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Thermochemical methylation of lignin to produce high value aromatic compounds

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Thermochemical methylation of lignin to produce high value aromatic compounds

by

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DEDICATION

I dedicate this work to my family—my wife, Whitney, for her support and endless patience while working on this dissertation; my daughters, Alice & Edie, for providing much needed breaks with movie therapy via Walt Disney; and lastly my parents, Pete & Becky, for the lifetime of guidance they have given me.

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NOMENCLATURE

DB – Dry Basis

DI – Deionized (water)

DMF – Dimethylformamide

CHNS – Carbon, Hydrogen, Nitrogen, and Sulfur

FID – Flame Ionization Detector

GC – Gas Chromatography

GHG – Green House Gas

HCA – Hydroxycinnamic Acid

HPLC – High Performance Liquid Chromatography

MS – Mass Spectrometer

MTPD – Metric Ton per Day

PEMC - Tetra-methyl Carbonate of Pentaerythritol

RFS – Renewable Fuel Standard

SF – Stage Fraction

TGA – Thermal Gravimetric Analyzer

TMAH – Tetramethylammonium Hydroxide

Wt. % - Weight Percent

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ABSTRACT

Technical lignin, the byproduct from cellulosic ethanol production, is a potential feedstock for producing coumaric and ferulic acids. These biorenewable aromatic hydroxycinnamic acids can be upgraded to high value chemicals for the manufacture of cosmetics, flavoring agents, and sunscreens. We hypothesize that during the fast pyrolysis of lignin, aromatic acid esters can be recovered from coumaric, ferulic and other hydroxycinnamic acids using a methylation agent. In this study, technical lignin from cellulosic ethanol production was pyrolyzed in a micropyrolyzer with a methylation agent to produce gas chromatography (GC) detectable aromatic acid esters. Corn stover lignins from enzymatic hydrolysis and the organosolv processing were combined with different methylation agents to produce, identify and quantify the hydroxycinnamic acid esters. High performance liquid chromatography (HPLC) with a diode array detector (DAD) was used to separate and quantify the extracted hydroxycinnamic acids from lignin for determination of yield recoveries.

To determine the actual total extractable yields of coumaric and ferulic acid in the lignin an alkaline extraction method was used. The yields on a mass basis of coumaric and ferulic acid in the enzymatic hydrolysis lignin was 5.6% and 1.9%, respectively. The yields on a mass basis of coumaric and ferulic acid in the organosolv lignin was 7.7% and 2.9%, respectively.

The recovery of hydroxycinnamic acids from enzymatic hydrolysis and organosolv lignin with different loading of methylation agent were compared using a micropyrolyzer at 500°C and the pyrolysis cup method. Using tetramethylammonium hydroxide (TMAH) or tetra-methyl carbonate of pentaerythritol (PEMC) at concentrations of 1μL –

10μL produced high yields of coumaric and ferulic acid that can be recovered at concentrations up to 89% and 97%, respectively.

The recovery of hydroxycinnamic acids from enzymatic hydrolysis and organosolv lignin with different loading of methylation agent were compared using a micropyrolyzer at 500°C and the micro reaction method. Using tetramethylammonium hydroxide (TMAH) or tetra-methyl carbonate of pentaerythritol (PEMC) at concentrations of 1μL – 10μL produced high yields of coumaric and ferulic acid that can be recovered at concentrations up to 97% and 90%, respectively.

Overall, both techniques and methylation agents produced a significantly high concentration of biorenewable chemical intermediates from the lignin byproduct of corn stover cellulosic ethanol production. The hydroxycinnamic acids that are produced can be further upgraded into high value chemical products. Utilizing lignin valorization could provide a substantial effect on the economics of cellulosic ethanol production via precursors or intermediates of high value commodity chemicals.

CHAPTER 1

INTRODUCTION

With the growing population and increased demand of fuels and chemicals the utilization of biorenewable byproducts of low value from biorefinery processes could be a way to reduce demand of petroleum imports and create better efficiencies and economics within these processes. One way to do this is through lignin valorization, or adding value to a product that is being underutilized, or if the product is only used in small markets. Ragauskas et al. (2014) has suggested that converting lignins to chemicals such as 1,4-butanediol and adipic acid has improved economics and reduced greenhouse gas emissions by an order of magnitude compared to just burning the byproduct for electricity.

There are many different types of lignin but all have three main phenolic structures or building blocks including *p*-hydroxphenyl, guaiacyl and syringyl denoted H, G, and S polymers respectively. The ratios of these polymers change depending on biomass type. Grasses contain all three phenolic polymers, whereas, hardwoods contain mainly G and S and soft woods only G (Boerjan, Ralph, & Baucher, 2003; Bruijnincx, Weckhuysen, Gruter, Westenbroek, & Engelen-Smeets, 2016). Unlocking these lignin building blocks are key to understanding the significance and product utilization as a direct petrochemical replacement.

The main lignin types that are currently being produced are from steam explosion (autohydrolysis), ammonia fiber explosion (AFEX), CO_2, ozonolysis, acid hydrolysis, oxidative delignification, organosolv, and biological pretreatment (Y. Sun & Cheng,

2002). Pyrolytic lignin from pyrolysis and solvent liquefaction processes is another type of lignin that could be utilized as biorenewable phenolic replacements. During these processes the insoluble organic lignin portion of the bio-oil produced is called pyrolytic lignin that contains a large molecular weight range of phenolic monomers through tetramers (Robert C. Brown & Brown, 2014). The technical lignin that this research will focus on is organosolv and enzymatic hydrolysis lignin. The organosolv process typically involves organic solvents and an organic acid catalyst to extract lignin from biomass (Sarkanen, 1980; Y. Sun & Cheng, 2002; Zhao, Cheng, & Liu, 2009). The lignin from enzymatic hydrolysis is produced using an inorganic acid and temperature treatment to remove and extract lignin from biomass. Extensive research has been conducted in this field (Haghighi Mood et al., 2013; Y. Sun & Cheng, 2002).

Corn stover lignin is a byproduct of cellulosic ethanol production. The lignin content of corn stover can range from 15-21% depending on location (Buranov & Mazza, 2008). It is estimated that approximately 225 million tons/year of lignin could be available by 2030 from cellulosic ethanol production (Sahoo, Seydibeyoğlu, Mohanty, & Misra, 2011). Producing second generation fuels such as cellulosic ethanol is a way to decrease our dependency on foreign fuels. Crude petroleum, unlike ethanol, can be used for a number of different petrochemical products besides fuels that include high value chemicals. Producing chemicals from lignin aligns with the Department of Energy's (DOE) strategy of "Replacing the Whole Barrel". Figure 1 displays the products of a barrel of crude oil. At least 7 % of the barrel is made up of petrochemicals that can produce high value products and currently only 42% of the barrel can be replaced by ethanol (USDOE, 2013). Developing chemicals from biorenewable

byproducts like lignin can help replace petrochemicals for future energy security in the United States.

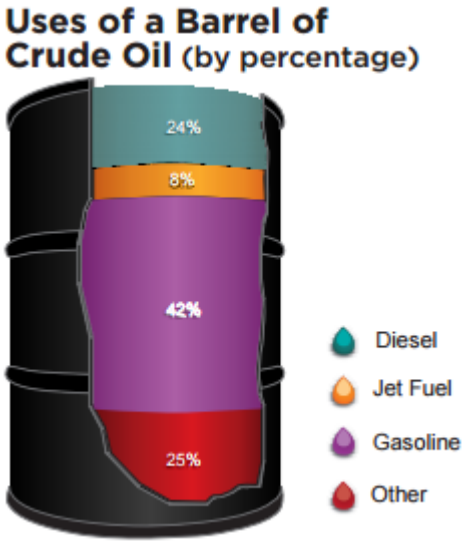


Figure 1. Uses of a barrel of crude oil from U.S. Department of Energy (USDOE, 2013).

There has been a push over the last decade to produce fuels from lignocellulosic materials. Mandates such as the second Renewable Fuel Standard (RFS2) require that 36 billion gallons of renewable transportation fuels are produced by 2022. The RSF2 requirement for advanced biofuels that includes cellulosic ethanol is 16 billion gallons. These fuels must meet a requirement of at least a 60% reduction in Green House Gas (GHG) emission compared to the baseline lifecycle GHG emission of gasoline in 2005 (EPA, 2010). Due to these mandates production of lignin will increase and add value to cellulosic ethanol production. If ethanol producers utilize this stream it could have an even greater potential impact on the reduction of GHG emissions since renewable chemicals are also being produced when considering a complete life cycle analysis (LCA). It would also improve the minimum selling price of ethanol. From previous studies, the ethanol economics improved considerably when producing commodity and

specialty chemicals from lignin (T. R. Brown & Brown, 2013; Kraus et al., 2015). The minimum fuel selling price of ethanol drops almost 50% (table 1).

Table 1. Minimum fuel selling price comparison of ethanol between typical ethanol production and ethanol product utilizing lignin for chemicals (T. R. Brown & Brown, 2013; Kraus et al., 2015).

Contribution to Ethanol Price	Minimum selling price for ethanol ($/gal)	
	Base Case	Chemical Products
Feedstock	$0.741	$0.741
Capitol	$0.638	$0.638
Materials and Utilities	$0.437	$0.437
Fixed	$0.274	$0.274
Income Tax	$0.159	$0.152
Specialty Chemicals	$0	-$0.691
Electricity	-$0.108	-$0.042
Commodity Chemicals	$0	-$0.345
Total	$2.142	$1.164

During cellulosic ethanol production a byproduct stream of lignin is produced from a pretreatment step of the corn stover. The lignin can account for up to 18% of this process depending on feedstock characteristics (Ragauskas et al., 2014). This pretreatment step consists of heat, enzymes, and/or acids to break down the lignin polymers before hydrolysis (Houghton, Weatherwax, & Ferrell, 2006; Wyman, 2007).

The complete process for producing fuel grade ethanol is outlined in figure 2. The lignin can be recovered from this process and some of the current uses are heat and electricity from combustion. Utilizing the lignin just as a fuel source is not very efficient. The costs associated with building a boiler or turbine system for a cellulosic ethanol plant that utilizes lignin as a source for heat or electricity could be at least 1/3 of the total installed equipment costs (Kazi et al., 2010). It has been found that utilizing lignin for chemicals can increase the economics of the process by an order of magnitude (Ragauskas et al., 2014).

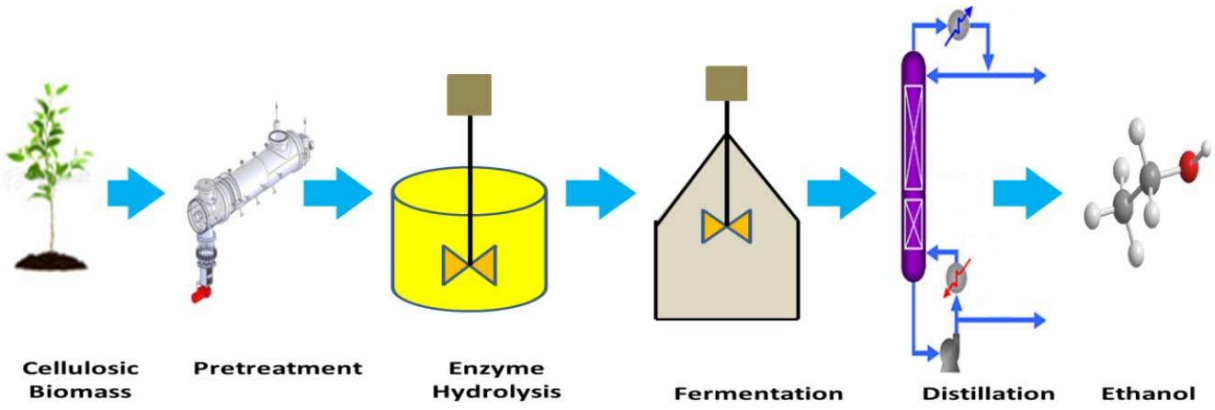


Figure 2. Typical cellulosic ethanol process from University of Florida IFAS Extension (Tong, Pullammanappallil, & Teixeira, 2012) – the four main steps include: 1. Acid Pretreatment; 2. Enzymatic hydrolysis; 3. Fermentation; 4. Distillation.

An alternative route to utilizing this lignin is by physical pretreatment or thermochemically upgrading with alternative technologies such as fast pyrolysis or solvent liquefaction to produce high value chemicals or precursors to high value chemicals. The current value for technical lignin is approximately $116.00/ton (Strassberger, Tanase, & Rothenberg, 2014). One valuable chemical that lignin can be upgraded to is vanillin. This can be done by using precursor compounds found in lignin

such as ferulic acid. Vanillin is a very high demand aromatic flavoring agent that has a production rate of 16 ktpa worldwide (Strassberger et al., 2014). The current retail price of reagent grade vanillin with a purity of at least 99% sells for $130/kg and natural vanillin sells for $1300/kg ("Sigma Aldrich," 2017). Other commodity chemicals that could possibly be produced from the hydroxycinnamic acid precursors are p-hydroxybenzioc acid, octinoxate, β-amino acid derivatives and polystyrenes (Kraus et al., 2015). This would be a more efficient and cost effective approach to utilizing lignin from ethanol production and producing a high value biorenewable chemical stream that could increase the economic value of cellulosic biofuels.

A feedstock sample of corn stover, red oak, and switchgrass from Iowa State University (ISU) was characterized at Celignis Limited (Limerick, Ireland) for carbohydrate and lignin content. The corn stover had 15.31% lignin content. The complete analysis of the feedstocks is found in Table 2 and Figure 3. The test methods were based off of National Renewable Energy Laboratory (NREL) TP-510-42618 protocol.

Table 2. Corn stover lignocellulosic Summary Data (% Dry Matter)

Sample	Total Sugars	Glucan	Xylan	Mannan	Arabinan	Galactan	Klason Lignin	Acid Soluble Lignin	Extractives	Ash
Corn Stover	60.55	36.41	20.13	0.24	2.68	0.98	13.25	2.06	10.55	8.24
Red Oak	58.56	40.00	15.66	1.30	0.34	0.92	20.29	2.95	6.85	0.40
Switchgrass	59.25	34.28	21.46	0.21	2.43	0.79	16.90	1.90	9.42	5.08

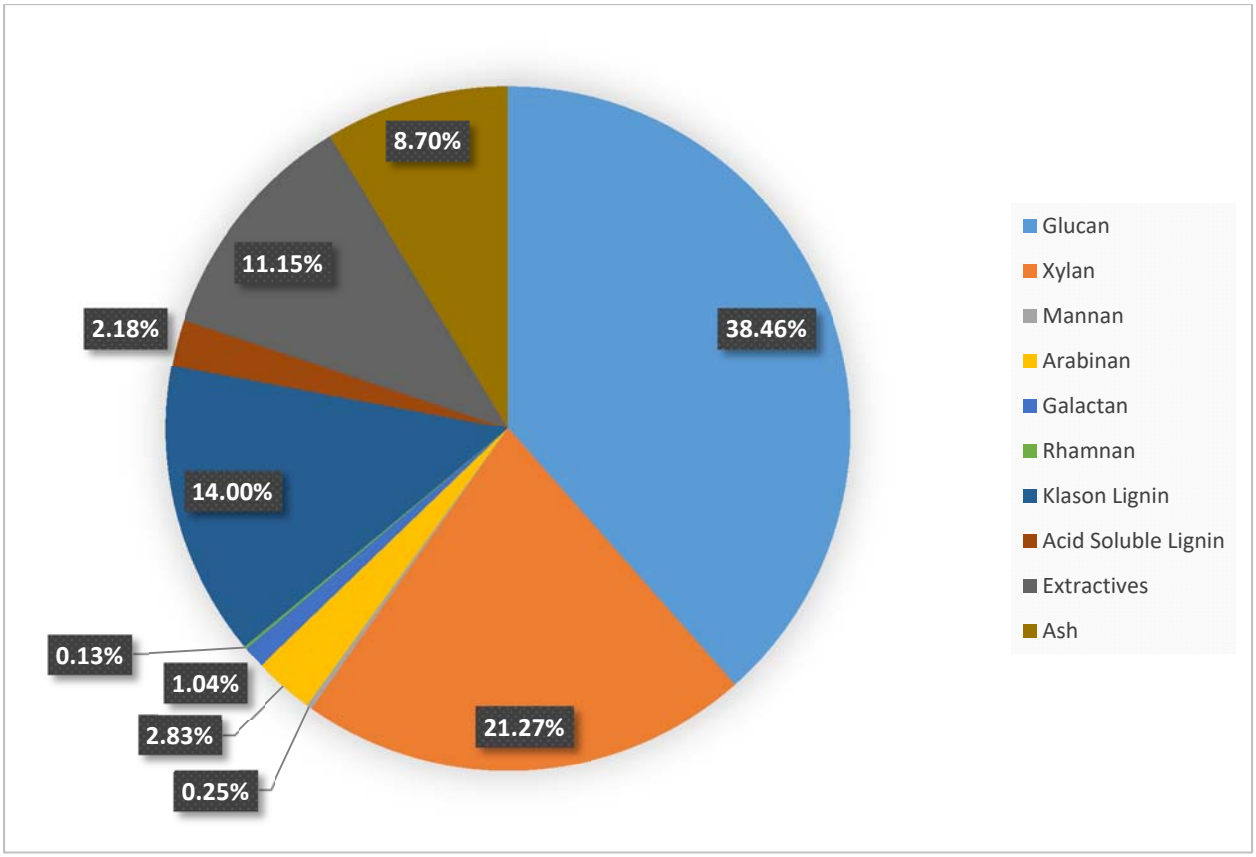


Figure 3. Complete lignocellulosic analysis of corn stover form Iowa State University.

Kato and Nevins found that ferulic content could be 3µg per 100µg of carbohydrate (Kato & Nevins, 1984, 1985). It has been reported that as high a 20% *p*-coumaric acid analyzed from ^{13}C NMR and 17.4% *p*-coumaric from saponification found in Maize rind stem lignin (Ralph et al., 1994).

Fast Pyrolysis Overview

Fast pyrolysis is a thermochemical process that is performed without the presence of oxygen at temperatures typically ranging from 400-600°C (A. V. Bridgwater, 1999; R. C. Brown, 2011). This process converts biomass and biomass constituents into gases, condensable vapors (liquids), aerosols, and char (Bai, Johnston, Sadula, & Brown,

2013). The process yields are: liquids (55-60%), gases (12-22%), and char (16-20%) (Agblevor, Besler, & Wiselogel, 1995; Mullen et al., 2010; Wright, Daugaard, Satrio, & Brown, 2010). The main liquid portion called bio-oil is a dark brown complex mixture of organic acids, alcohols, aldehydes, ketones, phenols, carbohydrates, esters, lignin oligomers, and water (R. C. Brown, 2011). The pyrolysis liquid yield can be as high as 50-80% depending on process conditions (A. V. Bridgwater, 1999; R. C. Brown, 2011; Robert C. Brown & Brown, 2014). Process conditions that favor pyrolysis liquid yields include short residence times, fast heating rates, temperatures of approximately 500°C and rapid cooling of gas vapors (A. V. Bridgwater, 1999; R. C. Brown, 2011; George W. Huber, Iborra, & Corma, 2006).

The liquid fraction can be upgraded further into fuels and/or chemicals. A potential issue with upgrading the oil is the very low pH of 2-3 and high organic acid content that makes the product very corrosive to certain metal surfaces (Czernik & Bridgwater, 2004). A second unfavorable chemical and physical property of pyrolysis oil includes high water content, low molecular weight, high oxygen content, high viscosity, low volatility and high reactivity (Robert C. Brown & Brown, 2014; Czernik & Bridgwater, 2004). Upgrading the pyrolysis oil by catalytic cracking can thermally stabilize and remove oxygen thus reducing the problematic chemical and physical characteristics (G. W. Huber & Corma, 2007). The gas and char can also be converted into heat, stream, and/or electricity as final products (A. Bridgwater, 1997). A detailed mass balance composition of fast pyrolysis bio-oil produced from corn stover is displayed in table 3.

There are many different types of fast pyrolysis reactors that can be used to thermochemically process biomass feedstocks. Table 4 displays a list of fast pyrolysis reactors and the main characteristics of these reactors.

Table 3. Corn stover pyrolysis oil composition (Wright et al., 2010).

Gas Compounds	**Composition (kg/100 kg of dry biomass)**
Carbon Dioxide	5.42
Carbon Monoxide	6.56
Methane	0.035
Ethane	0.142
Hydrogen	0.588
Propane	0.152
Ammonia	0.0121
Bio-Oil Compounds	
Acetic Acid	5.93
Propionic Acid	7.31
Formic Acid	0.61
Methoxyphenol	3.80
Ethylphenol	3.41
Propyl-Benzoate	16.36
Phenol	0.46
Toluene	2.27
Furfural	18.98
Benzene	0.77
Other Compounds	
Water	10.80
Char	16.39

Table 4. Fast pyrolysis reactors and characteristics (A. V. Bridgwater, 1999; R. C. Brown, 2011; Robert C Brown & Holmgren, 2009; George W. Huber et al., 2006).

Reactor Type	**Characteristics**
Bubbling Fluid Beds	a. Heat is supplied externally b. Good mass transfer c. Biomass needs to be uniform and very small 2-3mm d. Produces high yields of bio-oil
Circulating Fluid Beds	a. Hot sand is used as bed material b. Heating is supplied from the thermochemical processing of biomass c. Has high throughput d. Produces high yields of bio-oil
Auger Reactor	a. Hot sand is mixed by reciprocating augers b. Metal catalysts may be needed to get uniform heat transfer c. Easy to scale up process
Ablative Reactor	a. Biomass pyrolysis achieved by pressure on rotating reactor b. Particles can be heterogeneous and larger c. Very difficult to scale up process d. Produces high yields of bio-oil
Entrained Flow	a. High amounts of carrier gas need for process b. Easy to scale up process
Rotating-cone pyrolyzer	a. Sand and biomass are mixed together in rotating cone b. Will not need a carrier gas c. Biomass needs to be homogenous and very small
Vacuum Pyrolysis	a. Biomass is gravity fed into heated zone with multiple grates b. Particle can be heterogeneous and large c. Very difficult to scale up process

A fluidized bed reactor with fractionation capabilities would be ideal for thermochemically processing biomass into high value chemical products. A typical fluidized bed pyrolysis system with fractionation separation technology is displayed in figure 4. After the biomass has been thermochemically processed the particulate matter (char) is removed from the gas by two cyclones.

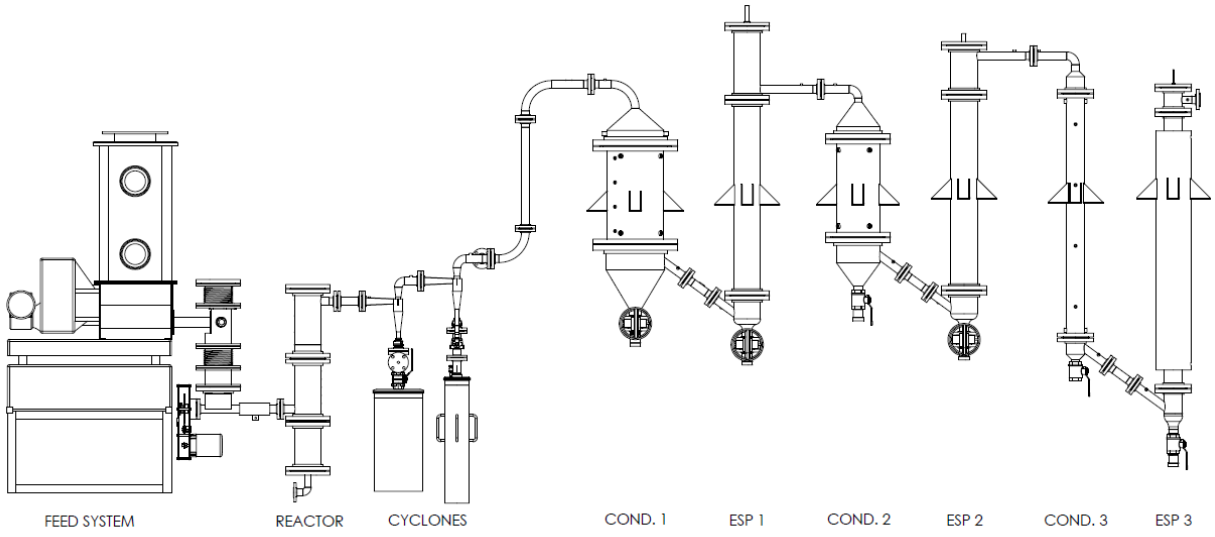


Figure 4. Schematic of 8 kg/hr fast pyrolysis fluidized bed reactor process development system: Stage Fraction 1 (SF1) – designed to collect anhydrosugars and other larger molecular weight compounds; Stage Fraction 2 –electrostatic precipitator (ESP) designed to capture aerosols; Stage Fraction 3 (SF3) – designed to capture phenols and other heterocyclic compounds; Stage Fraction 4 (SF4) –electrostatic precipitator (ESP) designed to capture any aerosols after condenser SF3; Stage Fraction 5 (SF5) – designed to capture water, acids, and other light oxygen containing compounds (Pollard, Rover, & Brown, 2012).

In the final stages the gases are condensed at different temperatures to produce six distinct fractions of pyrolysis oil. With the fractionation process removal of high value chemicals from biomass is achievable. In the future, this type of continuous system could possibly be utilized for separating the high value aromatics from the methylated

lignin feedstock. Figure 5a displays the wt. % of components that are currently being produced from biomass pyrolysis. Figure 5b further explains the separations that are currently needed to break these components down to value-added products.

a b

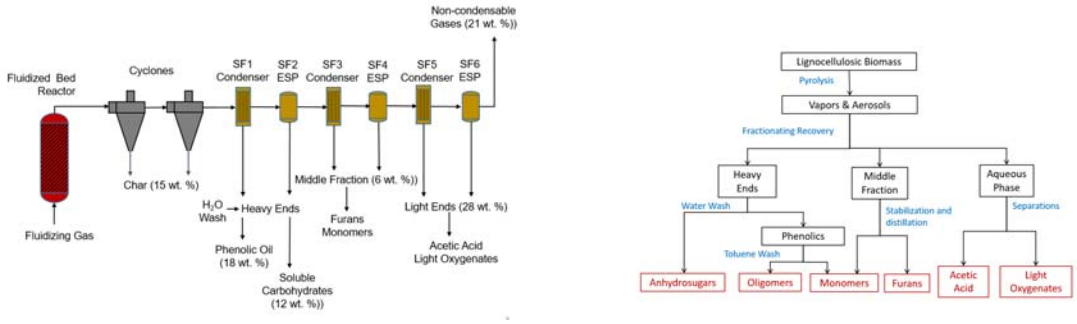


Figure 5. a) The wt. % of components that are currently being produced from biomass pyrolysis in fractionation system; b) Separations that currently are needed to break these components to value-added products.

Solvent Liquefaction Overview

This process combines the biomass with a solvent and reacts the mixture under pressure at moderately elevated temperatures to convert the biomass into predominately liquid product. Solvent liquefaction temperature are typically 150-450°C with pressure ranging from 55 – 720 bar (Elliot et al., 1991; Ghosh, Brown, & Bai, 2016; Vanasse, Chornet, & Overend, 1998). The solvent system used to separate the chemicals from biomass can be either be polar, nonpolar, or a combination of both (biphasic) (Robert C. Brown & Brown, 2014).

It has been reported that this process can produce solubilized carbohydrate at concentrations as high as 94% from cellulose (Ghosh et al., 2016). One main advantage of oil from solvent liquefaction compared to oil from pyrolysis is its lower moisture and oxygen content (Robert C. Brown & Brown, 2014). These characteristic advantages would be beneficial for upgrading into biofuels. A typical continuous pilot solvent liquefaction separation process is outlined in figure 6. Alternatively, this is another process that is able to produce renewable chemical compounds from biomass feedstocks.

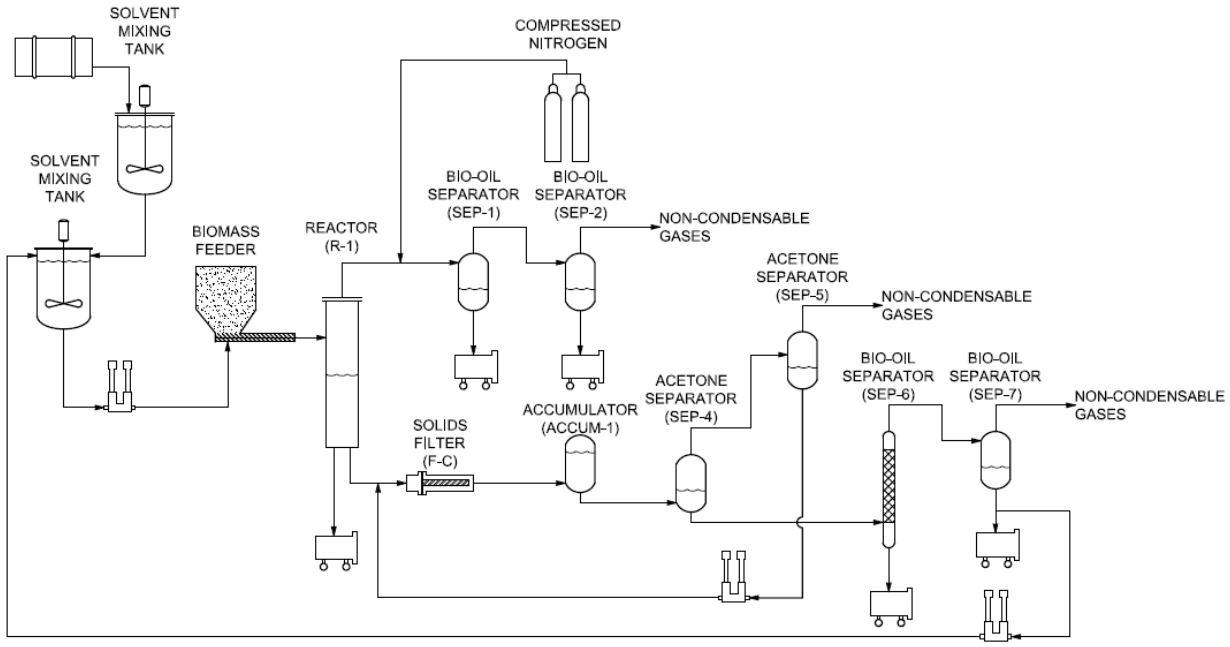


Figure 6. Solvent liquefaction separation schematic (Haverly, 2015).

CHAPTER 2

LITERATURE REVIEW

Lignin

It is estimated that 300 million metric tons of lignin is available on earth and almost 7% of this amount could be renewed on an annual basis (Argyropoulos & Menachem, 1998; Buranov & Mazza, 2008). Lignins account for 10-30% of the total biomass carbon on earth (Bai & Kim, 2016; Ralph, Brunow, & Boerjan, 2007). During the cellulosic ethanol process lignin remains as a byproduct. This accounts for approximately 225 million tons/year of lignin that could be available by 2030 from cellulosic ethanol production (Sahoo et al., 2011). Utilization of these waste streams are critical for the production of renewable chemicals that could overall decrease the amount of petrochemicals produced from crude oil. One way to produce chemicals from lignin is thermochemical depolymerization processes such as fast pyrolysis. This process employs high heating rates and high temperatures that can break down plant polymers into monomers. This reduction in petrochemicals would help boost the U.S. Energy Security and decrease dependence of foreign oil.

The lignin structure is mainly composed of "*aromatic polymers resulting from the oxidative combinatorial coupling of 4-hydroxyphenylpropaniods*" (Vanholme, Demedts, Morreel, Ralph, & Boerjan, 2010). The three main monolignol building blocks of lignin are *p*-coumaryl (H lignin), sinapyl (S lignin), and coniferyl (G lignin) alcohol (Boerjan et al., 2003; Freudenberg & Neish, 1968; Vanholme et al., 2010). The structures of these lignin building blocks are represented in figure 7. Figure 8 represents a NMR

adaptation of the structure of lignin from Stewart et al. (Stewart, Akiyama, Chapple, Ralph, & Mansfield, 2009). From previous research (Boerjan et al., 2003; Freudenberg & Neish, 1968; Ralph, 2009), lignin structure can be summarized into the following generalized groups:

1. Aromatic monomers – *p*-coumaryl, coniferyl, and sinapyl alcohols.
2. Phenolic polymers – *p*-hydroxyphenyl, quaiacyl, and syringyl.
3. Hydroxycinnamates – *p*-coumarate, ferulate, and sinapate.

These aromatic monomers become phenolic polymers in the lignin structure. Hydroxycinnamates provide the cross linkages between the lignin and carbohydrate (Ralph, 2009).

There are many possible high value chemicals that can be extracted from lignin. During fast pyrolysis these complex polymers can be thermochemically depolymerized into phenolic and other volatile aromatic compound. Chemicals such as vanillin, ferulic acid, coumaric acid, and vinyl guaiacol can be used for a variety of industrial applications or as a precursor to other high value chemicals (Buranov & Mazza, 2008). Potential applications for these chemicals include carbon fibers and resins (Baker & Rials, 2013). The most common bond types in lignin are n types with β-O-4-, β–5-, β–β-, 5–5-, and 5-O-4-linkages (Ralph, 2009; Ralph et al., 2004). Again, these compounds can be used as chemical replacements for petrochemicals. Bai and Kim, 2016, outlined the process of an integrated biorefinery that would produce both fuel and chemical replacements for petrochemicals and petroleum crude (figure 9) (Bai & Kim, 2016) .

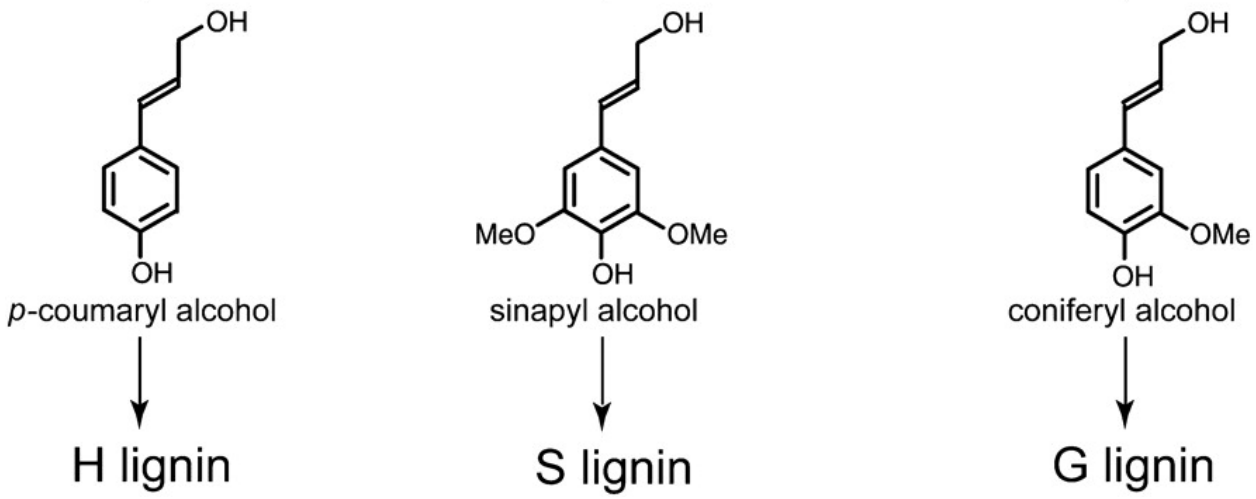


Figure 7. Representation of lignin aromatic monomeric building blocks from Vanholme et al. (Vanholme et al., 2010).

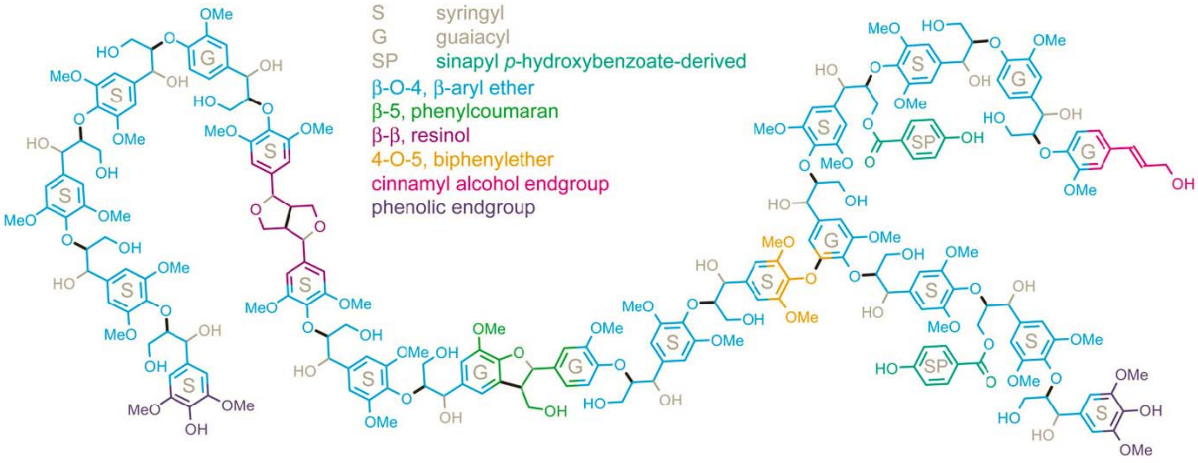


Figure 8. Representation of a Poplar lignin polymer from NMR analysis (Stewart et al., 2009; Vanholme et al., 2010).

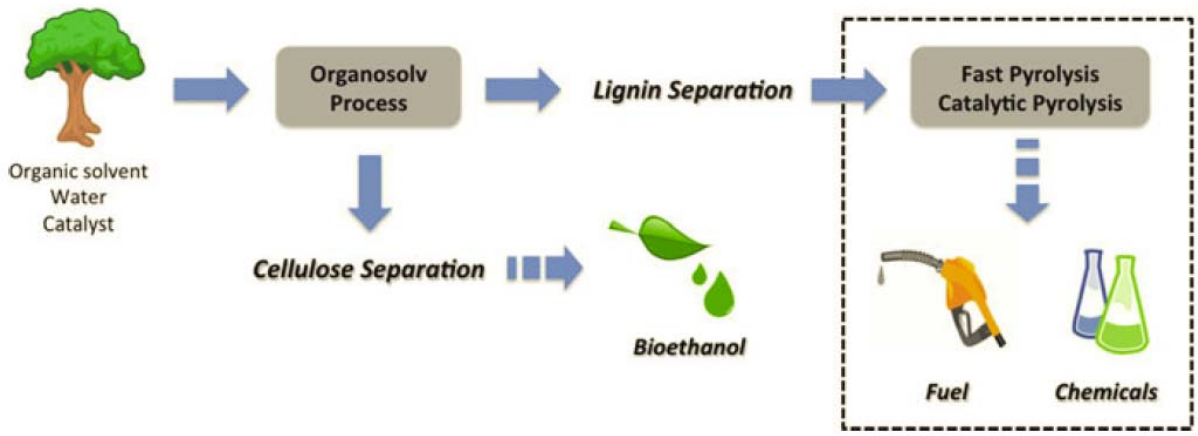


Figure 9. Integrated biorefinery concept from Bai and Kim, 2016 to produce fuels and chemicals (Bai & Kim, 2016).

Hydroxycinnamic Acids

Hydroxycinnamic acids in plant biomass consist mostly of ferulic and coumaric acids (figure 10). These acids are formed during the lignification process. This process has been extensively studied by Morrison et al. (1998) and Sun et al. (2002). They concluded that ferulic acid is formed and deposited in the cell walls in the early stages of cell wall formation and coumaric acid is deposited throughout the lignification process (Morrison, Jung, Buxton, & Hatfield, 1998; R. Sun, Sun, Wang, Zhu, & Wang, 2002). During the incorporation of hydroxycinnamic acids in lignin, coumaric acid is esterified (-COO) at lignin side chains and ferulic acid is linked to lignin side chains with ether (-O-) and ester (-COO) bonds (R. Sun et al., 2002). The total amount of hydroxycinnamic acid, depending on feedstock/lignin type, can range from less than 1% to as great as 15%, based on total lignin (Buranov & Mazza, 2009; Ralph, 2009; R. Sun et al., 2002).

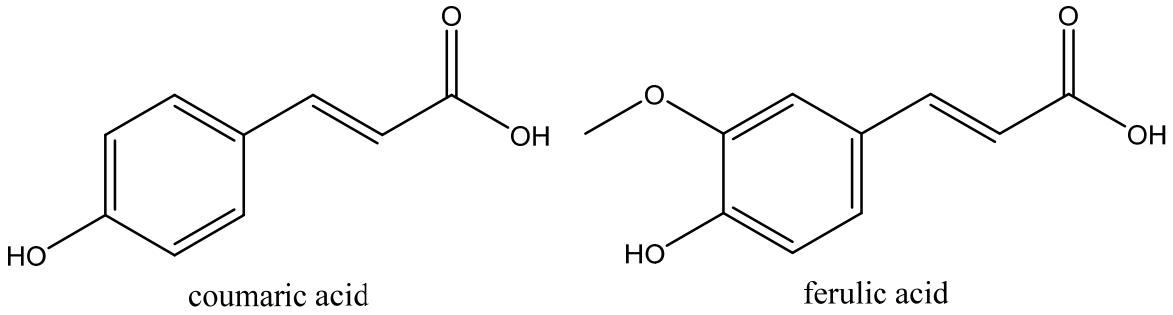


Figure 10. Structures of hydroxycinnamic acids.

Hydroxycinnamic acids exist in plants mostly in the *trans* form (R. Sun et al., 2002). The hydroxycinnamic acids can also crosslink with the cell wall polysaccharides during lignification (R. Sun et al., 2002). The concentration of these aromatic acids were determined by the methylation/derivatization process that is known to hydrolyze esters and methylate the resulting carboxylate ion ($RCOO^-$) and produce corresponding methyl esters ($RCOOCH_3$) that are GC detectable (Fabbri, Baravelli, Chiavari, Prati, & Finessi, 2007).

Lignin extraction and analysis is critical to determine high valve chemicals that can be retrieved. Lignin compounds can be analyzed by Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC). Extraction of hydroxycinnamic acids from lignin can either be completed by using an acidic or alkaline solution. In these two cases either hydrochloric acid or sodium hydroxide can be used. Figure 11 represents this extraction process and bond cleavage from depolymerization using an acid or base catalyst. This extract can then be analyzed with liquid chromatography for concentration determination. Concentrations of these acids can be directly determined by gas chromatography with a micropyrolyzer. The lignin sample can be methylated, pyrolyzed and volatile aromatic acid esters can be measured.

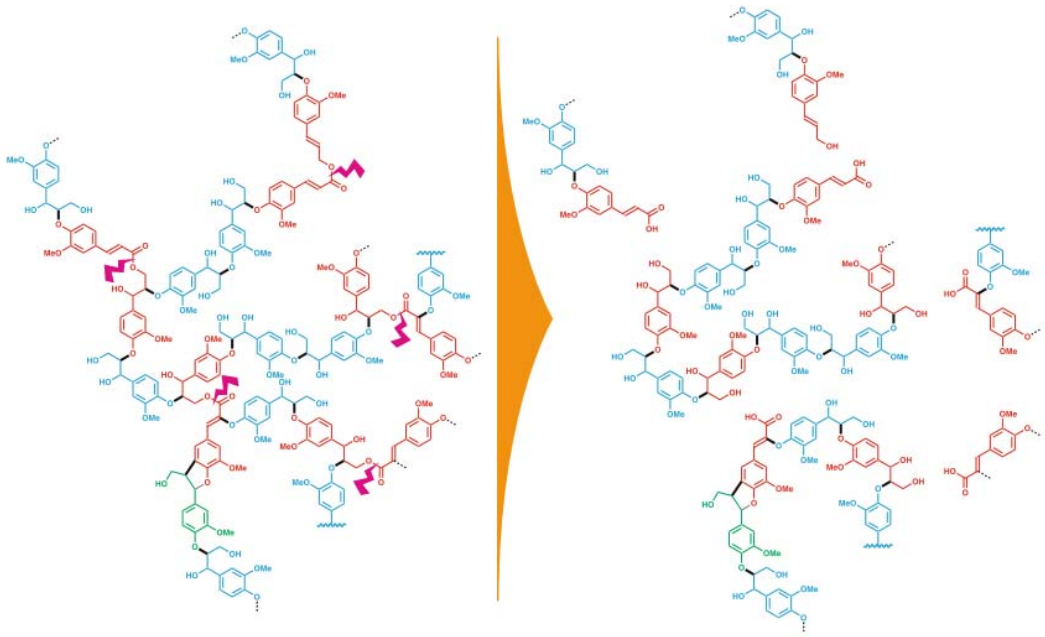


Figure 11. Lignin hydroxycinnamic acid extraction process with ester bond cleavage and depolymerization adapted from Ralph, 2009 (Ralph, 2009).

Hydroxycinnamic Acid Analysis

Methods for extracting chemicals from lignin can involve either acidic or alkaline conditions. Recovery of hydroxycinnamic acids from lignin can be a very difficult process which involves a base or acid extraction. Hertog et al. (1992), proposed using 3N or 6N hydrochloric acid to extract the acids (Hertog, Hollman, & Venema, 1992). Other recovery methods involve a base such as sodium hydroxide. For this extraction method, concentrations ranged from 0.5 - 4N sodium hydroxide with an extraction time of 2-4 hrs. (Buranov & Mazza, 2009; R. Sun et al., 2002). Both extraction methods involve refluxing the lignin in a reflux apparatus for a set time period. Once the

extraction is finished the analysis was performed by HPLC. There are numerous methods that have been used for the analysis.

Previous research on identification and quantification of hydroxycinnamic acids has been limited with varying preparation procedures and analytical methods to determine concentrations of these acids. Sun et al. (2002), quantified hydroxycinnamic acids from various feedstocks including wheat straw, rice straw, rye straw, barley straw, maize stems, and poplar wood. The lignin from these feedstocks were extracted using the organosolv process. Table 5 displays the concentrations of ferulic and coumaric acid found in these lignins (R. Sun et al., 2002).

The samples were prepared using the following four steps before high pressure liquid chromatography analysis (HPLC): 1) The samples were heated at 170°C for 2 hr in 4M NaOH; 2) They were then neutralized with 6M HCl to a pH of 2; 3) The samples were then extracted with chloroform; and 4) Quantified by HPLC (R. Sun et al., 2002). The HPLC program was setup using the following method and outlined in Sun et al., 2002: The HPLC column was a Phenomenex Hichrom H5ODC (250 X 4.60mm) and analysis was performed at room temperature. Solvent A was 89% water, 10% methanol, and 1% acetic acid while solvent B was 90% methanol, 9% water, and 1% acetic acid. The gradient was linear with a ramp from 0-40% B (100-60% A) over the course of 31 minutes. The detector was a UV at 320nm.

Table 5. Concentrations of hydroxycinnamic acids from organosolv lignin (wt. % dry basis) adapted from Sun et al. (R. Sun et al., 2002).

Compound	**Wheat Straw**	**Rice Straw**	**Rye Straw**	**Barley straw**	**Maize Stems**	**Poplar Wood**
p-Coumaric Acid	5.50	7.25	5.34	4.36	8.02	0.82
Ferulic Acid	3.22	4.35	5.31	3.28	3.84	0.31

Buranov et al. (2009), determined hydroxycinnamic acid concentration in lignin from flax shives, wheat gran, and corn bran. The samples were prepared using the following method: 1) The samples were heated at 50°C for 4 hr in 0.5M NaOH; 2) and neutralized with 6M HCl to pH of 7; 3) They were then separated with ethanol; 4) and quantified by HPLC (Buranov & Mazza, 2009). The results of this study are displayed in table 6.

The instrumentation system used in Buranov and Mazza's 2009 study was an Agilent 1100 HPLC with a diode array (DAD) detector, pump, and autosampler. The system was controlled by Agilent Chemstation Plus software (Agilent Technologies, Palo Alto, CA). The column used was a Zorbax SB-C18 (5µm, 3 X 250mm) with a guard column (4.6 X 12.5mm, Poly pore CA 10µm). The solvent mobile phases were as follows: Solvent A – 50 µM phosphoric acid and Solvent B – methanol. The gradient was ramped linearly with solvent A from 5-55% over the course of a 51 minute run time, 55-100% from 51 to 61 minutes, 100% from 61 to 68 minutes, 100-5% from 68 to 73 minutes, and at 5% from 73-83 minutes. A diode array detector was used to collect data between 210 and 400nm. The wavelength used for quantitation was 280nm (Buranov & Mazza, 2009).

Table 6. Concentrations of hydroxycinnamic acids in flax shives, wheat bran, and corn bran (wt. % basis). Table adapted from Buranov and Mazza (Buranov & Mazza, 2009).

Compound	**Flax Shives**	**Wheat Bran**	**Corn Bran**
p-Coumaric Acid	0.061	0.02	0.35
Ferulic Acid	0.025	0.391	2.51

Hertog et al. (1992), determined concentration of flavonoids in vegetables and fruits. These compounds are very similar in structure to hydroxycinnamic acids, as they contain two phenolic rings. The methodology used in their study was an extraction of these flavonoids with a strong acid solution (Hertog et al., 1992). Identification and quantification were completed using a HPLC. The following HPLC conditions were used by Hertog et al. (1992): The HPLC instrument was a Kratos (Kratos Analytical Systems, Ramsey, NJ) with a Spectroflow solvent pump and a Linear (Linear Instrument Corp., Reno, NV) Model 204 UV-vis detector at 370nm. The autosampler used for the injections was a Marathon (Spark Holland, The Netherlands) auto injector. The column used was a Nova-Pak C18 (Waters, Milford, MA) (3.9 X 150mm, 4pm) with a Perisorb RP-18 (3.9 X 40mm, 30-40pm) with a flow rate of 0.9mL/min utilizing a mobile phase consisting of either 25% acetonitrile or 45% methanol and 0.025M KH_2PO_4.

Preparation of lignin samples by Hertog et al. (1992) used an acid extraction method for extracting the compounds of interest. The extraction method was completed by

using 40mL of 62.5% methanol and 37.5% water added to 500 mg of lignin sample. An additional 10mL of 6 M hydrochloric (HCl) acid was then mixed into solution and refluxed at 90°C for 2 hr. After the extraction was complete, the sample was made up to 100mLwith methanol and sonicated for 5 minutes followed by filtering for HPLC analysis (Hertog et al., 1992).

Bahri et al. (2012) used HPLC analysis to identify and quantify hydroxycinnamic acids in the leaf tissue of Chicory. The acids were extracted using ethanol and acetic acid followed by HPLC analyses on the extract. The HPLC method was completed using the following parameters (Bahri et al., 2012):

Solvent A = Water

Solvent B = Acetonitrile and 2% Acetic Acid

Column = 150 X 4.6mm, 5uM LiChrospher RP-18 column

UV-vis detector = 280nm

The results for caftaric, chlorogenic, and chicoric acids were reported.

Fritz and Moore (1987), used high-resolution gas chromatography to determine phenolics including hydroxycinnamic acids in lignin (Fritz & Moore, 1987). The hydroxycinnamic acids were extracted using sodium hydroxide and then acidified to a pH of 2.5 and finally washed with ether and dried and combined with acetone for GC injection (Fritz & Moore, 1987). Identification was complete using gas chromatography mass spectroscopy (GC/MS). The GC column used for this study was a 25m X 0.2mm X 0.33µm 5% phenyl methyl capillary column.

The results for coumaric acids in orchard grass was 0.74 wt.% and for ferulic acid content was 1.46 wt.% (Table 7) (Fritz & Moore, 1987).

Table 7. Concentrations of coumaric and ferulic acids found in orchard grass cell walls. Table adapted from Fitz and Moore (Fritz & Moore, 1987).

Compound	Concertation (g/kg)	ppm	wt. %
cis-*p*-coumaric acid	0.75	750	0.075
cis-ferulic acid	0.48	480	0.048
trans-*p*-coumaric acid	6.63	6630	0.663%
trans-ferulic acid	14.15	14150	1.415%

CHAPTER 3

APPROACH AND METHODOLOGY

Problem Statement

Our research objective is centered on the problem of lignin byproduct from cellulosic ethanol. Currently the lignin is combusted and utilized as a heat source or used in small markets as binders. The Midwest region of the United States is known as the “corn belt” and has an abundance of corn stover that can contain as much as 19% lignin (Zhu, Lee, & Elander, 2005). The corn stover processed at Iowa State University contained 15.31% lignin. Lignin production from the cellulosic ethanol process could supply 225 million tons/year by 2030 (Sahoo et al., 2011) with the current selling price of lignin at an estimated $116.00/ton (Strassberger et al., 2014) . Our goal is to upgrade lignin with chemical pretreatment followed by thermochemical processing to produce high value chemicals or precursors. The biorenewable chemicals obtained from lignin upgrading can decrease the U.S. dependency of importing foreign crude oil by supplying renewable chemicals and replacing petrochemicals from crude petroleum. Ultimately, this aligns with the Department of Energy’s (DOE) strategy of “Replacing the Whole Barrel” of products made from crude oil.

Research Objectives

The research objective or goal of the project is to improve product yield of high value aromatics from lignin pyrolysis. This can be accomplished by using a methylation agent and thermochemically processing the sample at high temperatures producing hydroxycinnamic acids.

Hypothesis

Methylating lignin during fast pyrolysis will produce volatile acid methyl esters that can be recovered from coumaric, ferulic and other hydroxycinnamic acids.

Methylation and Pyrolysis of Lignin

Pure model compounds of ferulic and coumaric acid were tested with and without methylation agents. The methylation process is known to hydrolyze esters and methylate the resulting carboxylate ion ($RCOO^-$) that produce corresponding methyl esters ($RCOOCH_3$) that are GC detectable (Fabbri et al., 2007). Figure 12 displays the methylation thermochemical reaction process. The technical lignin from enzymatic hydrolysis and organosolv processes that are byproducts of cellulosic ethanol process were methylated with tetramethylammonium hydroxide (TMAH) and tetra-methyl carbonate of pentaerythritol (PEMC) using double-shot sampler and micropyrolyzer that simulated the fast pyrolysis process on a micro scale. The compounds were identified using a gas chromatography mass spectroscopy (GC/MS) and quantified using a gas chromatograph flame ionization detector (GC/FID). Methylation agent concentration was optimized to increase yields of aromatic acids.

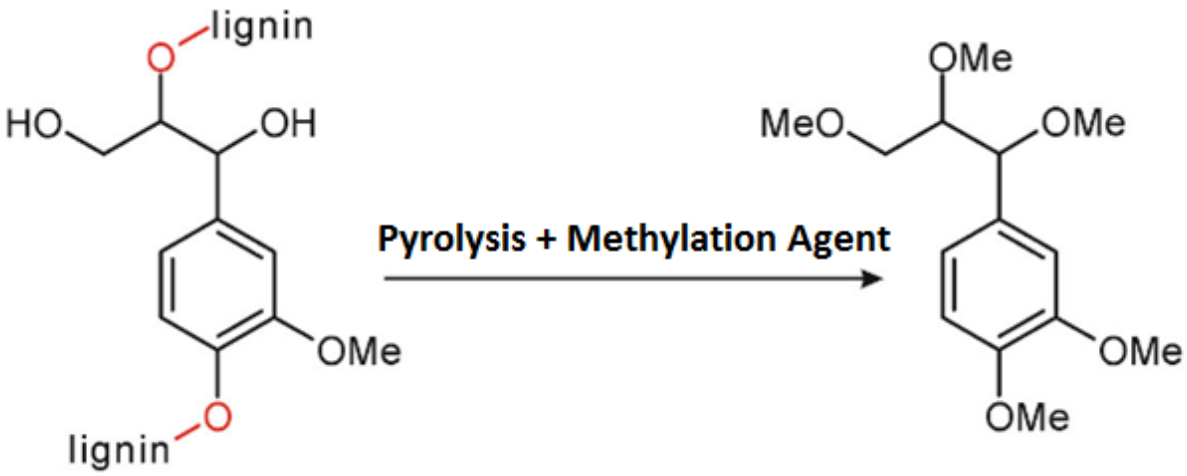


Figure 12. Methylation process adapted from Kaelin et al., followed by pyrolysis to form volatile acid esters (Kaelin, Huggett, & Anderson, 2006).

Methylation and Solvent Liquefaction of Lignin

Pure model compounds of ferulic and coumaric acid were tested with and without methylation agents. The technical lignin from enzymatic hydrolysis and organosolv processes were methylated with tetramethylammonium hydroxide (TMAH) and tetra-methyl carbonate of pentaerythritol (PEMC) using micro reaction sampler and micropyrolyzer that simulated a solvent liquefaction process. The compounds were identified using a gas chromatography mass spectrometer (GC/MS) and quantified using a gas chromatograph flame ionization detector (GC/FID). Methylation agent concentration and residence time was optimized to increase yields of aromatic acids.

Direct Hydroxycinnamic Acid Extraction from Lignin

Extracting the hydroxycinnamic acids from lignin was necessary to determine total extraction yields of these acids. The yields could then be compared to the amounts recovered utilizing the methylation process as previously described. The method conditions for extracting and quantifying these hydroxycinnamic acids use either a base or acid reflux process followed by HPLC analysis (Buranov & Mazza, 2009; Hertog et al., 1992; R. Sun et al., 2002). A 40mL mix of 62.5% HPLC grade methanol and 37.5% 18.2 MΩ deionized water was added with 500mg of lignin in a round bottom flask. Either 10mL of 6N hydrochloric acid or 4N sodium hydroxide was slowly added to the lignin solution depending on if using the acid or alkaline extraction. The lignin was refluxed for two hours at approximately 80°C and made up to 100mL with methanol. The solution was finally shaken and sonicated. After complete mixing the solution was filtered with a 0.45μm glass microfiber filter and analyzed using a high performance liquid chromatograph (HPLC).

Upgrading Hydroxycinnamic Acids to High Value Chemicals

Upgrading hydroxycinnamic acids into high value chemicals such as octinoxate for sunscreens, parabens for preservatives in cosmetics, and vanillin or vanillic acids for food flavoring agents can be achieved by the following routes depicted in figures 13 and 14. The approximated chemical retail costs per kilogram is displayed in table 8.

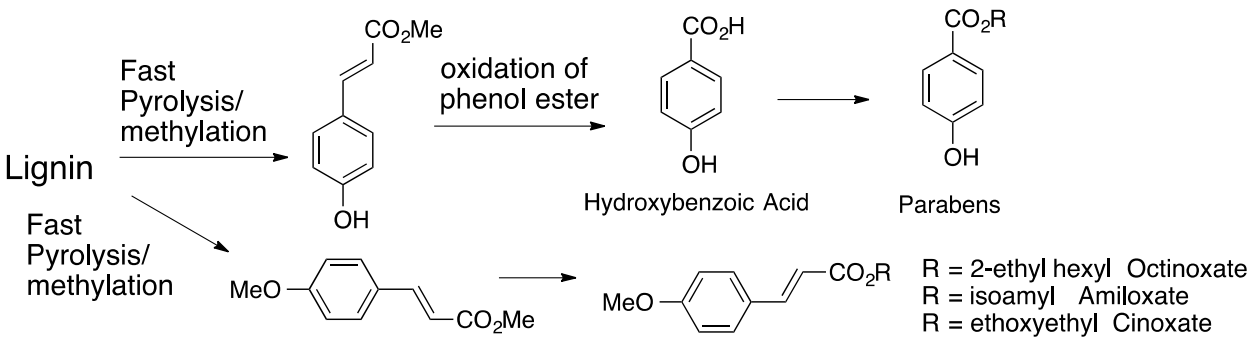


Figure 13. Upgrading coumaric acid to octinoxates and hydroxybenzoic acid (Kraus et al., 2015).

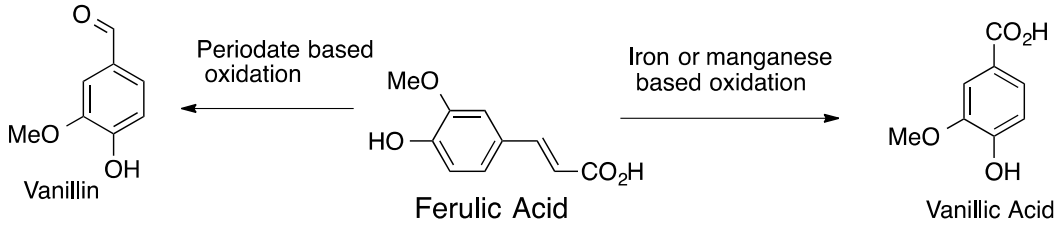


Figure 14. Upgrading ferulic acid to vanillin and vanillic acid (Kraus et al., 2015).

Table 8. Retail prices of commodity chemicals that can be produced from hydroxycinnamic acids ("Sigma Aldrich," 2017).

Chemical	Uses	Price/kg
Vanillin (phenolic aldehyde)	Food Flavoring Additive	$130
Parabens (Esters of Hydroxybenzoic Acids)	Cosmetic Preservatives	$300
Hydroxybenzoic Acid (Salicylic Acid)	Cosmetics – Acne Creams	$600
Vanillic Acid (4-hydroxy-3-methoxybenzoic acid)	Food Flavoring Additive	$900
Octinoxate (Octyl methoxycinnamate)	Sunscreens and Lip Balms	$30,000

CHAPTER 4

MATERIALS AND METHODS

Reagents and Standards

Coumaric and Ferulic acid were purchased from Sigma Aldrich (St. Louis, MO) and had purities of ≥99.0%. All samples and standards solutions were prepared using ultrapure 18.2 MΩ deionized water from a Barnstead E-Pure system Thermo Fisher Scientific (Waltham, MA, USA). The sodium hydroxide used in all experiments was certified 4N with an assay of 3.95−4.05 (Thermo Fisher Scientific). Ultra-high purity (Airgas) gas cylinders of hydrogen and helium with a minimum purity of 99.999% were used for all of the gas chromatography (GC) tests. The Ultra Zero Air (Airgas) had a purity of minimum purity of 99.9997%. All solvents used were HPLC grade and sub-micron filtered from Thermo Fisher Scientific.

Hydroxycinnamic Acid Extraction Method

The alkaline extraction method was used to determine total extraction yields of hydroxycinnamic acids in lignin on a mass basis and was based on Hertog et al. (1992), Sun et al. (2002) and Buranov and Mazza (2009) and is outlined as follows:

Hydroxycinnamic acid extraction and hydrolysis method for high performance liquid chromatography:

1. Methanol and water mix: mix 62.5% HPLC grade methanol and 37.5% 18.2 MΩ water by volume.
2. Add 40mL of the methanol solution to 500mg of lignin.
3. Slowly add 10mL of 4N sodium hydroxide to the solution.
4. Heat to 80°C for 2 hr.
5. Make up to 100mL with HPLC grade methanol and sonicate for at least 5 minutes.
6. Filter 2mL with 0.45um GMF filter into HPLC vial.
7. Quantitative yields using high performance liquid chromatography (HPLC) with diode array detector (DAD).

The samples were refluxed at approximately 80°C for 2 hr using a compact high efficiency reflux condenser tube (figure 15) from Chemglass part # CG-1217-20. A thermocouple attached to a Fluke temperature controller was used to take temperature measurements of the reflux reaction. A picture of the total system is located in figure 16. After refluxing, the samples were prepared for HPLC analysis using the above described extraction method.

Figure 15. High efficiency reflux condenser used to extract hydroxycinnamic acids from lignin ("Chemglass Life Sciences," 2013).

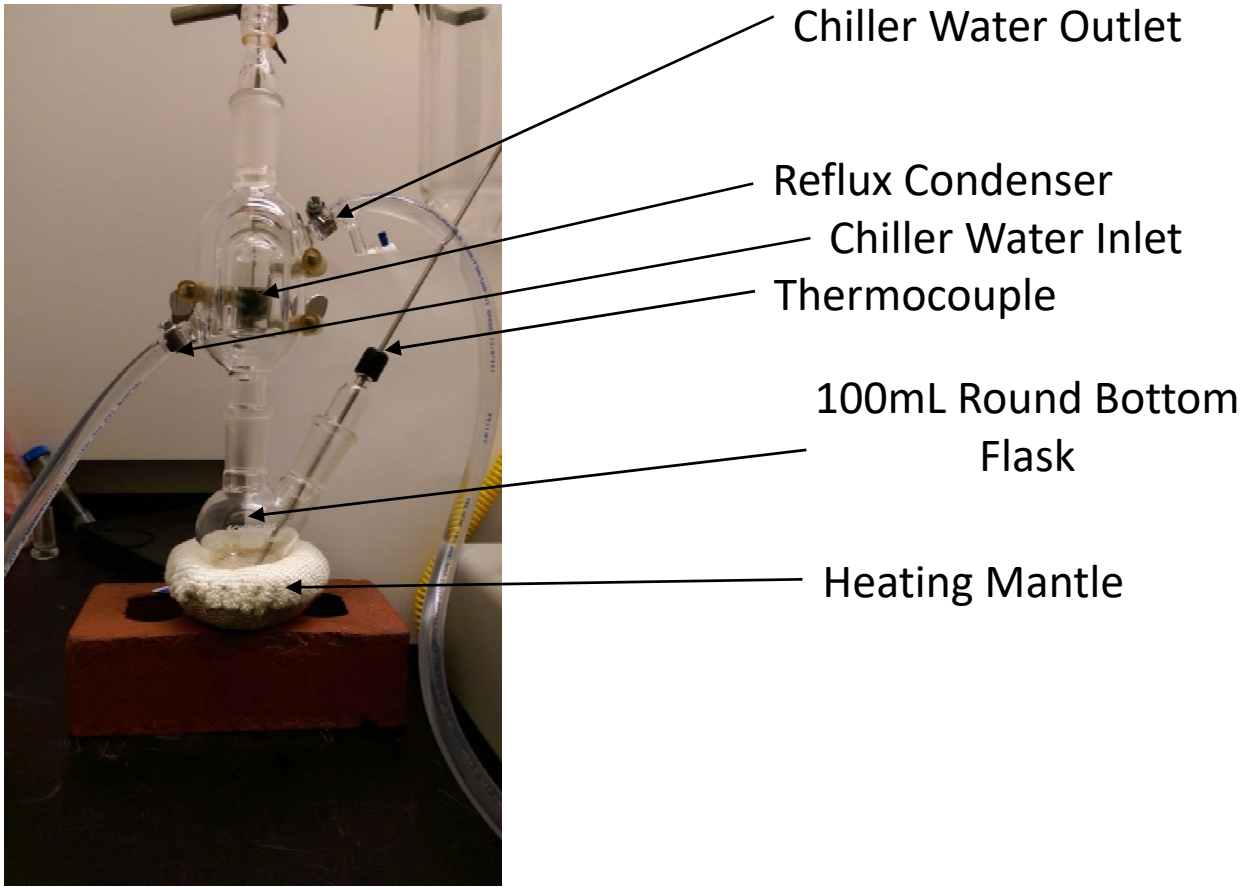


Figure 16. Complete reflux setup for extracting hydroxycinnamic acids from lignin.

Methylation Agents

For this study 25% tetramethylammonium hydroxide (TMAH) in methanol (Sigma-Aldrich) and 50% tetra-methyl carbonate of pentaerythritol (PEMC) in dimethylformamide (DMF) were used as methylation agents at concentrations of 1μL, 5μL, and 10μL. The PEMC, with a projected boiling point of 400°C, was synthesized at Iowa State University by Professor George Kraus, Department of Chemistry. Figure 17 displays the synthesis of PEMC.

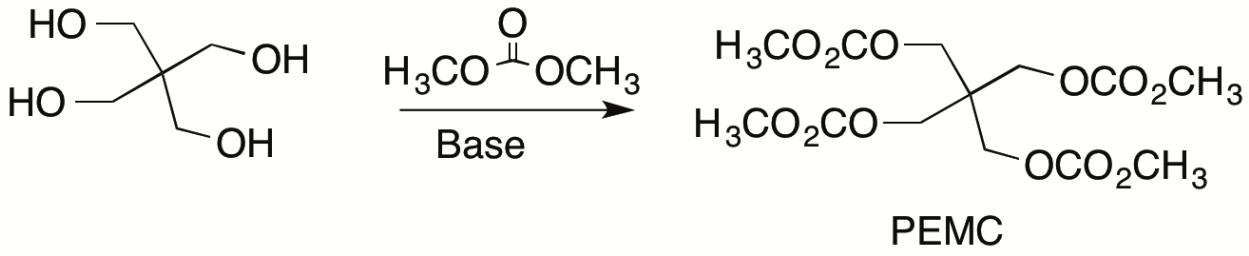


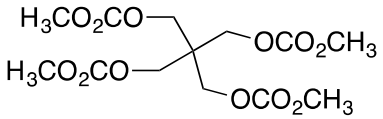
Figure 17. Synthesis of PEMC using pentaerythritol and dimethyl carbonate (Kraus, 2017).

Lignin Samples

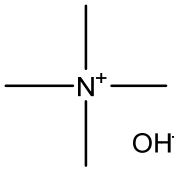
The corn stover lignin samples were obtained from either POET or Archer Daniels Midland (ADM). The POET lignin was from the enzymatic hydrolysis process and the ADM lignin was from the Organosolv/Acetosolv process. The cellulosic acid hydrolysis process typically involves using inorganic acids as a pretreatment step to breakdown the hemicellulose and cellulose into simple carbohydrates that can be readily fermented to alcohols. The Organosolv process also known as Acetosolov uses acetic acid and solvent to solubilize carbohydrate prior to enzymatic hydrolysis.

Methylation of Lignin Samples

Samples were methylated with TMAH or PEMC (figure 18). The methylation process is known to hydrolyze esters and methylate the resulting carboxylate ion ($RCOO^-$) and produce corresponding methyl esters ($RCOOCH_3$) that are GC detectable (Fabbri et al., 2007). The methylation agents TMAH and PEMC were used to methylate the lignin and form the aromatic acid esters, this was completed by adding between 1µL and 10µL of the methylation agent. The amounts of methylation agents were calculated according to Sen et al. (2015) journal article (Sen, Patil, & Argyropoulos, 2015) . It has been established that lignin contains approximately 4.66mmol of phenolic hydroxyl groups (Sen et al., 2015). Previous research published by Sen et al. (Sen et al., 2015) suggested using equivalents of between 1 eq. and 10 eq. of the phenolic hydroxyl group for methylation experiments. The eq. ranges in these experiments were very similar to what was suggested. The TMAH was added at corresponding amounts from ~1.6 eq. – 16.3 eq. based on the phenolic hydroxyl content of the lignin. The PEMC was added at ~1.03 eq. – 10.3 eq. based on the phenolic hydroxyl content of lignin. The PEMC was mixed with DMF at a 50:50 ratio not only to reduce viscosity but to enhance base catalyst activity and the TMAH and methanol was 25:75. Calculations are located in appendix A.



PEMC



Tetramethylammonium hydroxide

Figure 18. Structures of methylation agents used in this study.

Instrumentation

Gas chromatograph-flame ionization detector (GC/FID)

A Bruker 430 gas chromatograph – flame ionization detector (GC/FID) (Billerica, MA, USA) with a PolyArc (Activated Research, Eden Prairie, MN, USA) was used to quantify the hydroxycinnamic acids in the lignin. The column used was a Phenomenex (Torrance, CA, USA) 1701 - 60m length, 0.25um film thickness, and 0.25mm ID. The GC method parameters include an injector temperature of 280°C with the oven programmed to hold at 40°C for 3 minutes and then ramped at 10°C/min to 300°C and held for 4 minutes. The total runtime was 33 minutes with the detector temperature set at 300°C. The helium, hydrogen and air flow rates were 25, 1, and 350mL/min, respectively. A Polyarc (Activated Research, Eden Prairie, MN, USA) system was used in conjunction with the FID to convert all compounds into methane and keep the relative retention times of the individual compounds the same. The supplemental hydrogen and air flowrates were made up with the PolyArc flows set at 35mL/min and 2.5mL/min, respectively. The overall flows to the FID detector were 36mL/min of hydrogen and 352.5mL/min of zero air. Galaxie® software was used to identify and quantitatively determine concentrations.

Polyarc

The PolyArc (Activated Research, Eden Prairie, MN, USA) system contains a catalytic bed that is heated to 450°C. This system converts all organic compounds to methane before the FID detector. The relative retention time and elution order of the compounds stay the same. The hydrogen and air flow rates were 35mL/min and

2.5mL/min. Figure 19 displays a graphic of the Polyarc GC/FID system compared to a typical GC/FID. As you can see by the figure the Polyarc system is setup between the column and the FID, thus allowing the retention times to stay very similar to the retention time of the compounds on the standard GC/FID system. The equation used to calculate the concentration when using the Polyarc with a FID is as follows (Beach et al., 2016; Jones, 2016; Maduskar et al., 2015).

$$CA = \mathrm{CS}\left(\frac{\mathrm{AreaA}}{\mathrm{AreaS}}\right)\left(\frac{\mathrm{MwA}}{\mathrm{MwS}}\right)\left(\frac{\#\mathrm{CS}}{\#\mathrm{CA}}\right)$$

Where:

C_A = The mass concertation of the analyte.

C_S = The mass concentration of the standard

$Area_A$ = Integrated peak area of the analyte

$Area_S$ = Integrated peak area of the standard

Mw_A = Molecular weight of the analyte

Mw_S = Molecular weight of the standard

$\#C_S$ = Number of carbon atoms per molecule of standard

$\#C_A$ = Number of carbon atoms per molecule of analyte

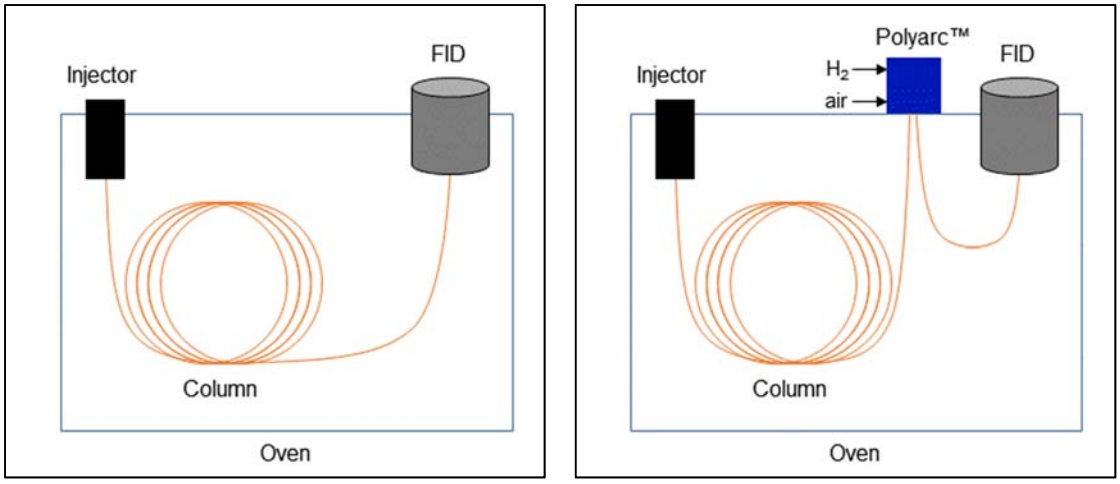


Figure 19. A) Typical standard GC/FID system. B) GC/FID system with Polyarc ("Activated Research Company," 2017)

Gas chromatograph/mass spectrometer (GC/MS)

An Agilent 7980 gas chromatograph (GC) with a 5977 Mass spectrometer (MS) (Santa Clara, CA, USA) with an additional extractor source was used to identify the hydroxycinnamic acids. This GC/MS instrument had a Phenomenex (Torrance, CA, USA) ZB-1701 60m length, 0.25um film thickness, and 0.25mm ID. The GC method parameters were as follows: the injector temperature was 280°C, with an oven program to hold at 40°C for 3 minutes followed with a ramp of 10°C/min to 300°C and hold of 4 minutes. The total runtime was 33 minutes and the detector temperature was set at 300°C. Agilent MassHunter® qualitative software was used to identify retention times and determine the peak areas.

Micropyrolyzer

A Frontier 3030D micropyrolyzer (Saikon, Koriyama, Fukushima, Japan) was used on both chromatographs - GC/MS and GC/FID. The pyrolysis tests were performed at 500°C unless otherwise noted with an interface temperature of 300°C. The

micropyrolyzer was equipped with two different samplers. The first sampler, standard double shot, was used to pyrolyze the lignin sample in a small stainless steel cup that was held above the heated zone with a small hook until the test was started. The diagram is outlined in figure 20. The double shot sampler could also utilize a long hook that could be lowered into the furnace with the cup attached and extracted after the test was finished. The second type of sampling system used for the micropyrolyzer was the online micro reaction sampler (figure 21). This sampler enables the sample to be sealed in a glass vial and heated with pyrolysis temperatures and a solvent liquefaction regime (figure 22). An ice bath was used to keep the methylation agent from volatilizing during the sample preparation. The residence times of the solvent can be varied using this type of sampler. The visual depiction of the complete system attached to a gas chromatograph is shown in figure 23.

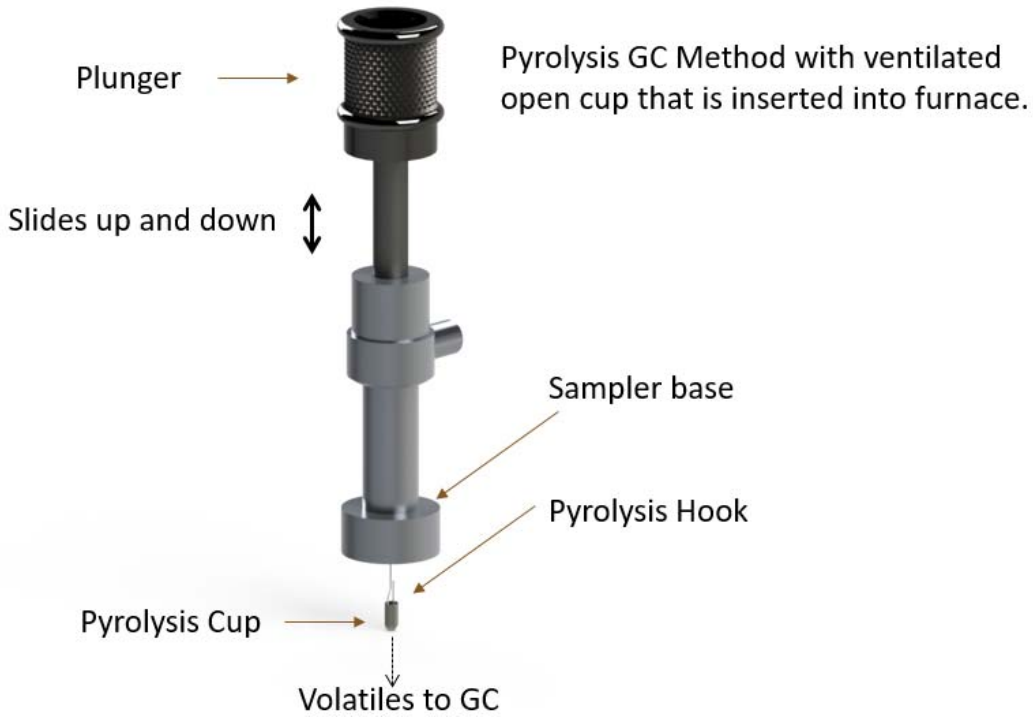


Figure 20. Schematic of the micropyrolzer double-shot sampler for pyrolysis cup method.

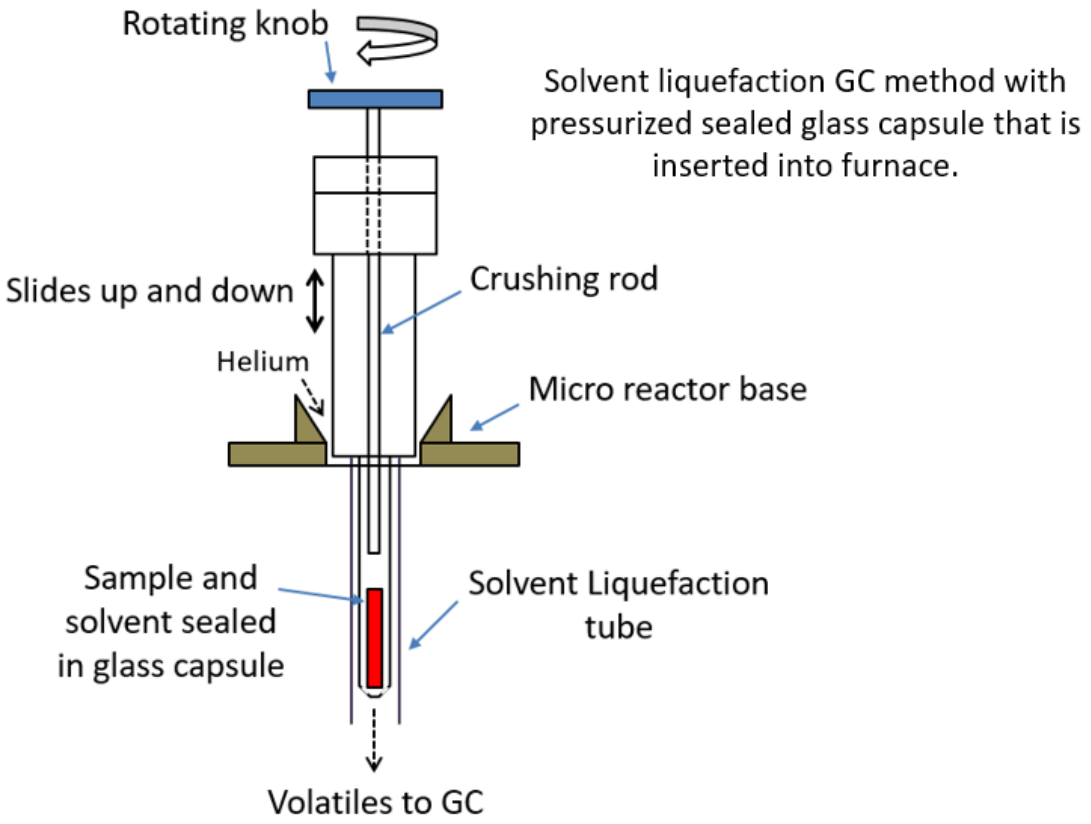


Figure 21. Micropyrolzer micro reaction sampler for micro reaction method.

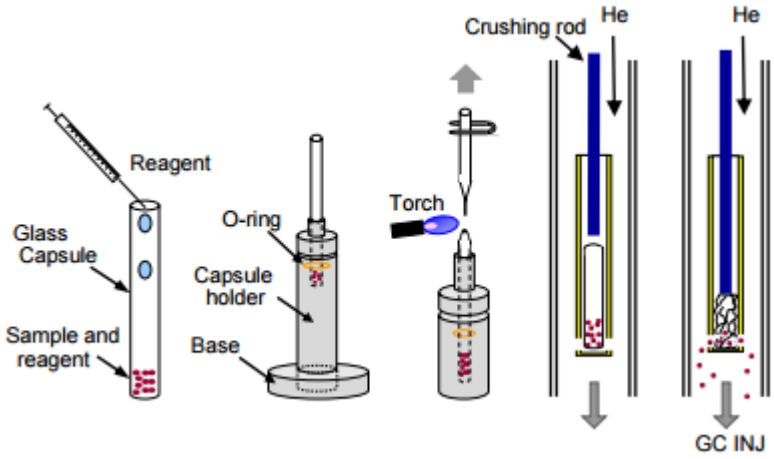


Figure 22. Sample preparation steps for micro reaction method from Frontier Laboratories LTD ("Frontier Laboratories On-line Micro Reaction Sampler ", 2017)

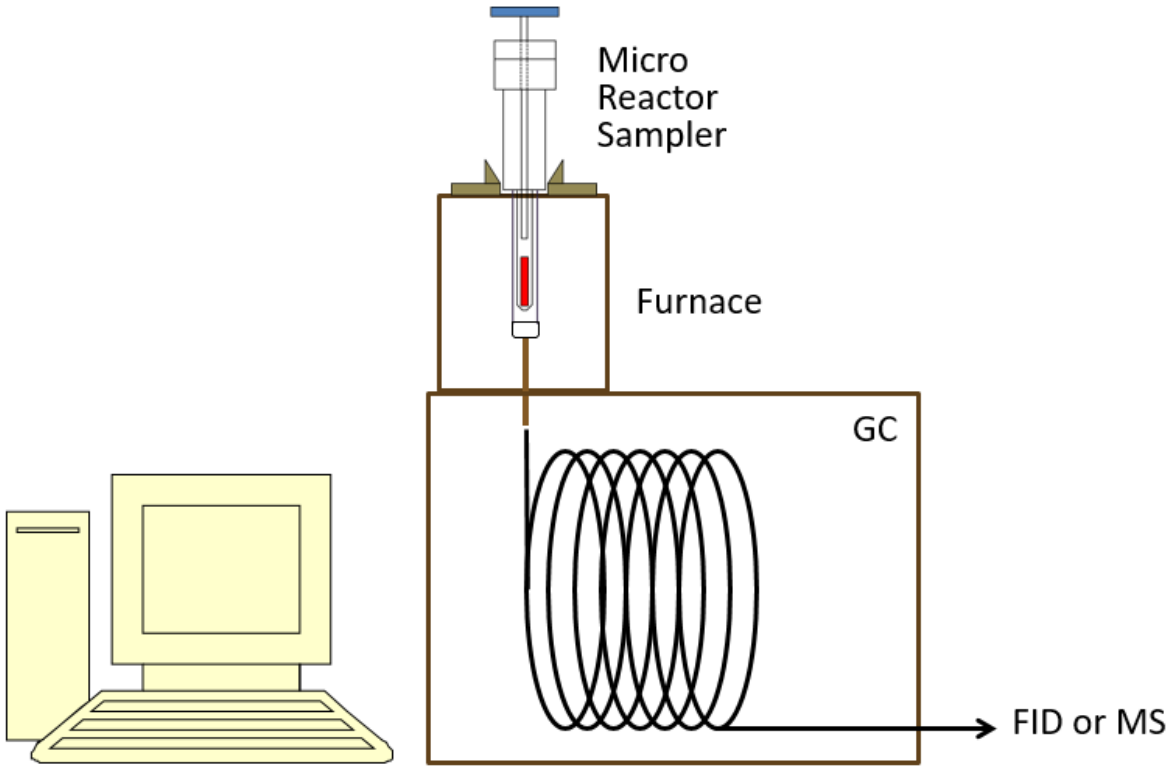


Figure 23. Micropyrolyzer attached to gas chromatography/mass spectrometer (GC/MS) or flame ionization detector (GC/FID).

High performance liquid chromatograph (HPLC)

The HPLC system used for the experiments was a Thermo Fisher Scientific/Dionex Ultimate 3000 LC system (Sunnyvale, CA, USA) with a quaternary analytical pump and a Diode Array Detector (DAD). The analytical column used was a 300mm x 7.7mm ID 8µm, EVO C18 (Phenomenex). The instrument parameters for the EVO C18 were as follows: the mobile phase was ultrapure 18.2 MΩ deionized water and 0.1 % formic acid and acetonitrile with a starting concentration of 95% water and 5% acetonitrile ramped to 100% acetonitrile, with a flowrate of 0.5mL/min, and column temperature of 30°C.

The DAD, set at 263nm, was used to determine hydroxycinnamic acid concentration. The HPLC was calibrated with a complete set of standards using ferulic acid and *p*-coumaric acid. The calibration curves contained 10 points - each concentration performed in duplicate. The range of the standards was approximately 100 – 2500 ppm. The coefficient of determination for both standards was >0.99 on the DAD. The wavelength that was used for quantification was 263nm.

Thermal Gravimetric Analyzer (TGA)

A Mettler Toledo TGA/DSC1 (Columbus, OH, USA) was used for proximate analysis of lignin samples. The modified ASTM method determined moisture content, volatile content, fixed carbon, and ash in the lignin feedstocks. The samples were tested in triplicate. The moisture results were used to determine the hydroxycinnamic acid content on a dry basis. The temperature profile/method for the TGA is displayed in table 9. At the beginning of the TGA tests, the sample is in an inert environment (nitrogen) for determination of the moisture and volatiles. At the end of the test, after a 20 minute hold at 900°C, the atmosphere is switched to air for determination of fixed carbon and ash.

Table 9. TGA method/program for proximate analysis.

Temperature Start (°C)	Temperature End (°C)	Temperature Ramp (°C/min)	Hold time (min)	Gas	Flow Rate (mL/min)
25	105	10	40	Nitrogen	100mL/min
105	900	10	20	Nitrogen	100mL/min
	900		30	Air	100mL/min

Elemental Analyzer

An Elementar vario MICRO cube elemental analyzer (Langenselbold, Hesse, Gemany) was used to characterize carbon, hydrogen, nitrogen, and sulfur (CHNS) content in lignin feedstocks. The CHNS content is usually described as ultimate analysis. The temperature settings and flow rates on this instrument were as follows: the temperature of the reduction tube was 1050°C and the combustion tube was 850°C. A thermal conductive detector (TCD) is used to quantify the amounts of carbon, hydrogen, nitrogen. An infrared (IR) detector is used to quantify sulfur. The complete list of parameters of the ultimate analysis method are in table 10.

Table 10. Elemental analyzer method parameters for CHNS ultimate analysis.

Temperature (°C)		
	Combustion Tube	1150
	Reduction Tube	850
	Ads.col.standby	40
	Ads.col.cooltemp	90
Time values (seconds)		
	Flush time	10
	O2 Delay	20
	Integrator reset delay peak N	10
	Integrator reset delay peak C	1
	Integrator reset delay peak H	1
	Integrator reset delay peak S	2

Table 10. (Continued)

Ball valve		
	Min. permitted ball valve current	20
	Max. permitted ball valve current	100
	Critical ball value	250
	Acoustic signal	no

Design of Experiments

A design of experiments was completed to determine qualitative peak identification and the starting conditions for this research. All tests were performed on an Agilent Technologies (Santa Clara, CA, USA) gas chromatograph with a quadrupole mass spectrometer (GC/MS). The design of experiments (DOE) was produced using JMP Pro 12 software. The experiments consisted of three loading concentrations of 1, 5, and 10μL and multiple residence times for the micro reaction tests. Following this initial set of experiments all other experiments were tested at 500°C with 1, 5, and 10μL of methylation agent making sure the equivalents (eq.) amounts corresponded correctly to the resulting phenolic groups in the lignin samples. The same method that was used on the GC/MS was implemented on a GC/FID with the PolyArc detector for quantification.

CHAPTER 5

RESULTS AND DISCUSSION

Proximate Analysis of Lignin

Enzymatic hydrolysis lignin and organosolv lignin were tested for proximate analysis using a TGA. The moisture results of this test were used to calculate the hydroxycinnamic acid content on a dry basis. The complete proximate analysis results are located in table 11. At the beginning of the TGA tests, the sample is in an inert environment (nitrogen) for determination of the moisture and volatiles. After a 20 minute hold at 900°C the atmosphere is switched to air for determination of fixed carbon and ash. The results show the organosolv lignin contained more moisture and fixed carbon content while the enzymatic hydrolysis lignin contained more volatiles and ash.

Table 11. TGA Proximate analysis of lignin.

Lignin Sample	Moisture %	Volatiles %	Fixed Carbon %	Ash %
Enzymatic Hydrolysis	3.41	60.25	22.85	13.49
Organosolv	6.10	57.45	30.11	6.33

Ultimate Analysis of Lignin

Ultimate analysis of the lignin samples was performed on an Elementar CHNS instrument. The results of the carbon, hydrogen, nitrogen and sulfur in the lignin feedstocks are located in table 12.

From these results we can conclude that the enzymatic hydrolysis lignin contained more carbon which correlates to the proximate analysis results. The hydrogen and nitrogen were very similar. The acid hydrolysis lignin had significantly higher sulfur results which is expected because of the sulfuric acid pretreatment in the enzymatic hydrolysis process.

Table 12. Ultimate analysis of lignin

Lignin Sample	Carbon %	Hydrogen %	Nitrogen %	Sulfur %
Enzymatic Hydrolysis	45.11	4.514	1.66	0.351
Organosolv	54.39	4.892	1.18	0.185

Hydroxycinnamic Acid Extraction Results from HPLC

The lignin samples were extracted with either an acid or base extraction method. For the acid method, 6N hydrochloric acid was used for the extraction of the enzymatic hydrolysis lignin and the organosolv lignin (figure 24). The samples were analyzed and quantified using HPLC. The hydroxycinnamic acid concentrations for both lignin types from the acid extraction method were unrealistic and actually less than the amounts produced from pyrolysis. This gives evidence that the acid extraction method was not effective, ultimately resulting in an incomplete extraction. After further investigation, the acid method was discontinued and all hydroxycinnamic acid concentrations were obtained using the alkaline extraction method, unless otherwise noted.

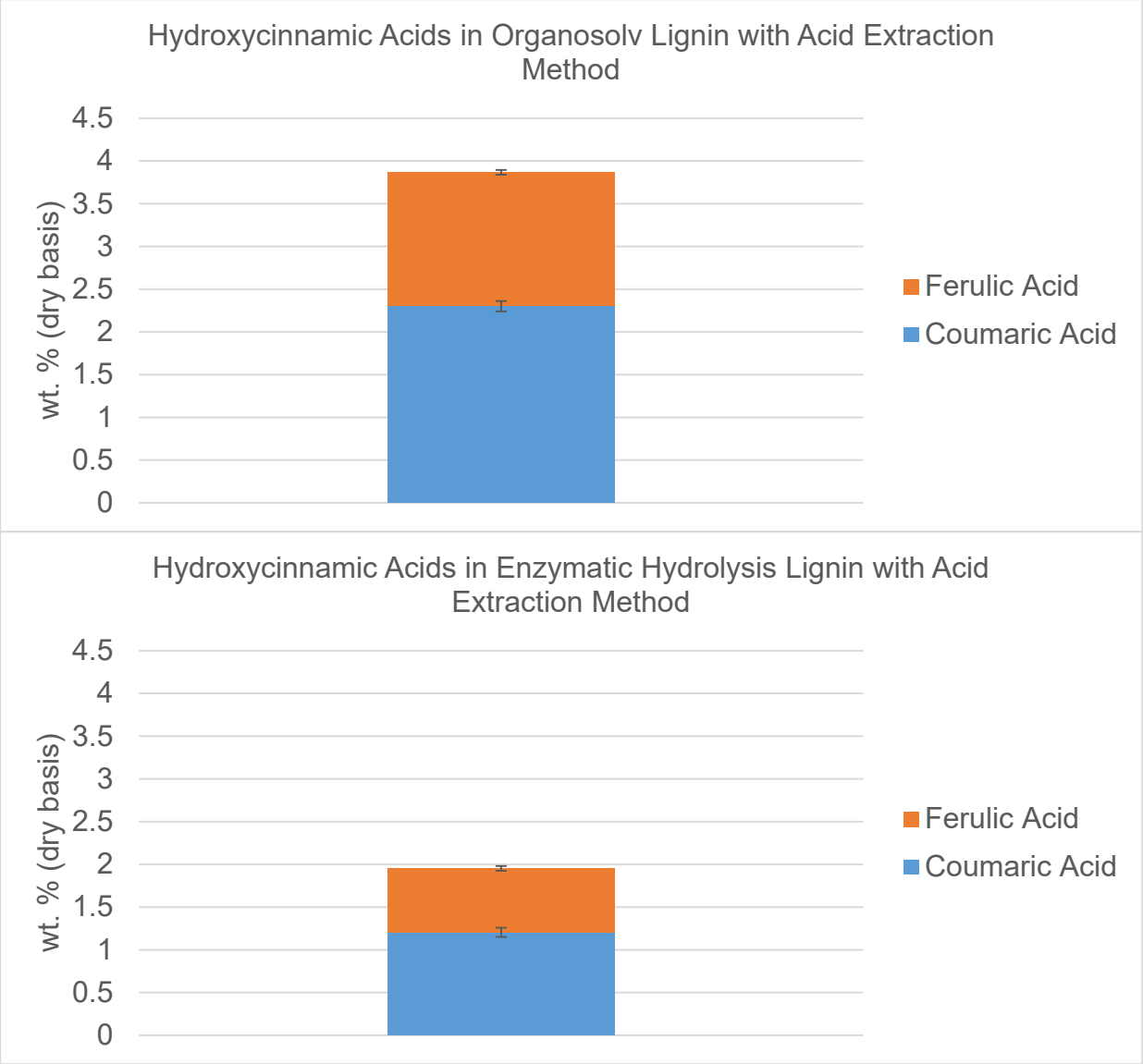


Figure 24. Acid extraction results for lignin samples.

The hydroxycinnamic acids were extracted using the alkaline extraction method with 4N sodium hydroxide and the results correlated well with results previously published in literature (figure 25). The total extraction yields of hydroxycinnamic acids on a mass basis in the enzymatic hydrolysis lignin using this extraction method were on average, 7.51% (5.65% coumaric and 1.86% ferulic). The total extraction yields of hydroxycinnamic acids on a mass basis in the organosolv lignin using the alkaline extraction method were on average 10.58% (7.71% coumaric and 2.87% ferulic). As described above, lignin was refluxed for 2 hr in 10mL of 4N sodium hydroxide and 40mL of 37.5% methanol and 62.5% water solution. The end product was diluted to a 100mL with HPLC grade methanol and filtered to 0.45 microns prior to HPLC analysis. After refluxing the liquid portion was decanted off. The initial lignin was extracted a second time to determine if the alkaline method was suitable. To determine whether this extraction method was able to completely remove hydroxycinnamic acids from lignin, a second test (after initial liquid was decanted) with the same lignin (after the initial reflux) was refluxed again using the same method parameters as described previously. The results from the second extraction showed negligible hydroxycinnamic acids by HPLC. Therefore, this method was used to determine the total extractable yields on a mass basis of hydroxycinnamic acid in lignin.

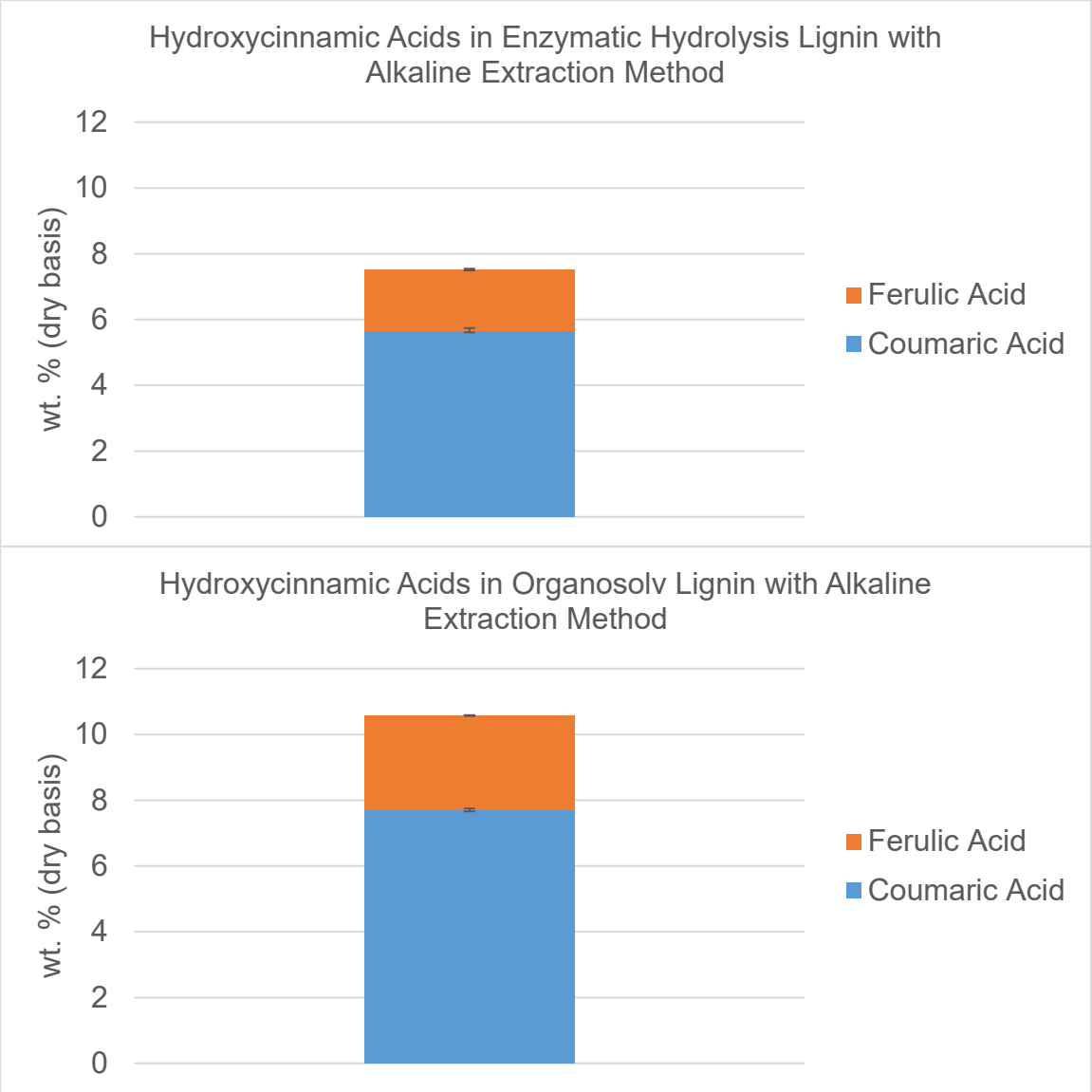


Figure 25. Alkaline extraction results for lignin samples.

Methylation of Pure Compounds

For determination and identification of GC detectable aromatic acid esters, testing was performed using pure compounds of coumaric and ferulic acids on a gas chromatograph/mass spectrometer (GC/MS) and micropyrolyzer. These tests would help to better understand the final products from methylation and pyrolysis. When using

a methylation agent such as tetramethylammonium hydroxide (TMAH) the compounds in figure 26 can be formed.

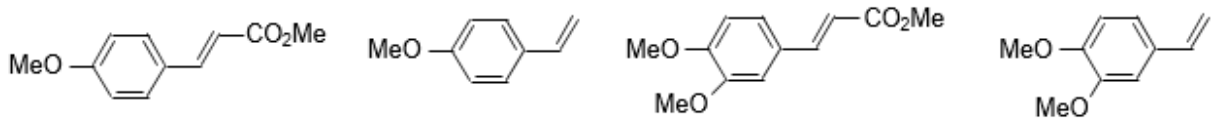


Figure 26. Compounds formed during 500°C micropyrolysis of pure coumaric and ferulic acid using a Frontier micropyrolyzer in the presence tetramethylammonium hydroxide (Kraus et al., 2015).

Initial testing was also performed on enzymatic hydrolysis lignin for the proof of concept that hydroxycinnamic acid esters could be produced from methylating lignin. A comparison of the lignin control and the lignin methylated with 5μL of tetramethylammonium hydroxide and then pyrolyzed at 500°C is displayed in figure 27. The compounds were identified using GC/MS and it was determined that the aromatic esters of the hydroxycinnamic acids were formed when using a methylation agent and the phenolics found in then initial control sample disappeared. The mechanism of the methylation of lignin occurs when the methylation agent cleaves the β-O-4 bonds resulting in the production of aromatic acid esters. In the absence of methylation, the pyrolysis of lignin will produce phenolics by decarboxylation. Figure 28 displays a representation of the mechanism of lignin during the methylation process. This proves the hypothesis that during methylation and the thermochemical processing of lignin hydroxycinnamic acid esters can be produced.

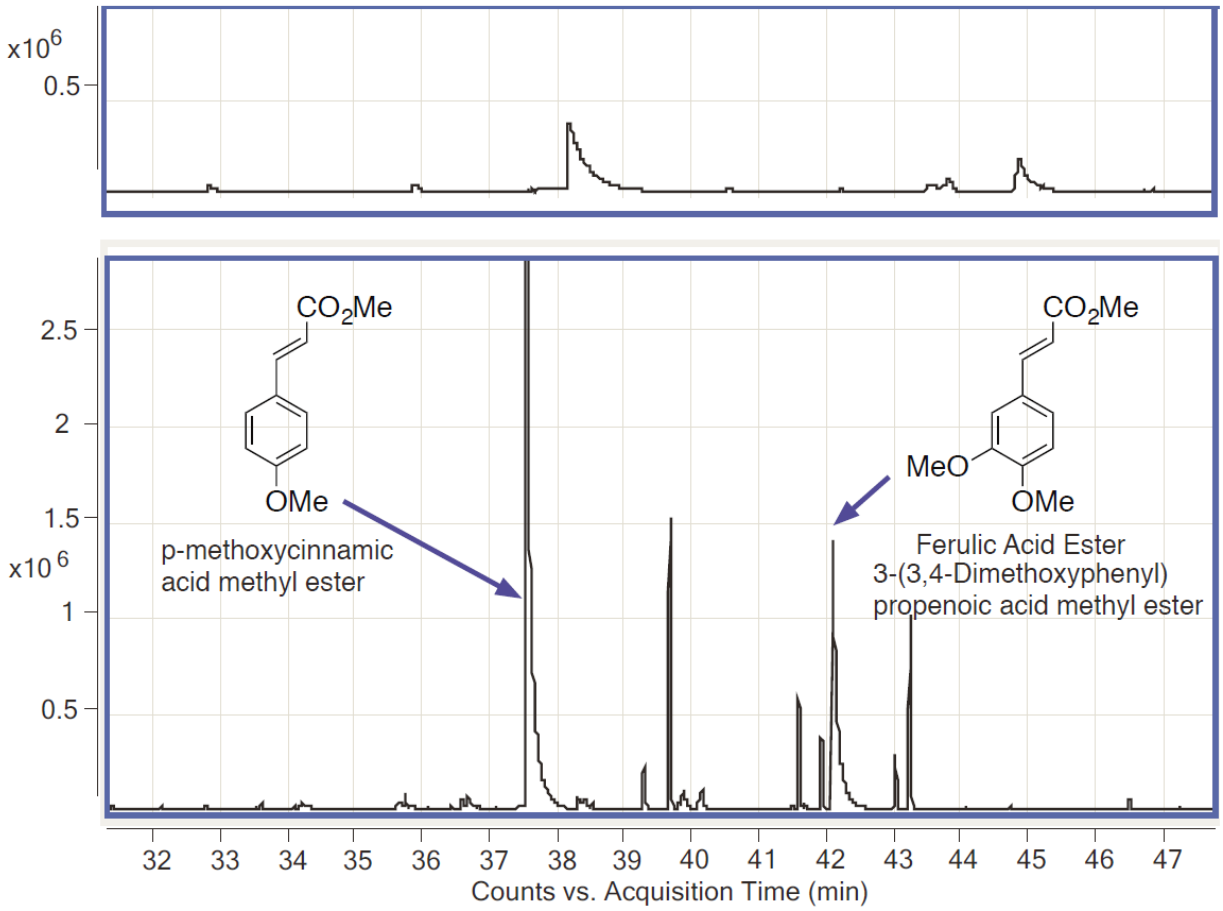


Figure 27. GC/MS chromatogram of A) lignin control pyrolyzed at 500°C and B) lignin pyrolyzed at 500°C with 5µL of tetramethylammonium hydroxide.

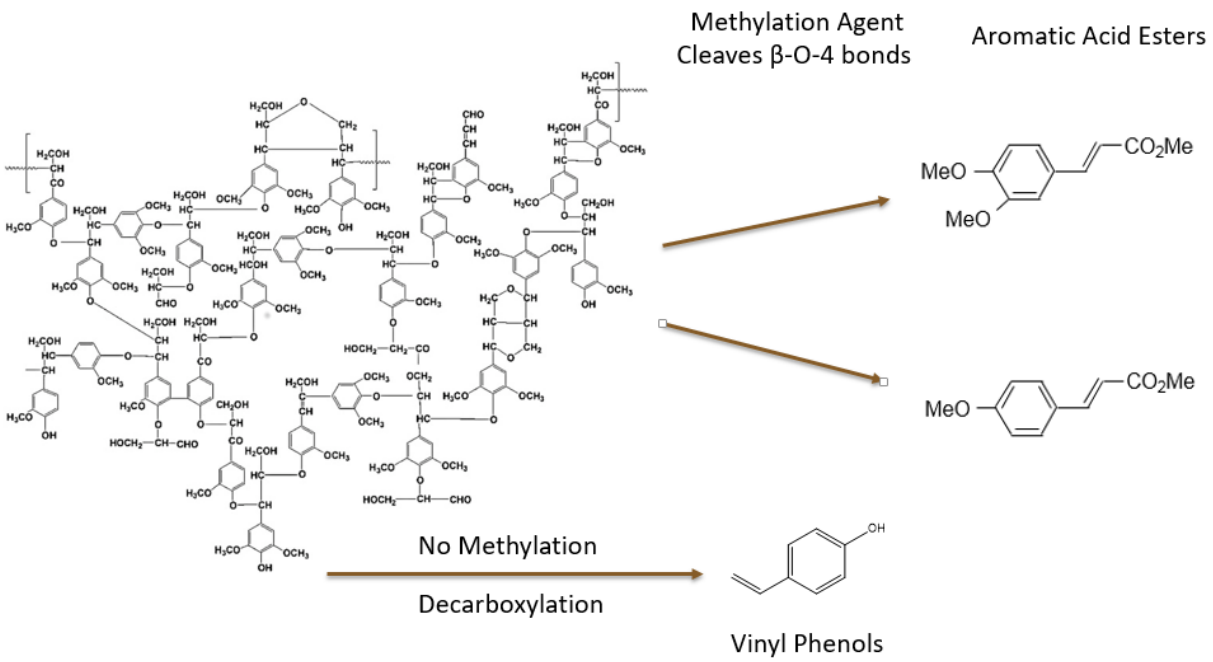


Figure 28. Partial lignin structure modified from Floch et al. (Le Floch, Jourdes, & Teissedre, 2015). Methylation of lignin during pyrolysis forms aromatic esters. If methylation does not occur the resulting pyrolysis compounds are phenols from decarboxylation.

GC/FID Micropyrolysis Results with Double-Shot Sampler

After initial qualitative analysis with GC/MS, quantitation of hydroxycinnamic acids was achieved by using a gas chromatograph/flame ionization detector (GC/FID) and a Polyarc system. The Polyarc as describe above converts all compounds into methane that can easily be quantified with a FID.

The enzymatic hydrolysis lignin and organosolv lignin hydroxycinnamic acid results after methylation followed by pyrolysis with 1µL, 5µL, and 10uL of TMAH are displayed below (Figures 29 and 30). The results where compared to the total yields of lignin from the alkaline extraction method. The recovered amounts of hydroxycinnamic acids from the micropyrolyzer compared to the total extraction yields are shown in table 13. What

we can conjecture from these test results is that the recovery of hydroxycinnamic acid from methylation and micropyrolysis are most significant in the 10μL loading of TMAH. At this level, we are recovering most of the total extractable amounts of the hydroxycinnamic acids. The enzymatic hydrolysis lignin and organosolv lignin hydroxycinnamic acid results, after methylation followed by pyrolysis with 1μL, 5μL, and 10uL of PEMC, are displayed below (Figures 31 and 32). Again loading the PEMC methylation agent at 10μL appeared to have the largest net positive effect on producing hydroxycinnamic acids from lignin. Table 14 displays the results for the PEMC methylation tests followed by pyrolysis.

In general, the pyrolysis regime highest recoveries were at the 10μL loading level with both TMAH and PEMC. The outcome of these tests were as expected—improved β-O-4 bond cleavage in lignin with increased amounts of methylation agent loading. The recoveries of the hydroxycinnamic acids from enzymatic hydrolysis lignin pyrolysis with 10μL TMAH produced coumaric and ferulic acid concentrations of 84% and 69%, respectively. The recoveries of the hydroxycinnamic acids from organosolv lignin pyrolysis with 10μL TMAH produced coumaric and ferulic acid concentrations of 89% and 64%, respectively. The recoveries of the hydroxycinnamic acids from enzymatic hydrolysis lignin pyrolysis with 10μL PEMC produced coumaric and ferulic acid concentrations of 85% and 85%, respectively. The recoveries of the hydroxycinnamic acids from organosolv lignin pyrolysis with 10μL PEMC produced coumaric and ferulic acid concentrations of 54% and 97%, respectively. This loading level would equal 16.3 eq. TMAH and 10.3 eq. PEMC that would correspond to the reacting phenolic hydroxyl group in the lignin samples.

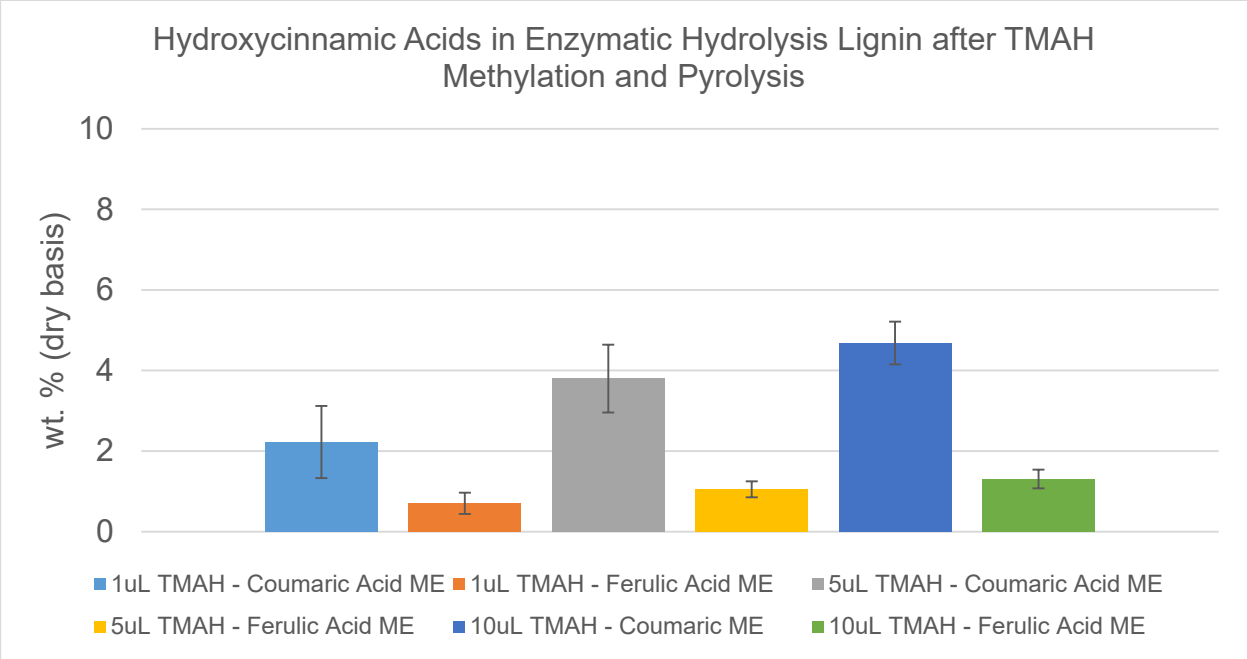


Figure 29. Hydroxycinnamic acids recovered from enzymatic hydrolysis lignin pyrolysis with TMAH as the methylation agent.

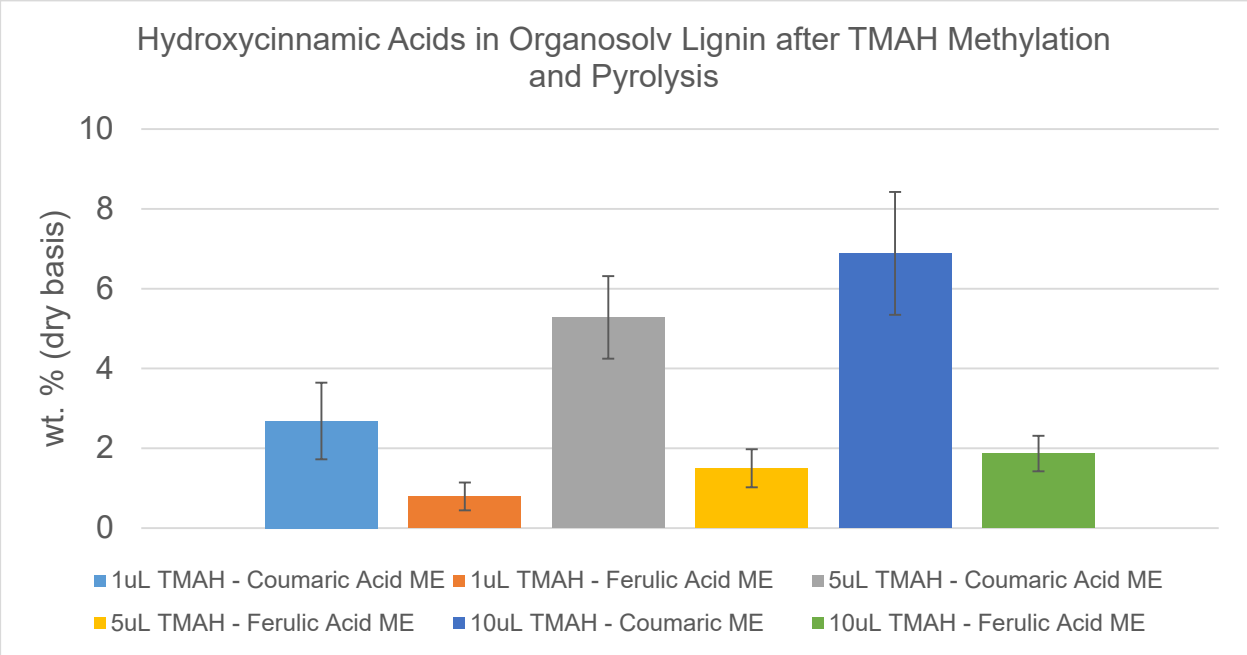


Figure 30. Hydroxycinnamic acids recovered from organosolv lignin pyrolysis with TMAH as the methylation agent.

Table 13. Recovery yield comparison of hydroxycinnamic acids from TMAH methylation and pyrolysis of enzymatic hydrolysis lignin and organosolv lignin.

Lignin Type	Coumaric Acid from Acid Extraction (db)	Ferulic Acid from Acid Extraction (db)	TMAH Amount	Recovery % Coumaric Acid from Pyrolysis	Recovery % Ferulic Acid from Pyrolysis
Enzymatic Hydrolysis Lignin	5.6%	1.9%	1uL 5uL 10uL	40% 68% 84%	37% 55% 69%
Organosolv lignin	7.7%	2.9%	1uL 5uL 10uL	35% 69% 89%	27% 52% 64%

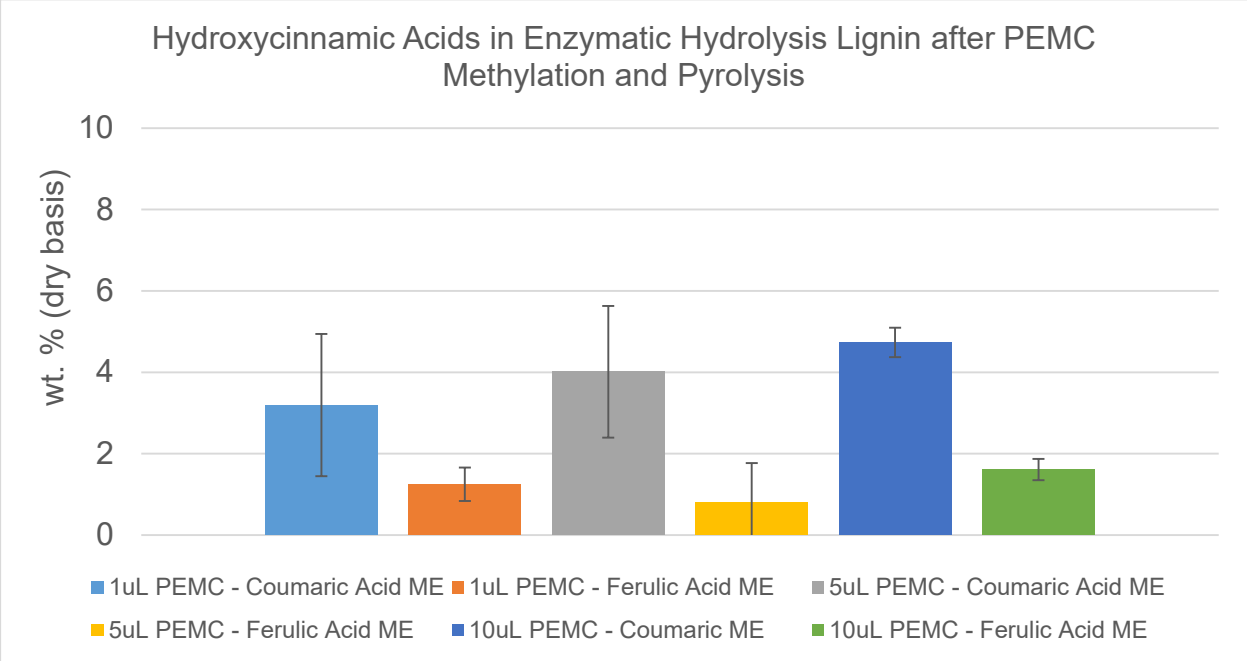


Figure 31. Hydroxycinnamic acids recovered from enzymatic hydrolysis lignin pyrolysis with PEMC as the methylation agent.

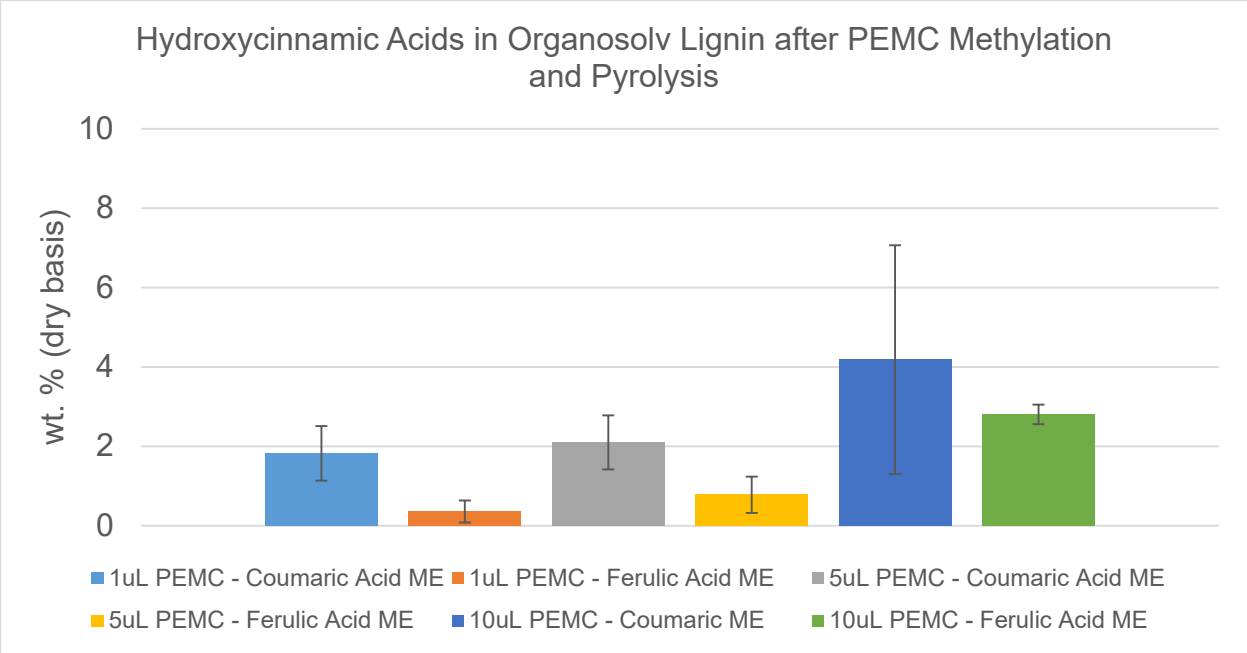


Figure 32. Hydroxycinnamic acids recovered from organosolv lignin pyrolysis with PEMC as the methylation agent.

Table 14. Recovery yield comparison of hydroxycinnamic acids from PEMC methylation and pyrolysis of enzymatic hydrolysis and organosolv lignin.

Lignin Type	Coumaric Acid from Acid Extraction (db)	Ferulic Acid from Acid Extraction (db)	PEMC Amount	Recovery % Coumaric Acid from Pyrolysis	Recovery % Ferulic Acid from Pyrolysis
Enzymatic Hydrolysis Lignin	5.6%	1.9%	1uL 5uL 10uL	57% 72% 85%	66% 42% 85%
Organosolv lignin	7.7%	2.9%	1uL 5uL 10uL	24% 27% 54%	12% 27% 97%

GC / FID Micropyrolysis Results with Micro Reaction Sampler

The Micro Reaction sampler was used to simulate the solvent liquefaction where residence time (exposure) in the solvent could be controlled. From previous testing the lowest residence (lowest exposure in the furnace) was optimum for production of

aromatic acid esters of hydroxycinnamic acids. All parameters (temperatures, methylation agent concentration, and GC method) were kept the same as with the previous tests with the micropyrolyzer and double-shot sampler. The results for the acid hydrolysis lignin using TMAH as the methylation agent with the micro reaction method are in figure 33 and the organosolv lignin results are in figure 34. Recovery yields for both types of lignin show very little statistical difference. Table 15 displays the recovery yields and the test parameter used in these trials. The overall best average results for the enzymatic hydrolysis lignin was with 10uL loading producing recoveries of 97% and 75% of coumaric and ferulic acid, respectively. The best averages for the organosolv lignin was at the 1uL loading, although there was no significant statistical difference between all methylation concentrations. The recoveries of the hydroxycinnamic acids from enzymatic hydrolysis lignin using PEMC at 1μL produced recoveries of 88% coumaric and 90%. The recoveries with 5μL PEMC produced coumaric and ferulic acid concentrations of 40% and 55%, respectively. The recoveries with 10μL PEMC produced coumaric and ferulic acid concentrations of 55% and 51%, respectively. Figures 35 and 36 display the concentrations obtained from the micro solvent liquefaction tests. Table 16 display the yields recovered when compared to the total mass yields of hydroxycinnamic acid in lignin. The recoveries of the hydroxycinnamic acids from organosolv lignin using PEMC at 1μL produced recoveries of 41% coumaric and 45% ferulic acid. The recoveries with 5μL PEMC produced coumaric and ferulic acids concentrations of 34% and 32%, respectively. The recoveries with 10μL PEMC produced coumaric and ferulic acid concentrations of 75% and 79%, respectively.

In general, the overall hydroxycinnamic acid averages were more variable using the solvent liquefaction regime. The best net recoveries for enzymatic hydrolysis lignin using TMAH were at the 10µL loading level and for PEMC it was the 1µL loading level. These loading levels would equal 16.3 eq. TMAH and 1.03 eq. PEMC that would react with the corresponding phenolic groups in the lignin. The best net recoveries for the organosolv lignin using TMAH was at the 1µL loading level. The best net result for using PEMC were at the 10µL loading level. These loading levels would equal 1.6 eq. TMAH and 10.3 eq. PEMC that would react with the corresponding phenolic groups in the lignin. The outcome from this testing indicates marginal higher net hydroxycinnamic acid recovery using the micro reaction sampler compared to the typical pyrolysis cup method. The possibility exists that the results are higher during the solvent liquefaction regime because the methylation agent had a slightly longer exposure time to the lignin biomass in the furance. This does not explain the variability within the results using the micro reaction sample. There was not a clear loading concentration that could be defined as optimal for both lignin types. This could be due to multiple factors during the sample preparation and analytical analysis of the lignin samples. One possible cause is the fact that the lignin feedstocks are non-homogeneous and the samples are on a microgram scale utilizing a glass reaction vial that is 2mm x 30mm (ID x L). Any small variation within the sample could skew the results. Overall, solvent liquefaction is a more demanding process and even with the marginal increased hydroxycinnamic acid recovery, the economics may be better using the pyrolysis process.

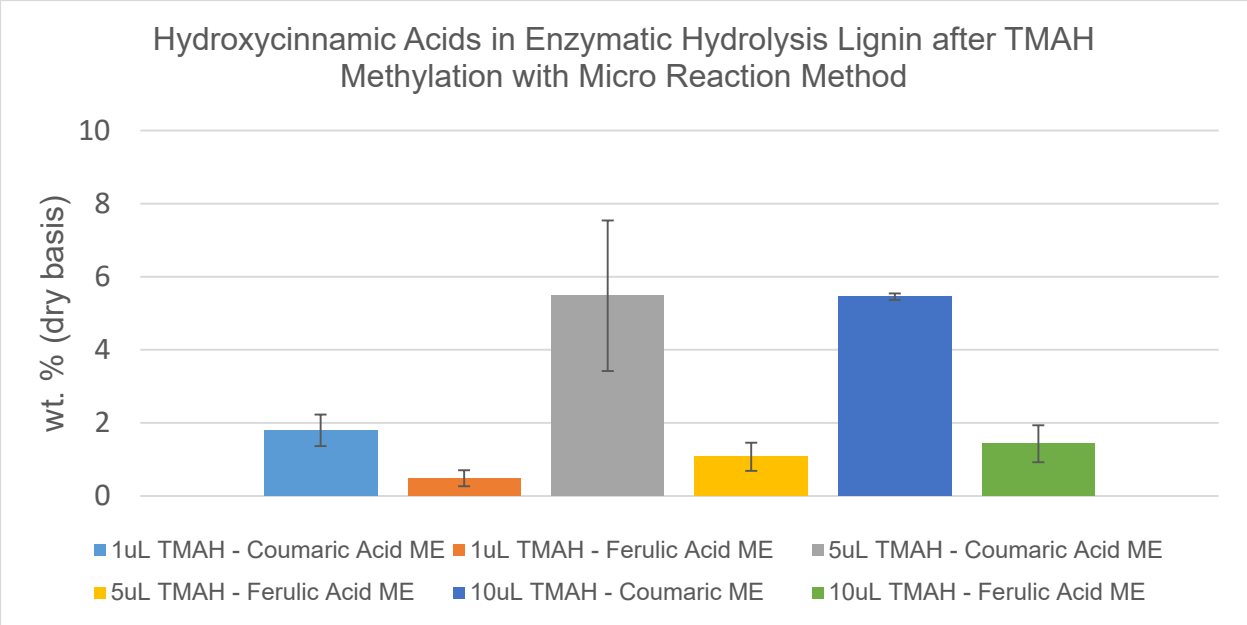


Figure 33. Hydroxycinnamic acids recovered from enzymatic hydrolysis lignin with TMAH as the methylation agent using micro reaction (solvent liquefaction) method.

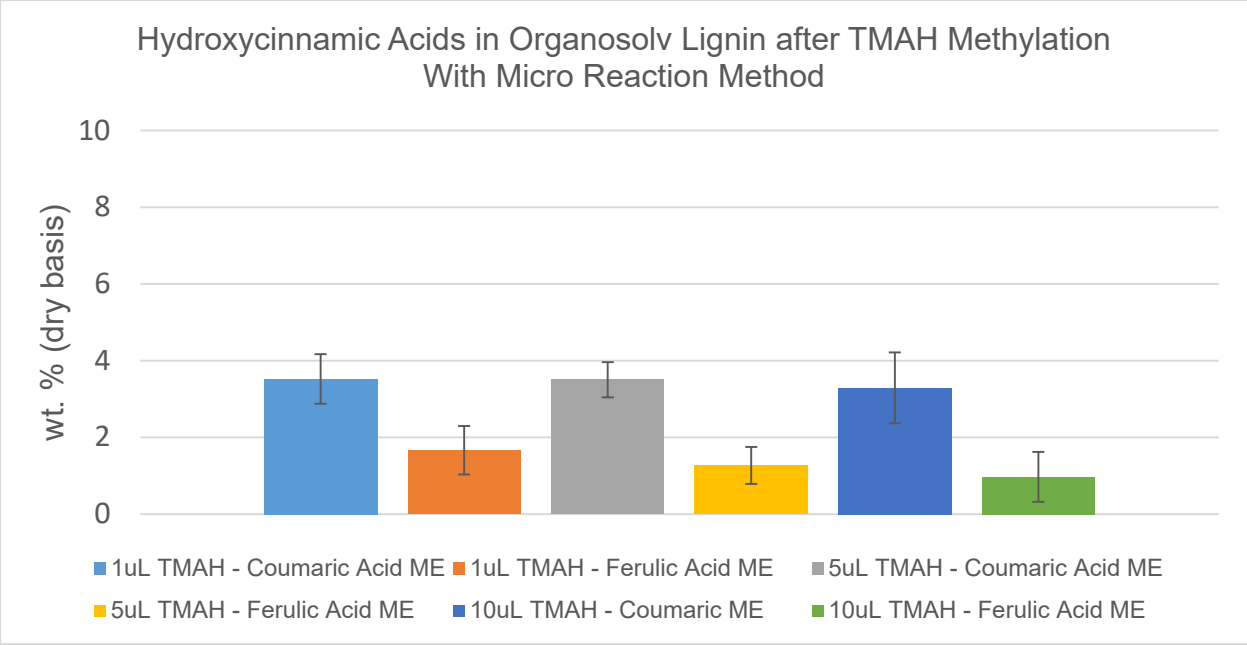


Figure 34. Hydroxycinnamic acids recovered from organosolv lignin with TMAH as the methylation agent using micro reaction (solvent liquefaction) method.

Table 15. Recovery yield comparison of hydroxycinnamic acids from TMAH methylation and solvent liquefaction of enzymatic hydrolysis and organosolv lignin.

Lignin Type	Coumaric Acid from Acid Extraction (db)	Ferulic Acid from Acid Extraction (db)	TMAH Amount	Recovery % Coumaric Acid from Solvent Liquefaction	Recovery % Ferulic Acid from Solvent Liquefaction
Enzymatic Hydrolysis Lignin	5.6%	1.9%	1uL 5uL 10uL	32% 98% 97%	26% 57% 75%
Organosolv lignin	7.7%	2.9%	1uL 5uL 10uL	46% 45% 43%	57% 44% 33%

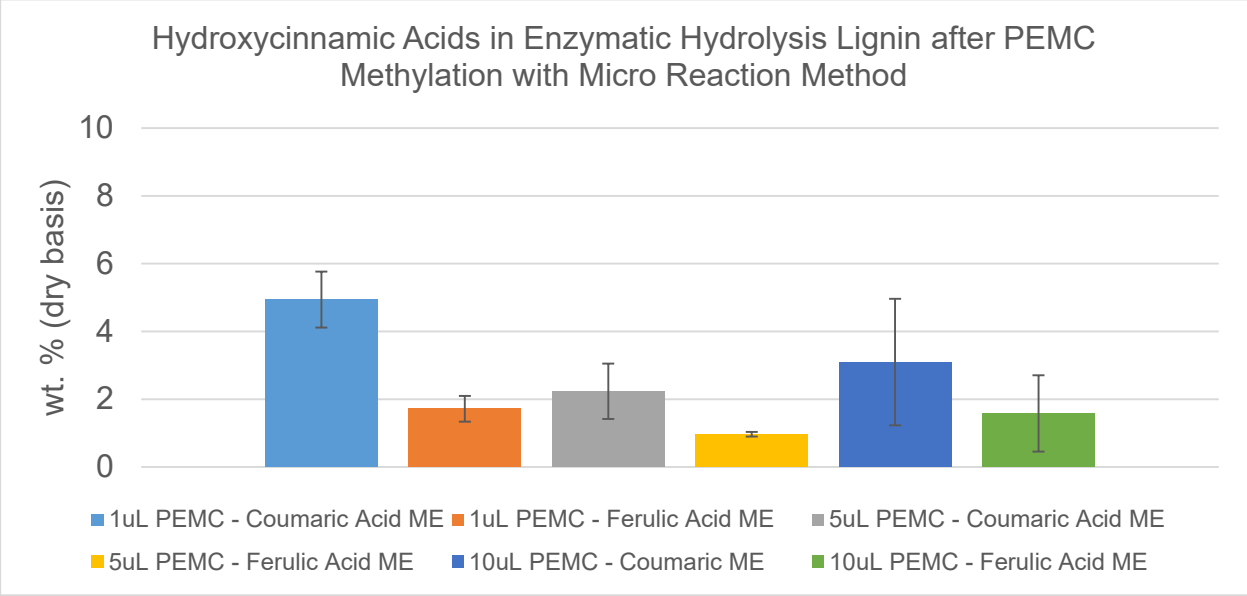


Figure 35. Hydroxycinnamic acids recovered from enzymatic hydrolysis lignin with PEMC as the methylation agent using micro reaction (solvent liquefaction) method.

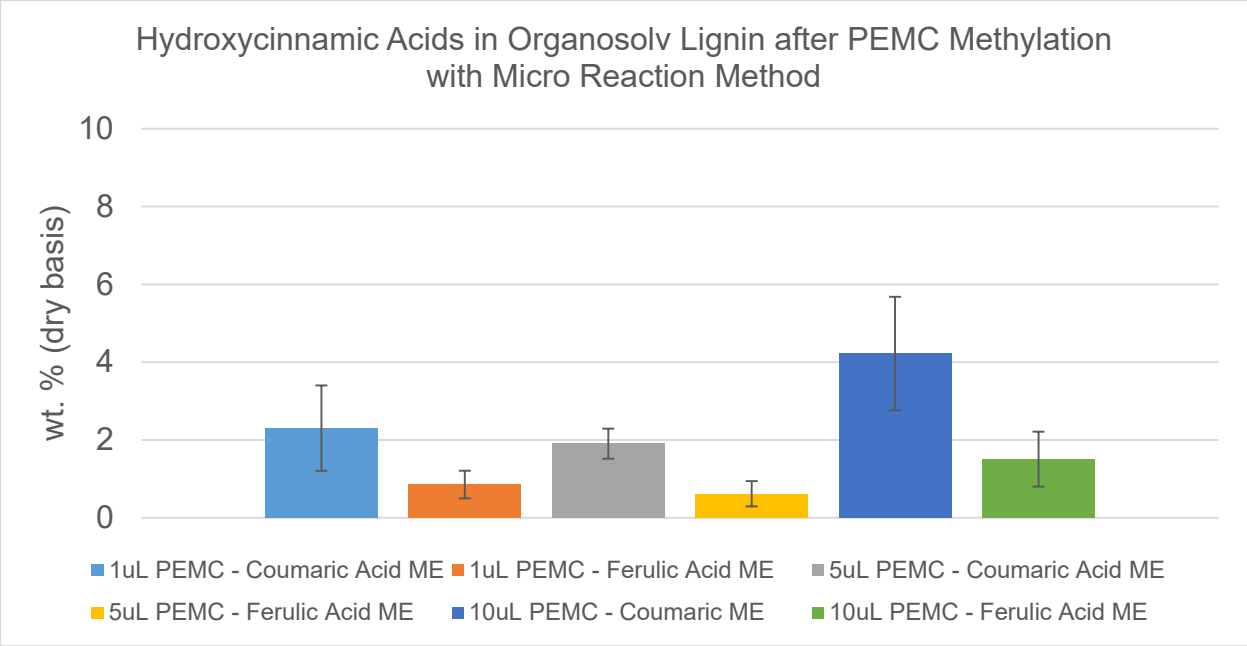


Figure 36. Hydroxycinnamic acids recovered from organosolv lignin with PEMC as the methylation agent using micro reaction (solvent liquefaction) method.

Table 16. Recovery yield comparison of hydroxycinnamic acids from PEMC methylation and solvent liquefaction of enzymatic hydrolysis and organosolv lignin.

Lignin Type	Coumaric Acid from Acid Extraction (db)	Ferulic Acid from Acid Extraction (db)	PEMC Amount	Recovery % Coumaric Acid from Solvent Liquefaction	Recovery % Ferulic Acid from Solvent Liquefaction
Enzymatic Hydrolysis Lignin	5.6%	1.9%	1uL 5uL 10uL	88% 40% 55%	90% 51% 51%
Organosolv lignin	7.7%	2.9%	1uL 5uL 10uL	41% 34% 75%	45% 32% 79%

CHAPTER 6

TECHNO-ECONOMIC ANALYSIS

A techno-economic analysis was performed on upgrading lignin via methylation to hydroxycinnamic acid precursors utilizing both thermochemical routes and the best recovery yields from the micropyrolysis tests. The scenario analyzed in this study is from a 2000 dry metric ton per day (MTPD) plant of lignin from corn stover biomass. Figure 37 displays a flow diagram of hydroxycinnamic acids produced though fast pyrolysis and solvent liquefaction. Important assumptions that were used by Wenqin Li (Li, Mba-Wright, Dang, & Johnston, 2017) in this analysis are located in table 17.

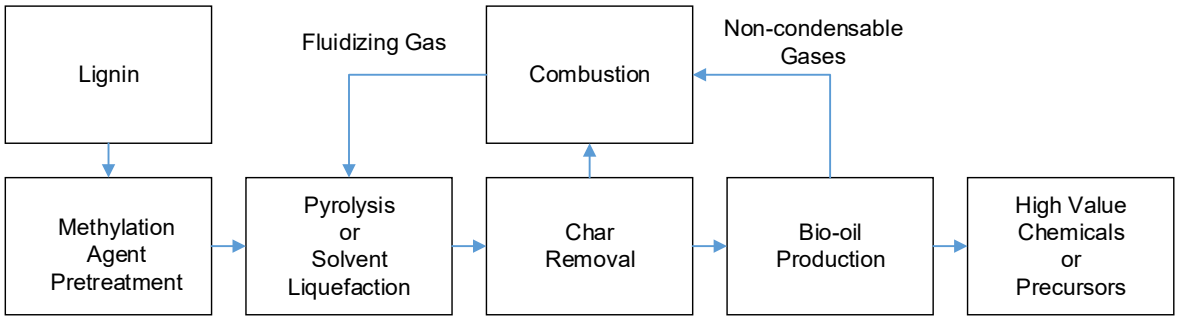


Figure 37. Simple schematic of hydroxycinnamic production via lignin pyrolysis or solvent liquefaction to produce high value chemicals or precursors to high value chemicals.

Table 17. Important assumption used in this techno-economic study (Li et al., 2017).

Lignin price ($/t)	120
Chemical usage	
Methylation agent (uL/ug lignin)	1/250
Tetralin solvent (kg/kg lignin)	0.875
Naphthalene solvent (kg/kg lignin)	2.625
Recycle rate	
Methylation agent	0
Tetralin solvent	95%
Naphthalene solvent	100%
Bio-oil yield (wt.% of dry biomass)	
Liquefaction	51%
Fast pyrolysis	60%

The installed equipment cost for both fast pyrolysis and solvent liquefaction are located in table 18 and 19. The overall costs for both processes are very similar. Using TMAH as the methylation agent in fast pyrolysis and solvent liquefaction are $298 million and $301 million, respectively. Using PEMC as the methylation agent in fast pyrolysis and solvent liquefaction are $316 million and $329 million, respectively.

Table 18. Capital costs for solvent liquefaction (Li et al., 2017).

	Liquefaction with TMAH	Liquefaction with PEMC
Unit	MM$	MM$
Hydrothermal Liquefaction	236.50	251.67
Co-generation	55.07	55.07
Cooling Plant	3.12	3.12
Balance of Plant	6.17	6.17
Total	**$300.86**	**$316.03**

Table 19. Capital costs for fast pyrolysis (Li et al., 2017).

	Pyrolysis with TMAH	Pyrolysis with PEMC
Unit	MM$	MM$
Pretreatment and Pyrolysis	$272.32	$303.26
Fractionation	$11.07	$11.07
Co-generation	$1.96	$1.96
Cooling plant	$3.92	$3.92
Balance of Plant	$9.18	$9.18
Total	**$298.46**	**$329.39**

The operating costs and minimum chemical selling price for both processes are found in table 20 and 21, which shows that fast pyrolysis in combination with PEMC as methylation agent provide the lowest minimum chemical selling price of $137/kg (Li et al., 2017). Alternatively, PEMC with the solvent liquefaction process would have a minimum chemical selling price of $161/kg (Li et al., 2017). Both thermochemical pathways have a high potential considering selling price of products that can be produced from hydroxycinnamic acids such as vanillic acid, hydroxybenzoic acid, parabens and octinoxate, which all have current retail prices of above the minimum

chemical selling price. Table 22 displays the retail selling prices of chemical products that can be upgraded from the hydroxycinnamic acid intermediates. Using TMAH as a methylation agent is marginally higher for the minimum chemical selling price at $233/kg and $199/kg for solvent liquefaction and fast pyrolysis, respectively. Both options would require further optimization to reduce the minimum chemical selling price when using TMAH and PEMC as methylation agent.

Table 20. Operating costs and minimum chemical selling price for solvent liquefaction process (Li et al., 2017).

	Liquefaction with TMAH	Liquefaction with PEMC
Unit	$/kg product	$/kg product
Dry Biomass	$0.234	$0.234
Methylation Agent	$227.853	$157.266
Solvent	$1.281	$1.281
Waste Disposal	$0.012	$0.012
Electricity and other utilities	$0.001	$0.001
Credits	$0.003	$0.003
Fixed Costs	$0.064	$0.066
Capital Depreciation	$0.058	$0.061
Average Income Tax	$0.588	$0.408
Average Return on Investment	$3.196	$2.162
Minimum chemical selling price	**$233**	**$161**

Table 21. Operating costs and minimum fuel selling price for fast pyrolysis process (Li et al., 2017).

	Pyrolysis with TMAH	Pyrolysis with PEMC
Unit	$/kg product	$/kg product
Dry Biomass	$0.200	$0.200
Methylation Agent	$195.364	$134.200
Catalysts & Chemicals	$0.000	$0.000
Waste Disposal	$0.000	$0.000
Electricity and other utilities	$0.014	$0.014
Credits	$0.000	$0.000
Fixed Costs	$0.054	$0.058
Capital Depreciation	$0.033	$0.036
Average Income Tax	$0.501	$0.346
Average Return on Investment	$2.684	$1.771
Minimum chemical selling price	**$199**	**$137**

Table 22. High value chemical products that can be upgrading from hydroxycinnamic acid intermediates ("Sigma Aldrich," 2017).

Chemical	Uses	Price/kg
Vanillin	Food Flavoring Additive	$130
Parabens	Cosmetic Preservatives	$300
Hydroxybenzoic Acid	Cosmetics – Acne Creams	$600
Vanillic Acid	Food Flavoring Additive	$900
Octinoxate	Sunscreens and Lip Balms	$30,000

Sensitivity Analysis

The sensitivity analyses demonstrate the minimum chemical selling price to 20% variations in key techno-economic parameters for the fast pyrolysis and solvent liquefaction pathways with PEMC and TMAH methylation agents. Figures 38 and 39 display the sensitivity analysis for solvent liquefaction using TMAH and PEMC as methylation agents. Figures 40 and 41 display the results of the sensitive analysis for

fast pyrolysis using TMAH and PEMC. As shown, product chemical yields and PEMC/TMAH agent prices are the most critical parameters. The high operating cost contribution from the PEMC/TMAH agents skews results such that a 20% decrease in agent cost has a lower impact than a 20% increase in product yield. However, a 20% decrease in product yield would have a higher impact than a 20% increase in methylation agent price.

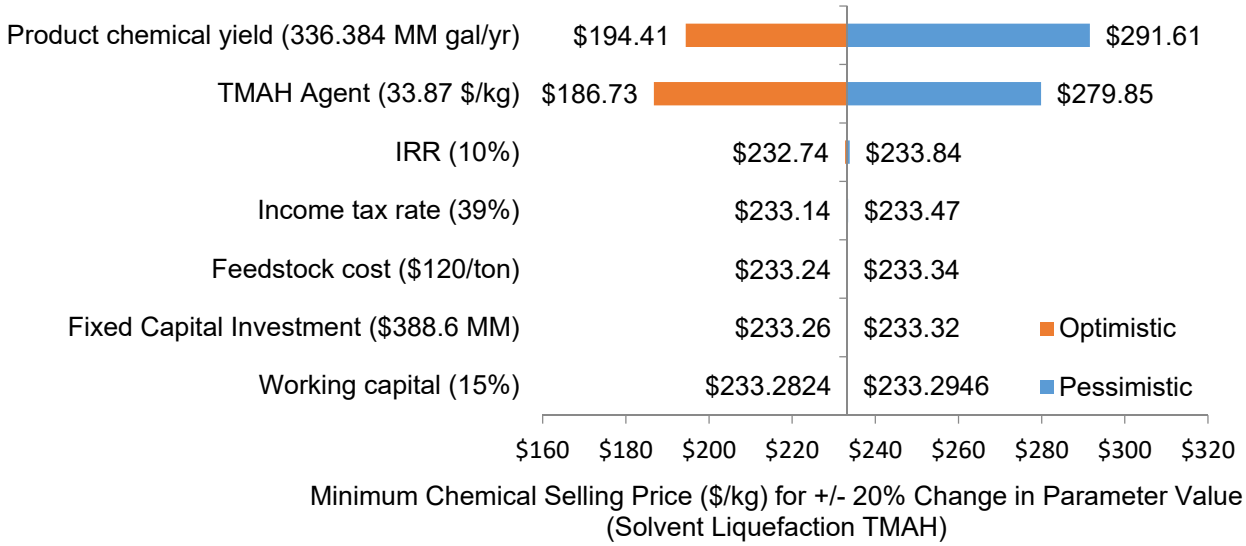


Figure 38. Sensitivity analysis for TMAH lignin methylation using the solvent liquefaction pathway (Li et al., 2017).

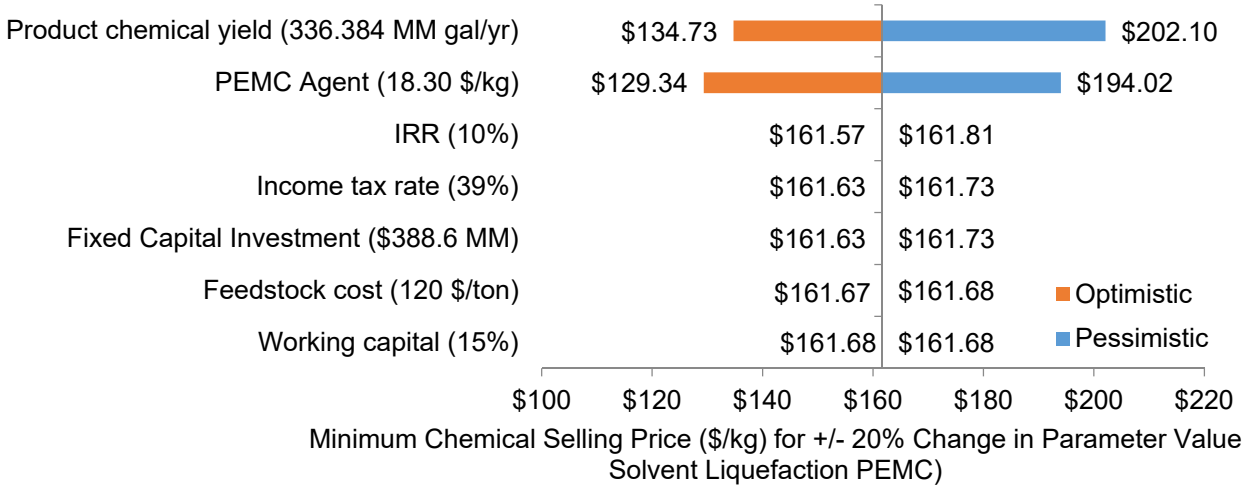


Figure 39. Sensitivity analysis for PEMC lignin methylation using the solvent liquefaction pathway (Li et al., 2017).

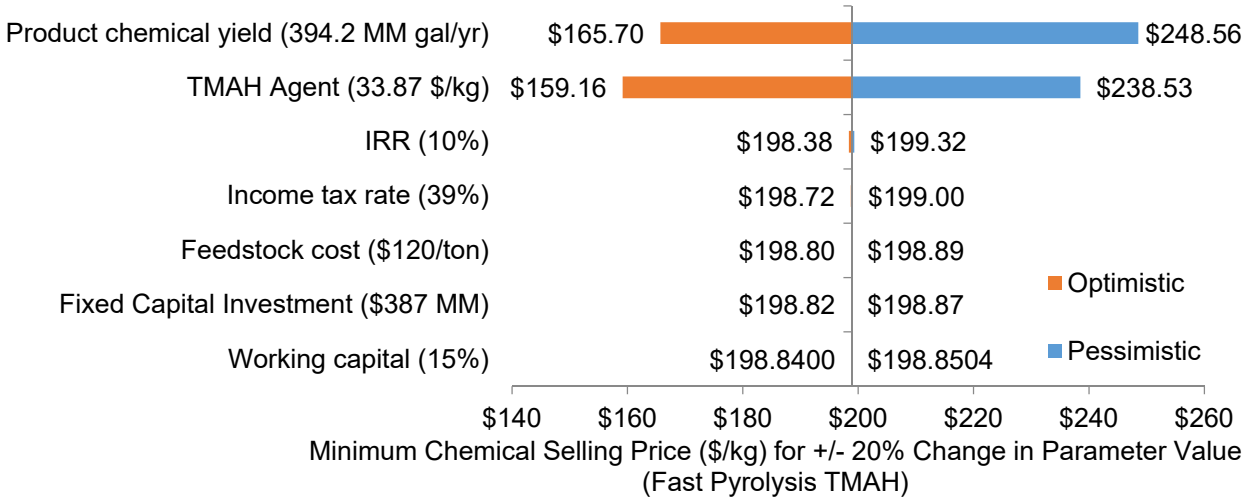


Figure 40. Sensitivity analysis for TMAH lignin methylation using the fast pyrolysis pathway (Li et al., 2017).

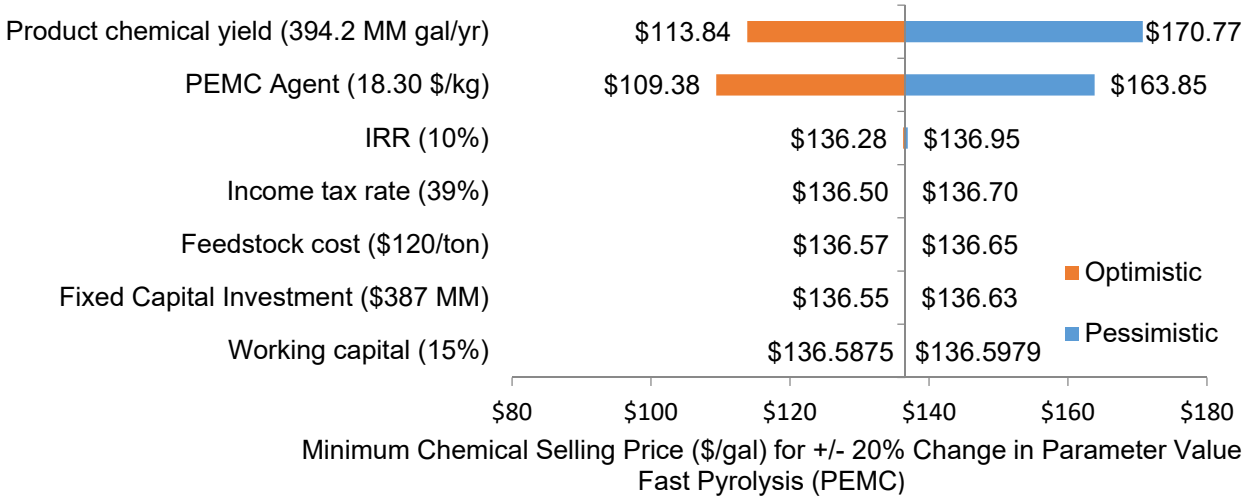


Figure 41. Sensitivity analysis for PEMC lignin methylation using the fast pyrolysis pathway (Li et al., 2017).

CHAPTER 7

CONCLUSIONS AND FUTURE WORK

Hydroxycinnamic acid extraction and recovery from lignin to determine total mass yields works best when using an alkaline base (sodium hydroxide) method. Using acidic extraction solutions did not work for recovering hydroxycinnamic acids. The acidic solution extraction method had yields that were significantly less than the recovered yields from the methylation thermochemical processes. This research suggests that the acid was incapable of breaking the β-O-4 bonds in the lignin molecule.

The hydroxycinnamic acid esters can be readily produced from lignin pyrolysis and solvent liquefaction using methylation agents. These volatile acid esters can easily be identified and quantified using GC. Addition of a base solvent (catalyst) is recommended to produce hydroxycinnamic acid esters from carbonate methylation compounds. When comparing hydroxycinnamic acid recoveries among different thermochemical technologies, i.e. pyrolysis vs. solvent liquefaction, pyrolysis would be the method of choice due to the complex nature of biomass feedstock processing in a solvent liquefaction regime.

The alkaline extraction method was successfully used to determine total extraction yield of hydroxycinnamic acids in the lignin samples. The yields of coumaric and ferulic in the enzymatic hydrolysis lignin was 5.6% and 1.9%, respectively. The total extracted yields on a mass basis of coumaric and ferulic in the organosolv lignin was 7.7% and 2.9%, respectively.

In the case of the pyrolysis regime the highest recoveries were at the 10μL loading level with both TMAH and PEMC. The recoveries of the hydroxycinnamic acids from enzymatic hydrolysis lignin pyrolysis with 10μL TMAH produced coumaric and ferulic acid concentrations of 84% and 69%, respectively. The recoveries of the hydroxycinnamic acids from organosolv lignin pyrolysis with 10μL TMAH produced coumaric and ferulic acid concentrations of 89% and 64%, respectively. The recoveries of the hydroxycinnamic acids from enzymatic hydrolysis lignin pyrolysis with 10μL PEMC produced coumaric and ferulic acid concentrations of 85% and 85%, respectively. The recoveries of the hydroxycinnamic acids from organosolv lignin pyrolysis with 10μL PEMC produced coumaric and ferulic acid concentrations of 54% and 97%, respectively. This loading level would equal 16.3 eq. TMAH and 10.3 eq. PEMC that would correspond to the reacting phenolic hydroxyl group in the lignin samples.

In the case of the solvent liquefaction regime there was more variability within the results. The best loading levels for the enzymatic hydrolysis lignin with TMAH was 10μL that produced coumaric and ferulic acid concentrations of 97% and 75%, respectively. This loading level would equal 16.3 eq. TMAH. The best loading levels for the organosolv lignin with TMAH was 1μL that produced coumaric and ferulic acid concentrations of 46% and 57%, respectively. This loading level would equal 1.6 eq. TMAH that would correspond to the reacting phenolic hydroxyl group in the lignin samples. The best loading levels for the enzymatic hydrolysis lignin with PEMC was 1μL that produced coumaric and ferulic acid concentrations of 88% and 90%, respectively. This loading level would equal 1.03 eq. PEMC. The best loading levels

for the organosolv lignin with PEMC was 10μL that produced coumaric and ferulic acid concentrations of 75% and 79%, respectively. This loading level would equal 10.3 eq. PEMC that would correspond to the reacting phenolic hydroxyl group in the lignin samples.

Overall, the recoveries of hydroxycinnamic acids in the majority of levels is very significant. The variability in the micro reaction (solvent liquefaction) test could be due to the difficulty in preparing the samples on the microscale in very small glass vials that have an ID and length of 2mm X 30mm and are fused shut with a torch before analysis.

This research demonstrated that it is possible to produce hydroxycinnamic acids at significant levels using thermochemical processes such as solvent liquefaction and pyrolysis. Coumaric and ferulic acid are high value chemical intermediate compounds that can be used to produce a variety of chemicals for cosmetics, sunscreens, plastics and food flavoring agents.

The techno-economic analysis of this research project shows that the operating costs and minimum chemical selling price using fast pyrolysis and PEMC as methylation agent would provide the lowest minimum chemical selling price of $137/kg (Li et al., 2017). Alternatively PEMC with the solvent liquefaction process would have a minimum chemical selling price of $161/kg (Li et al., 2017). Both of these options have a high potential considering costs of products that can be produced from hydroxycinnamic acids such as vanillic acid, hydroxybenzoic acid, parabens and octinoxate which all have current retail prices of above the minimum chemical selling price ("Sigma Aldrich," 2017). Using TMAH as a methylation agent is marginally higher for the minimum chemical selling price at $233/kg and $199/kg for solvent liquefaction and fast pyrolysis,

respectively. Both options should require further optimization to reduce the minimum chemical selling price when using TMAH and PEMC as methylation agents.

Future Work

This research presents many possible challenges before commercialization. In order to upscale this methylation process it would be necessary to perform the experiments on a continuous reactor. At first a small continuous lab scale pyrolyzer would be optimal to test parameters of temperature, residence times and methylation agent loading. Once optimized on the small scale system the experiments could be performed on a pilot/demo system to produce larger quantities of hydroxycinnamic acids and advance this process towards commercialization. The pyrolysis systems available at Iowa State University would be superior in separating these hydroxycinnamic acids from lignin with the current stage fractionation technology available. It would be interesting to see these methylation experiments completed on a continuous lab scale pyrolysis system. Additionally, a more in-depth techno-economic analysis could be performed after all conditions have been scaled-up and optimized on larger pyrolysis system.

Extracting hydroxycinnamic acids directly from biomass has also been examined. Preliminary results show that higher yields of acids can be obtained utilizing corn stover as the starting material before the cellulosic ethanol process. In this case of the methanol extraction coumaric acid was 19.3% on a dry lignin basis and ferulic was 6.39% on a dry lignin basis (figure 42). In this case of the ethanol extraction coumaric acid was 20.1% on a dry lignin basis and ferulic was 7.80% on a dry lignin basis (figure 43).

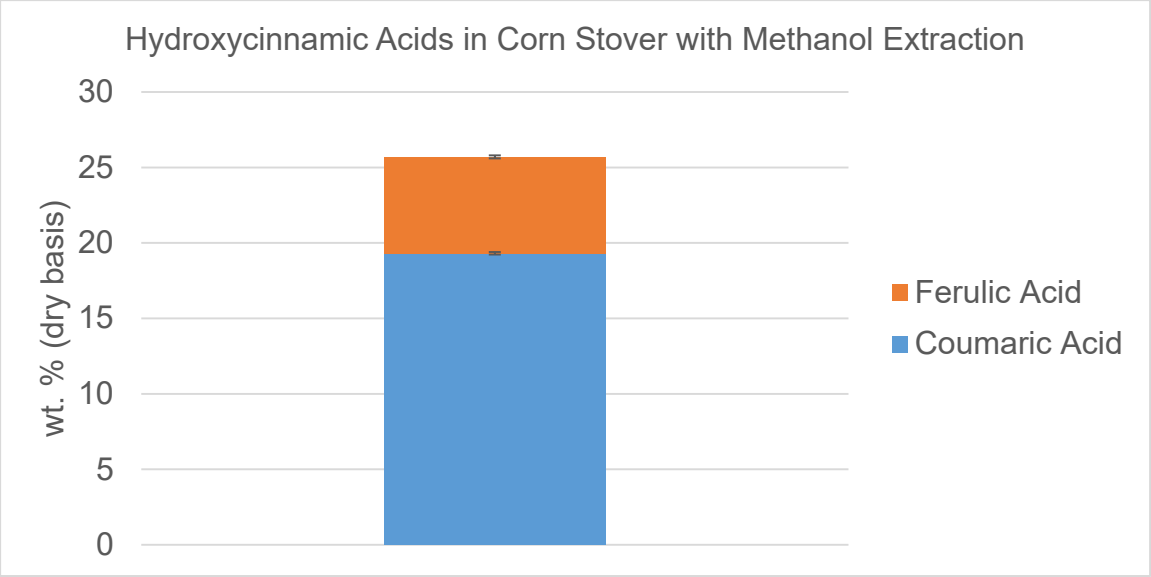


Figure 42. Hydroxycinnamic acid concentrations in corn stover with methanol extraction.

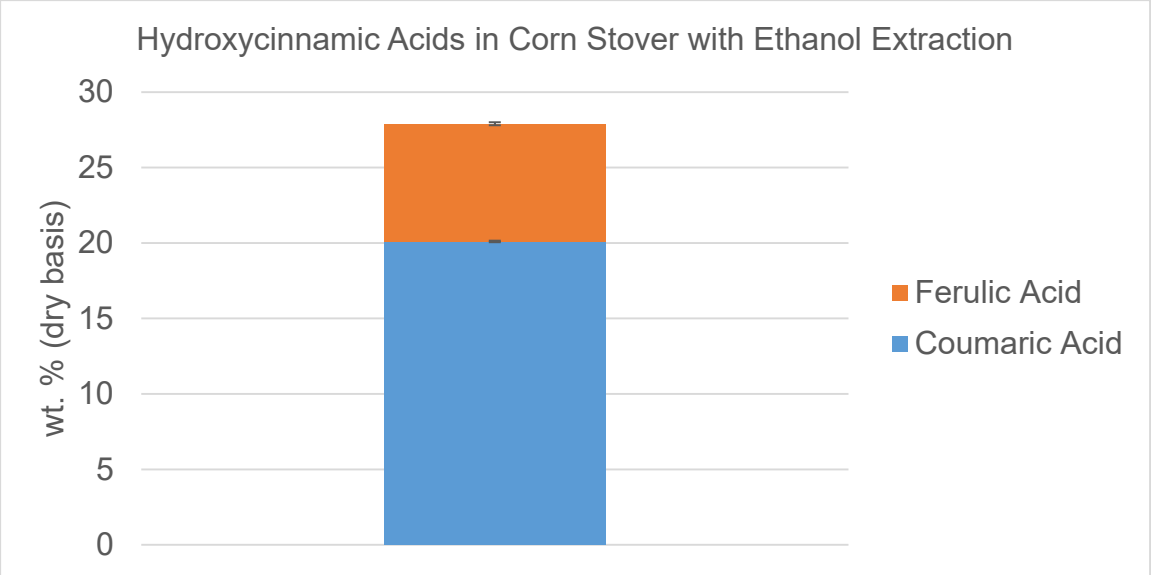


Figure 43. Hydroxycinnamic acid concentrations in corn stover with ethanol extraction.

This preliminary research is exciting and requires a more in depth study that could result in ethanol producers possibly having an alternative route to produce high value chemicals in conjunction with fuel production that would in general provide a more economic process. I would propose a scenario that would provide an alkaline

hydroxycinnamic acid extraction before the typical pretreatment process of corn stover for cellulosic ethanol. The hydroxycinnamic acid free lignin then could be further upgrading to chemicals via fast pyrolysis or solvent liquefaction route. The process is outline in figure 44. Overall the effects of this alkaline extraction pretreatment step would need to be studied in depth to determine the potential effects (sugar removal) on the biomass feedstocks before cellulosic pretreatment steps and not inhibit ethanol production. Preliminary HPLC testing shows negligible removal of sugars.

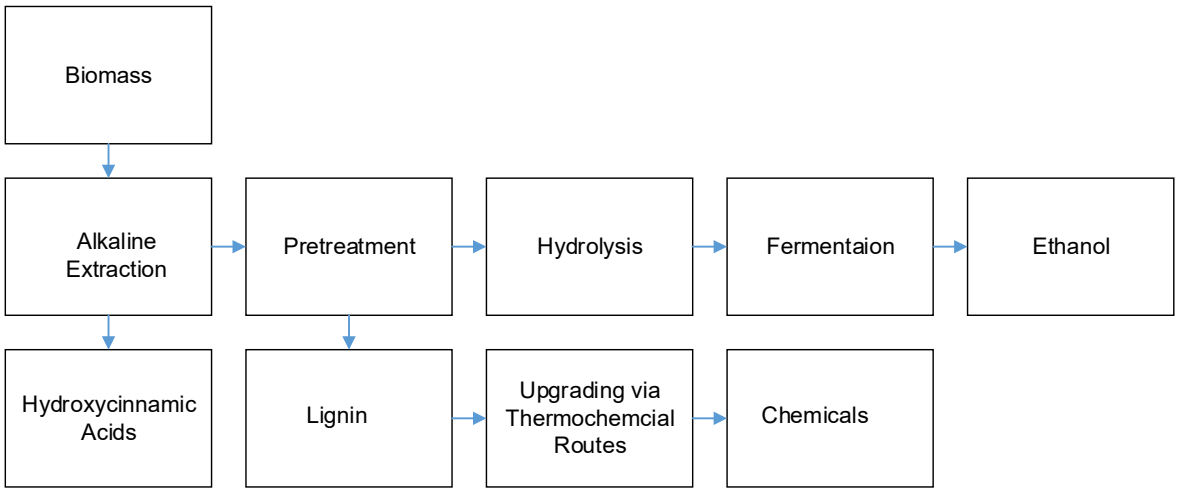


Figure 44. Process for extracting hydroxycinnamic acids before cellulosic ethanol pretreatment.

Another possible scenario to test for production of hydroxycinnamic acids is direct methylation of the corn stover followed by fast pyrolysis. This process is displayed in figure 45. As previously described above with the lignin methylation procedure, corn stover could be directly methylated and pyrolyzed before the enzymatic hydrolysis or organosolv process. A techno-economic analysis would need to be performed to see if this would be a preferred extraction route compared to removing the hydroxycinnamic acids after the cellulosic ethanol pretreatment.

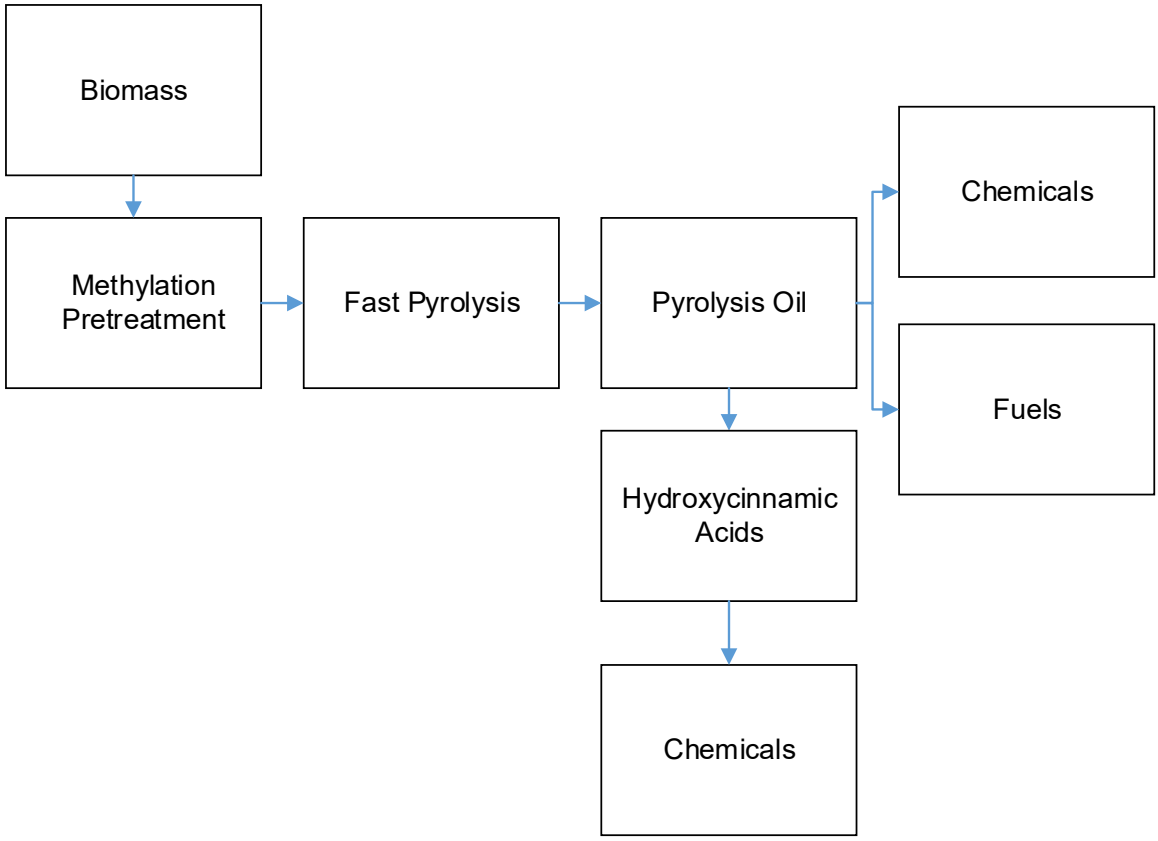


Figure 45. Direct thermochemical methylation of biomass.

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APPENDIX

SUPPLEMENTARY CALCULATIONS

Calculations for Equivalents (eq.) of Methylation Agent to Phenolic Hydroxyl Groups in Lignin

Tetramethylammonium Hydroxide (TMAH)

800mg of lignin contains 4.66mmol of phenolic hydroxyl groups (Sen et al., 2015).

TMAH as methylation agent

TMAH = 91.15 g/mol

Density = 0.866g/mL (25% in MeOH)

1 eq. = (4.66mmol) (91.15g/mol) = 424.76mg/800 mg of lignin

1 eq. for 0.25 mg of lignin = 0.1327 mg = 0.0001327 g

(0.0001327g) / (0.866g/mL) = 0.000153 mL = 0.153 µL = 1 eq. TMAH

Actual amounts of TMAH used in 25% MeOH (75:25 dilution):

1µL = 0.25µL of TMAH = 1.6 eq.

5µL = 1.25µL of TMAH = 8.2 eq.

10µL = 2.5µL of TMAH = 16.3 eq.

Tetra-Methyl Carbonate of Pentaerythritol (PEMC)

800mg of lignin contains 4.66mmol of phenolic hydroxyl groups (Sen et al., 2015).

PEMC as methylation agent

PEMC = 368.29 g/mol

Density = 1.10g/mL (50% in DMF)

1 eq. = (4.66mmol) (368.29g/mol) = 1716.23mg/800 mg of lignin

1 eq. for 0.25 mg of lignin = 0.536 mg = 0.000536 g

(0.000536g) / (1.10g/mL) = 0.000487mL = 0.487μL = 1 eq. PEMC

Actual amounts of PEMC used in 50% DMF (50:50 dilution):

1μL = 0.50μL of PEMC = 1.03 eq.

5μL = 2.5μL of PEMC = 5.13 eq.

10μL = 5.0μL of PEMC = 10.3 eq.