). Emission of volatile organic compounds from softwood pellets during storage. *Forest Products Journal*, *55*(12), 132–135.

Azmir, J., Zaidul, I., Rahman, M., Sharif, K., Mohamed, A., Sahena, F., Jahurul, M., Ghafoor, K., Norulaini, N., & Omar, A. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, *117*(4), 426–436. https://doi.org/10.1016/j.jfoodeng.2013.01.014

Biorenewables Education Laboratory. (2011). *Essential Oils from Steam Distillation Student Guide*. https://www.engineering.iastate.edu/brl/files/2011/10/brl_essentialoils.pdf

Charles, D. J., & Simon, J. E. (1990). Comparison of extraction methods for the rapid determination of essential oil content and composition of basil. *Journal of the American Society for Horticultural Science*, *115*(3), 458–462. https://doi.org/10.21273/jashs.115.3.458

Chen, Z. (2011). Research on new technology for preparation of terpine hydrate from turpentine. *Quality and Technical Supervision Research*. Published. http://en.cnki.com.cn/Article_en/CJFDTOTAL-FJXX201106015.htm [In Chinese]

Cheremisinoff, N. P. (2003). *Industrial Solvents Handbook, Revised And Expanded (Chemical Industries Book 94)* (2nd ed.). CRC Press.

Comelli, N., Avila, M., Volzone, C., & Ponzi, M. (2013). Hydration of α-pinene catalyzed by acid clays. *Open Chemistry*, *11*(5), 689–697. https://doi.org/10.2478/s11532-013-0217-4

Di Martino, P., Piva, F., Conflant, P., & Guyot-Hermann, A. M. (1999). Thermal analysis and powder X-ray diffraction study of terpin: Evidence of a eutectic ‘hydrate/anhydrous form’. *Journal of Thermal Analysis and Calorimetry*, *57*(1), 95–109. https://doi.org/10.1023/a:1010120703183

Eriksson, D., Arshadi, M., Kataria, R., & Bergsten, U. (2018). Lipophilic extractives in different tree fractions and forestry assortments of *Pinus sylvestris* due for thinning or final cutting. *Scandinavian Journal of Forest Research*, *33*(6), 594–602. https://doi.org/10.1080/02827581.2018.1434235

Feng, S., Cheng, S., Yuan, Z., Leitch, M., & Xu, C. C. (2013). Valorization of bark for chemicals and materials: A review. *Renewable and Sustainable Energy Reviews*, *26*, 560–578. https://doi.org/10.1016/j.rser.2013.06.024

Finar, I. L. (1973). *Organic Chemistry,* (6th ed.). Longman Publishing Group.

Fuller, G. T., Considine, T., MacGibbon, A., Golding, M., & Matia-Merino, L. (2018). Effect of tween emulsifiers on the shear stability of partially crystalline oil-in-water emulsions stabilized by sodium caseinate. *Food Biophysics*, *13*(1), 80–90. https://doi.org/10.1007/s11483-017-9514-3

Guo, Y., Simpson, J. R., & Pignatiello, J. J. (2007). Construction of efficient mixed-level fractional factorial designs. *Journal of Quality Technology*, *39*(3), 241–257. https://doi.org/10.1080/00224065.2007.11917691

Handrick, G. R., Duffley, R. P., Lambert, G., Murphy, J. G., Dalzell, H. C., Howes, J. F., Razdan, R. K., Martin, B. R., Harris, L. S., & Dewey, W. L. (1982). 3'-Hydroxy- and (.+-.)-3',11-dihydroxy-.DELTA.9-tetrahydrocannabinol: (THC) biologically active metabolites of .DELTA.9-tetrahydrocannabinol. *Journal of Medicinal Chemistry*, *25*(12), 1447–1450. https://doi.org/10.1021/jm00354a011

Hassan, M. M., Mossa, J. S., & Taragan, A. H. U. K. (1985). Terpin hydrate. *Analytical Profiles of Drug Substances*, 273–323. https://doi.org/10.1016/s0099-5428(08)60584-5

Huang, F., Singh, P. M., & Ragauskas, A. J. (2011). Characterization of milled wood lignin (MWL) in loblolly pine stem wood, residue, and bark. *Journal of Agricultural and Food Chemistry*, *59*(24), 12910–12916. https://doi.org/10.1021/jf202701b

Hussain, F., Chaudhry, M. N., & Batool, S. A. (2014). Assessment of key parameters in municipal solid waste management: a prerequisite for sustainability. *International Journal of Sustainable Development & World Ecology*, *21*(6), 519–525. https://doi.org/10.1080/13504509.2014.971452

Jackson, B. E. (2008, October). *Chemical, Physical, and Biological Factors Influencing Nutrient Availability and Plant Growth in a Pine Tree Substrate*. Virginia Polytechnic Institute and State University. http://hdl.handle.net/10919/29491

Koch, P. (1972). *Utilization of the Southern Pines; Agricultural Handbook 420*. USDA Forest Service: Washington, DC. https://www.srs.fs.usda.gov/pubs/ah/ah420_vol1.pdf

Kovals'skaya, S. S., Kozlov, N. G., & Tikhonova, T. S. (1989). Stereoselective synthesis of N,N′-diacyl-p-menthane-1,8-diamines. *Chemistry of Natural Compounds*, *25*(5), 552–557. https://doi.org/10.1007/bf00598074

Kubiak, E. J. (1968). Determination of terpin hydrate by gas-liquid chromatography. *Journal of Pharmaceutical Sciences*, *57*(3), 473–475. https://doi.org/10.1002/jps.2600570323

Kurlansik, L., Damon, C., & Salim, E. F. (1967). Determination of terpin hydrate by gas chromatography. *Journal of Pharmaceutical Sciences*, *56*(9), 1158–1160. https://doi.org/10.1002/jps.2600560922

Lakshmikumaran & Sridharan. (2016). *Global Impact of the Modern Pine Chemical Industry*. Pine Chemicals Association, Inc. P.O. Box 17136, Fernandina Beach, FL 32035 USA.

https://cdn.ymaws.com/www.pinechemicals.org/resource/resmgr/Studies/PCA-_Global_Impact_of_the_Mo.pdf

Lee, L. L., Niknafs, N., Hancocks, R. D., & Norton, I. T. (2013). Emulsification: Mechanistic understanding. *Trends in Food Science & Technology*, *31*(1), 72–78. https://doi.org/10.1016/j.tifs.2012.08.006

Li, Y., Li, S., Yin, G., & Zheng, M. (2009). Hydration of pinene and analysis of its by-products. *Guangdong Chemical Industry*, *7*. http://en.cnki.com.cn/Article_en/CJFDTOTAL-GDHG200907090.htm [In Chinese]

Liu, X., Xin, W., & Zhang, J. (2009). Rosin-based acid anhydrides as alternatives to petrochemical curing agents. *Green Chemistry*, *11*(7), 1018. https://doi.org/10.1039/b903955d

Loxley, T. A. (2018, May). *Within-tree Fuel Quality of Loblolly Pine (Pinus taeda)*. Auburn University. https://etd.auburn.edu/bitstream/handle/10415/6072/thesis_TALoxley.pdf?sequence=2&isAllowed=n

Megalos, M., Parajuli, R., & Lambert, C. (2019, January 1). *Managing Forests for Biomass Potential*. NC State Extension Publications. Retrieved October 11, 2021, from https://content.ces.ncsu.edu/managing-forests-for-biomass-potential#section_heading_13072

Mensah, J. (2019). Sustainable development: Meaning, history, principles, pillars, and implications for human action: Literature review. *Cogent Social Sciences*, *5*(1). https://doi.org/10.1080/23311886.2019.1653531

Mitchell, P. W. D., & Sasser, D. E. (1987). *Improved method of preparing cineoles* (EP0270023A1). European Patent Office. https://patents.google.com/patent/EP0270023A1/en

Mohammad Azmin, S. N. H., Abdul Manan, Z., Wan Alwi, S. R., Chua, L. S., Mustaffa, A. A., & Yunus, N. A. (2016). Herbal processing and extraction technologies. *Separation & Purification Reviews*, *45*(4), 305–320. https://doi.org/10.1080/15422119.2016.1145395

Nawaz, H., Shad, M. A., Rehman, N., Andaleeb, H., & Ullah, N. (2020). Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (Phaseolus vulgaris) seeds. *Brazilian Journal of Pharmaceutical Sciences*, *56*. https://doi.org/10.1590/s2175-97902019000417129

Oreopoulou, A., Tsimogiannis, D., & Oreopoulou, V. (2019). Extraction of polyphenols from aromatic and medicinal plants: An overview of the methods and the effect of extraction parameters. *Polyphenols in Plants*, 243–259. https://doi.org/10.1016/b978-0-12-813768-0.00025-6

ÖRså, F., & Holmbom, B. (1994). A convenient method for determination of wood extractives in papermaking process waters and effluents. *Journal of Pulp and Paper Science*, *20*(12), 361–366.

Osborne, N. (2013, January 1). *Woody Biomass Utilization Study* [Slides]. NC State Extension Publications. https://content.ces.ncsu.edu/woody-biomass-utilization-study

Pilz, S., & Bart, H. (2011). *Industrial Scale Natural Products Extraction* (1st ed.). Wiley-VCH.

Pokharel, R., Grala, R. K., Latta, G. S., Grebner, D. L., Grado, S. C., & Poudel, J. (2019). Availability of logging residues and likelihood of their utilization for electricity production in the US south. *Journal of Forestry*, *117*(6), 543–559. https://doi.org/10.1093/jofore/fvz047

Prakoso, T., Hanley, J., Soebianta, M. N., Soerawidjaja, T. H., & Indarto, A. (2017). Synthesis of terpineol from α-Pinene using low-price acid catalyst. *Catalysis Letters*, *148*(2), 725–731. https://doi.org/10.1007/s10562-017-2267-2

Restrepo, H. I., Bullock, B. P., & Montes, C. R. (2019). Growth and yield drivers of loblolly pine in the southeastern U.S.: A meta-analysis. *Forest Ecology and Management*, *435*, 205–218. https://doi.org/10.1016/j.foreco.2018.12.007

Rizzo, G., Norton, J., & Norton, I. (2015). Emulsifier effects on fat crystallisation. *Food Structure*, *4*, 27–33. https://doi.org/10.1016/j.foostr.2014.11.002

Saeidnia, S. (2014). Turpentine. *Encyclopedia of Toxicology*, 860–865. https://doi.org/10.1016/b978-0-12-386454-3.01034-4

Scandinavian Pulp, Paper and Board Testing Committee. (2003). *Wood Chips for Pulp Production and Pulp – Content of Extractable Lipophilic Matter* (Test Method SCAN-CM 67:03). Scandinavian Pulp, Paper and Board Testing Committee, Stockholm, Sweden. http://www.kemesta.fi/document.php/1/104/cm_67-03.pdf/66e76155cb4179ef39acbc06e124181a

Sharma, S., Adams, J. P., Sakul, R., Martin, E. M., Ricke, S. C., Gibson, K. E., & Carrier, D. J. (2016). Loblolly pine (*Pinus taeda L.*) essential oil yields affected by environmental and physiological changes. *Journal of Sustainable Forestry*, *35*(6), 417–430. https://doi.org/10.1080/10549811.2016.1192046

Sjöström, E., & Alen, R. (2010). *Analytical Methods in Wood Chemistry, Pulping, and Papermaking (Springer Series in Wood Science)* (Softcover reprint of hardcover 1st ed. 1999 ed.). Springer.

Sluiter, A., Ruiz, R., Scarlata, C., Sluiter, J., & Templeton, D. (2008, January). *Determination of Extractives in Biomass Laboratory Analytical Procedure (LAP)* (NREL/TP-510-42619). National Renewable Energy Laboratory. https://www.nrel.gov/docs/gen/fy08/42619.pdf

Smith, W. B., Vissage, J. S., Darr, D. R., & Sheffield, R. M. (2001). Forest resources of the United States, 1997. *General Technical Report*. Published. https://doi.org/10.2737/nc-gtr-219

Snyder, L. R., Kirkland, J. J., & Glajch, J. L. (2010). *Practical HPLC Method Development (Appendix II: Properties of Solvents Used in HPLC)* (2nd ed.). Wiley-Interscience. https://doi.org/10.1002/9781118592014

South, D. B., & Harper, R. A. (2016). A decline in timberland continues for several southern yellow pines. *Journal of Forestry*, *114*(2), 116–124. https://doi.org/10.5849/jof.15-006

Stinson, S. (1984). Organic chemistry. *Chemical & Engineering News Archive*, *62*(6), 18–19. https://doi.org/10.1021/cen-v062n006.p018

United States Department of Energy (US DoE). (2011). *U.S. Billion-Ton Update: Biomass Supply for a Bioenergy and Bioproducts Industry* (R. D. Perlack & B. J. Stokes, Eds.; ORNL/TM-2011/224). Oak Ridge National Laboratory, Oak Ridge, TN. 227p.

United Nations Statistics Division (UNSD). (2018). *SDG indicators global database* [Dataset]. https://unstats.un.org/sdgs/unsdg van Stralen, J., Kraan, C., Uslu, A., Londo, M., & Mozaffarian, H. (2016). Integrated assessment of biomass supply chains and conversion routes under different scenarios. *D7. 3 S2Biom Project*. Published. https://doi.org/10.13140/RG.2.2.26538.21441

Wang, L., Chen, X., Wei, X., & Zhu, Y. (2003). Study on synthesis of turpine hydrate from turpentine with aid of ultrasound. *Natural Gas Chemical Industry (C1 Chemistry and Technology)*. Published. http://en.cnki.com.cn/Article_en/CJFDTOTAL-TRQH200301013.htm [In Chinese]

Wear, D. N., & Greis, J. G. (2012). *The Southern Forest Futures Project: Summary Report*. Asheville, NC: USDA-Forest Service, Southern Research Station. https://www.srs.fs.usda.gov/pubs/42526

Welton, T., & Reichardt, C. (2010). *Solvents and Solvent Effects in Organic Chemistry* (4th ed.). Wiley-VCH. https://doi.org/10.1002/9783527632220

Wilbon, P. A., Chu, F., & Tang, C. (2012). Progress in renewable polymers from natural terpenes, terpenoids, and rosin. *Macromolecular Rapid Communications*, *34*(1), 8–37. https://doi.org/10.1002/marc.201200513

Wold, S., Esbensen, K., & Geladi, P. (1987). Principal component analysis. *Chemometrics and Intelligent Laboratory Systems*, *2*(1–3), 37–52. https://doi.org/10.1016/0169-7439(87)80084-9

Yang, G., Jaakkola, P., & Saimaa. (2012). *Wood chemistry and isolation of extractives from wood Literature study for BIOTULI project.* https://www.semanticscholar.org/paper/Wood-chemistry-and-isolation-of-extractives-from-Yang-Jaakkola/8da6397e7b858c44bf53fcf11de9f0b4682c04fa?sort=relevance

Yi, X., Xu, S., & Zhao, Z. (2015). Progress on preparation and application of p-Menthane-1,8-diol monohydrate. *Proceedings of the 3rd International Conference on Material, Mechanical and Manufacturing Engineering*. Published. https://doi.org/10.2991/ic3me-15.2015.89

Zhang, Q., Lin, L., & Ye, W. (2018). Techniques for extraction and isolation of natural products: a comprehensive review. *Chinese Medicine*, *13*(1). https://doi.org/10.1186/s13020-018-0177-x

Zhao, Z., Liu, X., Xian, H., Mei, L., & Sun, Z. (2000). Study on synthesis of terpenediol hydrate in higher yields. *Journal of Chemical Industry of Forest Products (Bimonthly)*. Published. http://en.cnki.com.cn/Article_en/CJFDTOTAL-LCHG200004000.htm [In Chinese]

APPENDICES

Appendix A Moisture content in the samples

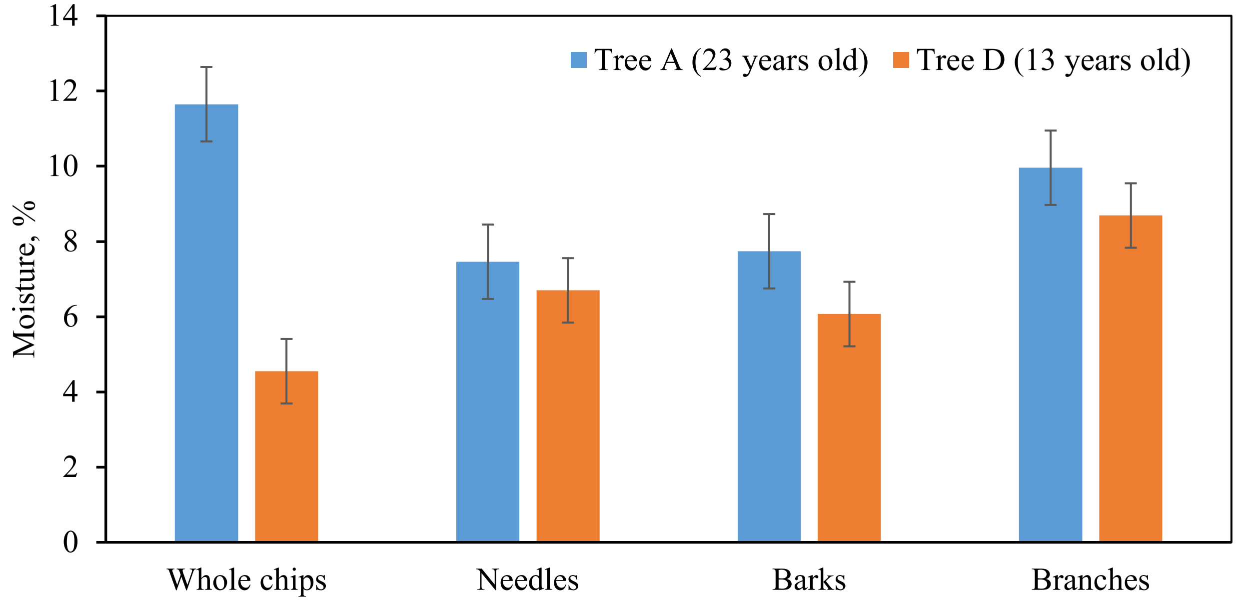

Figure A.1 Moisture content percent over the ages by different fractions for the Soxhlet extraction.

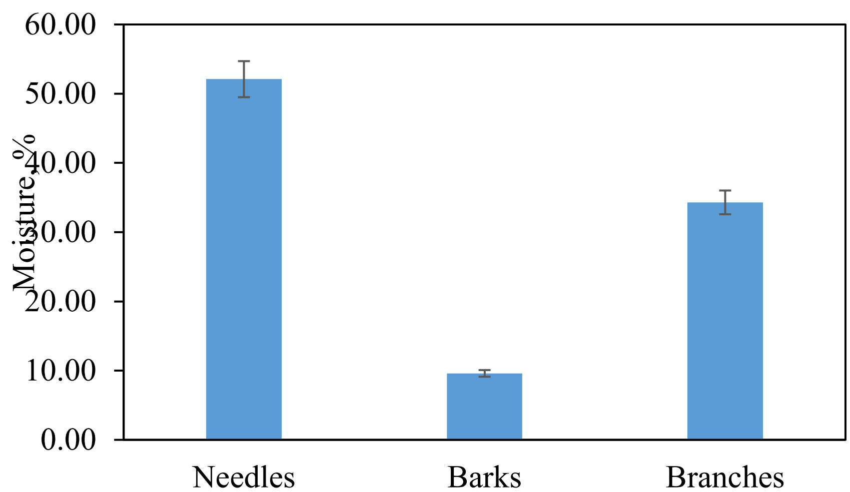

Figure A.2 Moisture content percent over different fractions for the steam distillation.

Appendix B Weight loss results for the Soxhlet extraction

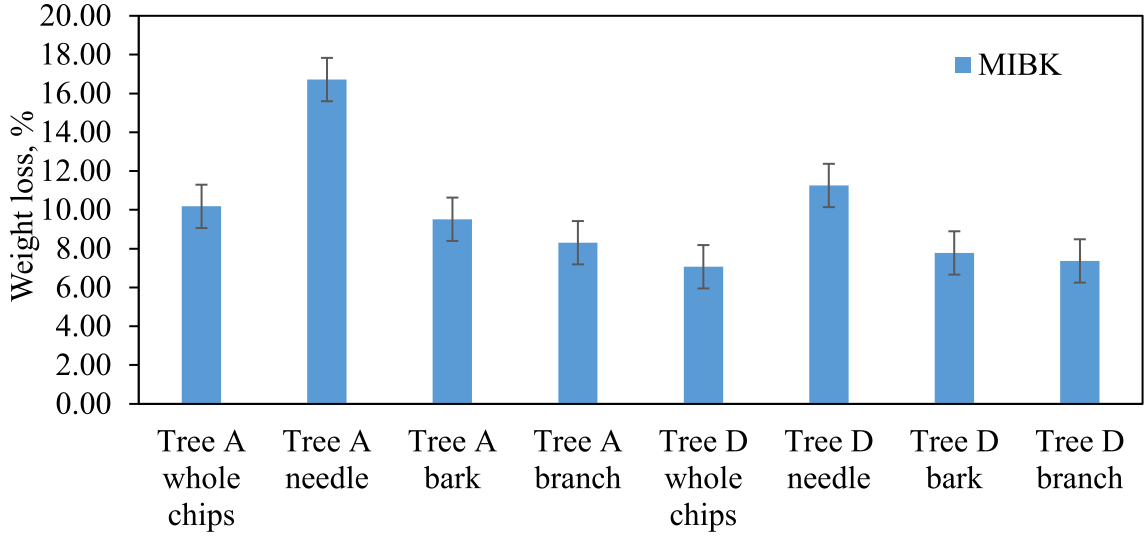

Figure B.1 Weight loss percent comparison between different fractions and trees [MIBK].

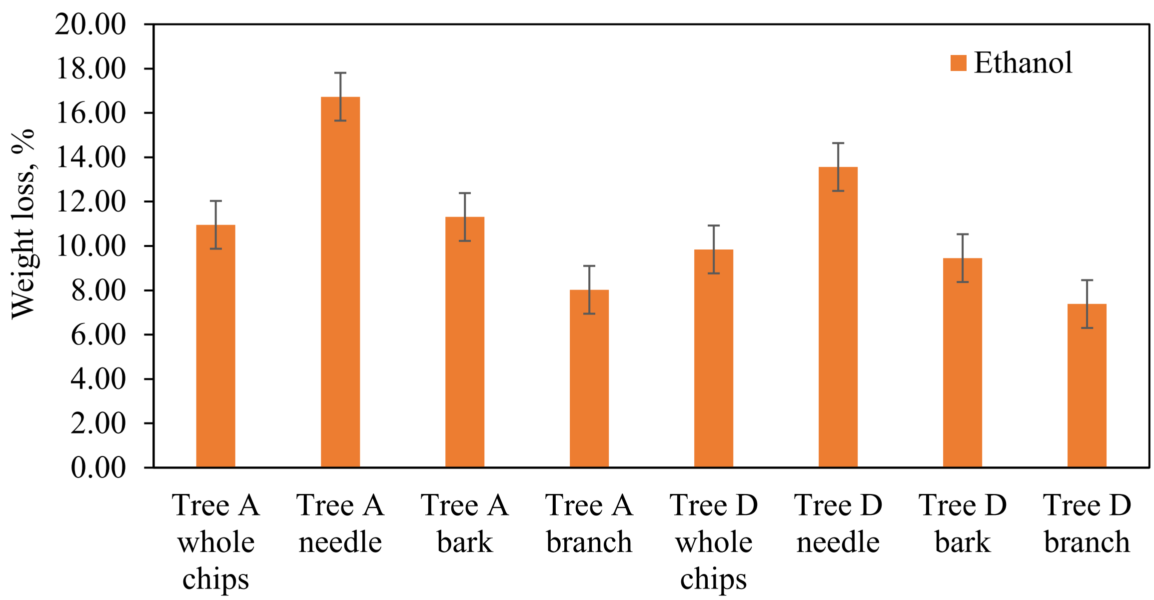

Figure B.2 Weight loss percent comparison between different fractions and trees [Ethanol].

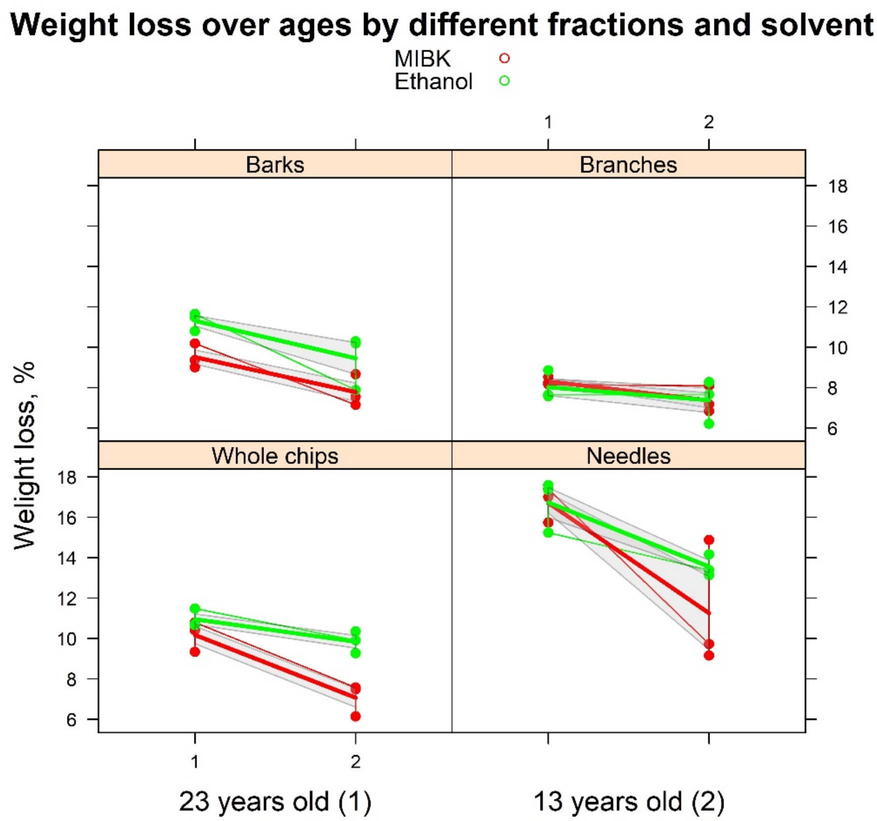

Figure B.3 Overall mean response for the weight loss percent. Grey zone indicates the 95% confidence interval for the group means.

Table B.1 Analysis of variance for weight loss percent

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Response Weight loss					
fractions	**1**	**0.3088**	**0.3088**	**9.8076**	**0.0032**
ages	**1**	**0.2862**	**0.2862**	**9.0881**	**0.0045**
solvent	1	0.0833	0.0833	2.6469	0.1116
fractions:ages	1	0.0238	0.0238	0.7567	0.3895
fractions:solvent	1	0.0248	0.0248	0.7881	0.3800
ages:solvent	1	0.0237	0.0237	0.7518	0.3911
fractions:ages:solvent	1	0.0132	0.0132	0.4190	0.5211
Residuals	40	1.2595	0.0315		

Appendix C Weight loss results for the steam distillation

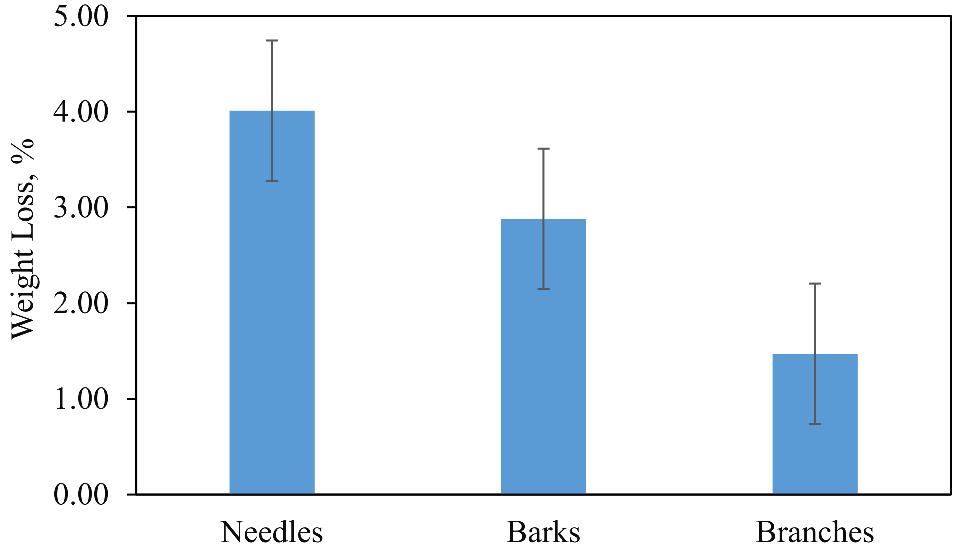

Figure C.1 Weight loss percent for the residues from different fractions.

Appendix D Other clustered PC score plots for the Soxhlet extraction

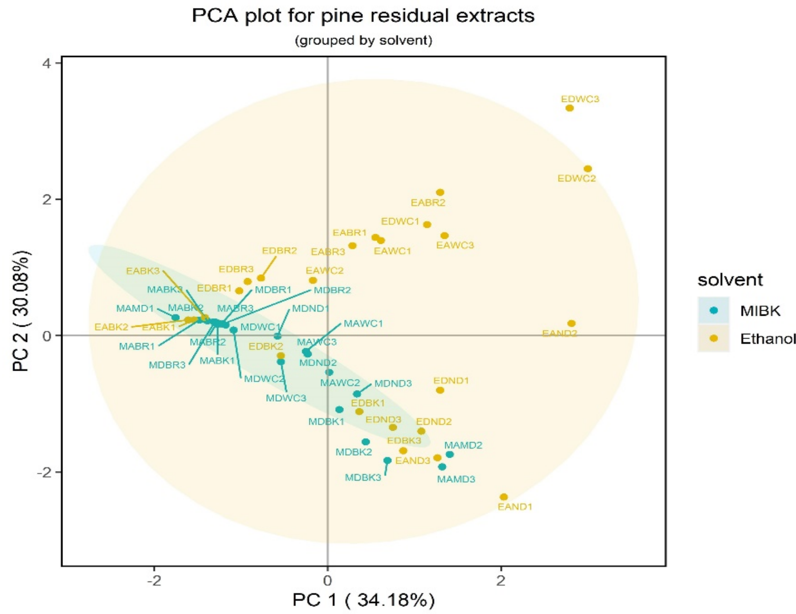

Figure D.1 PC 1 & 2 score plot for the pine extractives over different solvent. Colors indicate different fractions, and ellipses represent 95% confident intervals.

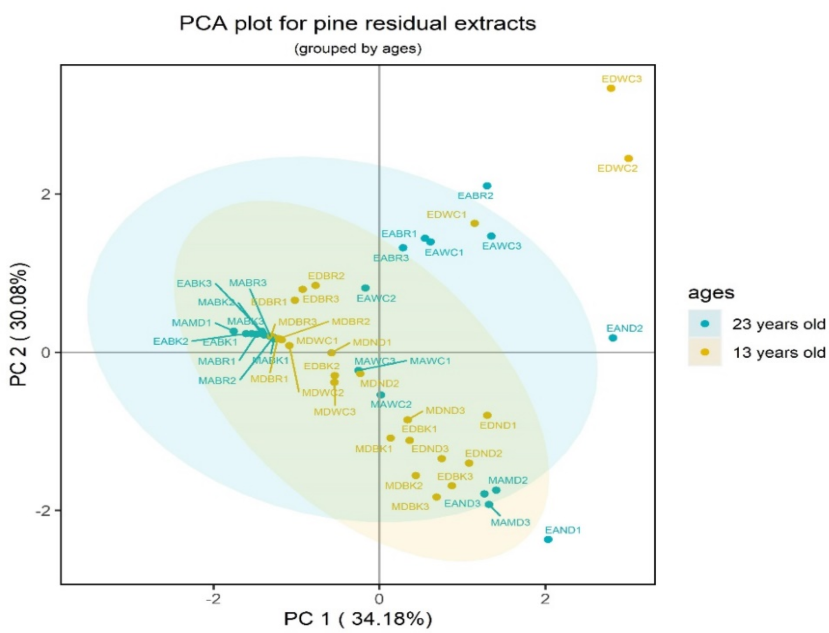

Figure D.2 PC 1 & 2 score plot for the pine extractives over different ages. Colors indicate different fractions, and ellipses represent 95% confident intervals.