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Analysis of sugars and phenolic compounds in bio-oil

Marjorie Ruth Rover
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Analysis of sugars and phenolic compounds in bio-oil

by

Marjorie Ruth Rover

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Biorenewable Resources and Technology

Program of Study Committee:
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Iowa State University
Ames, Iowa
2013

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DEDICATION

I dedicate this dissertation to my husband, James. Without his love, support, and understanding this endeavor would not have been possible. He has sacrificed a great deal to make this possible for me.

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EXTENDED ABSTRACT

The overall goal of this research is to develop methods for analyzing and recovering sugars and phenolic compounds from bio-oil. Specific objectives include (1) adapting analytical methods developed for sugar analysis in the food industry to measure total water-soluble sugars in the aqueous phase of bio-oil; (2) adapting analytical methods developed for total phenolic analysis of wine to measure total phenol content of bio-oil; (3) separate the heavy fraction of bio-oil into a concentrated sugar solution and a phenolic oligomer-rich raffinate; and (4) determine the effect of pyrolysis temperature on the yield of sugars and phenolic compounds in bio-oil.

Recent research at Iowa State University suggests that bio-oil may be the most economical approach to advanced biofuels production. Produced from the fast pyrolysis of biomass, bio-oil contains hundreds of chemical compounds that complicate their accurate and cost-effective chemical analysis. Among the most commercially important components of bio-oil are sugars and phenolic compounds. Both are difficult to analyze because of the large number of variations that can occur and potential interferences with other bio-oil components.

The approach to this research was to adapt chemical analysis methods developed by food chemistry to quantify total sugars and phenolic compounds using standardized test methods. The Association of Analytical Communities, International (AOAC) Official Method of Analysis 988.12 (44.1.30) Phenol-Sulfuric Acid Assay for Total Carbohydrate Determination was utilized to quantify water-soluble sugars and the Folin-Ciocalteu (FC) colorimetry method was used to quantify total phenolic compounds in bio-oil.

Bio-oil produced from fast pyrolysis of biomass contains sugars originating from cellulose. Traditional quantification of sugars in bio-oil is accomplished by gas chromatography/mass

spectroscopy (GC/MS) via derivatization, high-performance liquid chromatography (HPLC), ion-exchange chromatography (IC), or nuclear magnetic resonance (NMR) methodologies. These techniques are highly specific for each sugar, tedious to perform, expensive, and involve the use of hazardous solvents.

A standardized test method developed for food and agriculture applications, the Association of Analytical Communities, International (AOAC) Method 988.12 (44.1.30) Phenol-Sulfuric Acid Assay for Total Carbohydrate Determination, was utilized to quantify total sugars in the water-soluble fraction of bio-oil. This study investigated accuracy relative to matrix effects caused by non-sugar compounds using positive and negative controls. Positive controls included levoglucosan, D-glucose, D-mannose, D-xylose, D-fructose, D-galactose, L-arabinose, L-fucose, and cellobiosan. Negative controls included phenol, acetic acid, formic acid, propionic acid, glycolic acid, acetol, furfural, 5-hydroxymethylfurfural (5HMF), furfuryl alcohol, 2-methylfuran and 2(5H)-furanone.

Potential interference with the quantification of total water-soluble sugars by the AOAC Method 988.12 (44.1.30) was calculated for all positive and negative controls by using data obtained when adding the contributor (positive controls) and the interferent (negative controls) into the water-soluble fraction of bio-oil with typical concentrations found in bio-oil. It was found that furfural, 2(5H)-furanone, 5HMF, and furfuryl alcohol influenced results with a range of potential errors of 9.56-29.7%, 9.52-29.8%, 2.91-24.8%, and 1.34-11.9%, respectively. A correction factor of 0.76 wt% was established to reduce or eliminate this influence. Total water-soluble sugars content in bio-oil detected by AOAC Method 988.12 (44.1.30) was comparable to the quantity of sugars detected using hydrolysis with quantification by HPLC. The uncertainty of

measurement of water-soluble sugars in bio-oil at 95% confidence was $\pm 1.7\%$ using AOAC Method 988.12 (44.1.30) when the correction factor was employed.

Bio-oil from fast pyrolysis of biomass contains phenolic compounds derived from the lignin portion of the biomass. Traditional testing for total phenolic compounds in bio-oil is based on either a rough estimate of the weight percent water-insolubles in bio-oil or on tedious liquid-liquid extraction methods. The Folin-Ciocalteu (FC) colorimetry method used for quantifying total phenols in wine was used to determine total phenols in bio-oil. This method, based on the oxidation of phenolic compounds by the FC reagent, is fast and easy to perform. This study evaluated its accuracy relative to interferences by the use of positive and negative controls.

Positive controls included phenol, 4-methylphenol, 3-ethylphenol, guaiacol, 2,6-dimethoxyphenol and eugenol. The negative controls included sugars, furfural, and acids. Potential interferences with the quantification of total phenols by the FC method was calculated for all positive and negative controls by using data obtained when adding the contributor (positive controls) and the interferent (negative controls) into bio-oil using typical concentrations found in bio-oil. The positive and several of the negative controls produced strongly correlated linear relationships between the indicated phenolic content of the bio-oil and the amount of contributor or interferent added. However, the slopes of these relationships for the negative controls were much smaller than those for the positive controls, indicating that the error in the prediction of phenol content was small even for large concentrations of interferent compounds.

For typical concentrations of non-phenolic compounds in bio-oil, the error in predicted phenolic content as a result of their presence was $\leq 5.8\%$. Total phenolic content in bio-oil detected by the FC method was comparable to the quantity of total phenolic compounds obtained by liquid-liquid extraction. All results fell within the margin of error and the uncertainty of the

measurement by the FC method indicating there was no significant difference in the results between the two methods. The FC method uncertainty of measurement was $\pm 1.1\%$ at the 95% confidence level.

An investigation of sugar and phenolic oligomer recover from the heavy-ends of fractionated bio-oil is performed. This study explores the separate recovery of sugars and phenolic oligomers produced during the fast pyrolysis of lignocellulosic biomass. The experiments were conducted in an 8 kg/h fluidized bed pyrolysis process development unit. Bio-oil fractionation was accomplished with a five-stage system that recovers bio-oil according to “condensation points” of the constituent compounds. The first two stages capture “heavy-ends” consisting mostly of water soluble sugars derived from polysaccharides and water insoluble phenolic oligomers derived from lignin. Exploiting differences in water solubility, a sugar-rich aqueous phase and a phenolic-rich raffinate were recovered. The soluble sugars were effectively washed from the phenolic oligomers allowing the production of “pyrolytic sugars” and a carbohydrate-free raffinate comprised of phenolic oligomers that readily flowed at room temperature. Over 93 wt% sugars were removed with two wash stages for stage fractions (SF) 1 and 2.

The separated sugars from SF 1 and 2 are suitable for either fermentation or catalytic upgrading to biofuels. The phenolic oligomer-rich raffinate, which represents 44-47 wt% dry basis (db) of both SF 1 and 2, is less sticky and viscous than the unwashed SFs. It has potential for production of fuels, aromatic chemicals, unique polymers, resins, binders, coatings, adhesives, asphalt, and preservatives.

Iowa State University’s fluidized bed pyrolysis process development unit (PDU) with a condenser collection system is utilized to evaluate physicochemical properties of bio-oil produced at 350, 400, 450, 500, and 550 °C. A study of temperature effects on the production

and distribution of specific chemical families and or chemicals is pertinent to quality bio-oil with specific end-use applications. The red oak biomass gave maximum bio-oil yield at 400°C, highest non-condensable gases (NCG) yield at 550°C, with the highest char yield at 350°C. Carbon monoxide increased at the expense of carbon dioxide at 550°C. There was a slight increase in methane as well. A higher conversion of cellulose and hemicellulose content to sugars resulted at 400°C and was condensed in stage fractions (SF) 1 and 2. Total phenolic compound production was highest at 350°C with the majority being larger lignin derived oligomers which condensed in SF 1-2. The phenolic monomers were the most prevalent at 550°C with the highest concentration condensed in SF 3. The insolubles ranged from 40-45 wt% at 500-550°C in SF 1-2. Moisture content was highest at 550° in SF 5.

CHAPTER 1. OVERVIEW

1.1 Introduction

Chemical composition of bio-oil is dramatically different from that of petroleum-derived oil. Bio-oils are multi-component mixtures derived from cellulose, hemicellulose, and lignin. Therefore, its chemical make-up resemble that of biomass and not petroleum oils [8]. Produced from the fast pyrolysis of biomass, bio-oil contains hundreds of chemical compounds that complicate their accurate and cost-effective chemical analysis and separation.

Among the most commercially important components of bio-oil are sugars and phenolic compounds. Both are difficult to analyze because of the large number of variations that can occur and potential interferences with other bio-oil components. The approach to this research is to adapt chemical analyses methods developed by food chemistry to quantify total water-soluble sugars and phenols using standardized test methods. The Association of Analytical Communities, International (AOAC) Official Method of Analysis 988.12 (44.1.30) Phenol-Sulfuric Acid Assay for Total Carbohydrate Determination is utilized to quantify bio-oil water-soluble sugars and the Folin-Ciocalteu colorimetry method is used to quantify total phenolic compounds in bio-oil.

Development of a method for recovering two value-added products from the fast pyrolysis of lignocellulosic biomass: a concentrated sugar-rich solution and phenolic oligomer-rich raffinate is investigated. Effective removal of these two products offers an array of industrial opportunities. Pyrolytic sugars can be used for direct upgrading to liquid transportation fuels and/or fermentation. Successful sugar removal also has potential for pharmaceutical applications. Phenolic oligomers have potential to be used in resins, binders, asphalt, etc. The opportunity also exists for removal of specific phenolic compounds for use as valuable chemicals.

Lastly, the fast pyrolysis process can be adjusted to favor bio-char, bio-oil, or non-condensable gas (NCG) formation during the thermochemical conversion of biomass. Often research on fast pyrolysis has been carried out with the goal of maximizing the production of bio-oil and bio-oil constituents without being aware of the process conditions that optimize higher quality bio-oil [9-11].

Bio-oil quality is essential for specific targeted applications [9]. Higher molecular weight oligomers are important if bio-oil end-use is asphalt, resins, polymers, etc. If sugar production is the goal; temperature control for the production of sugars is important. Yield and the structure of chemical constituents would be essential in a biorefinery scenario utilizing direct upgrading to liquid fuels. Maximizing bio-oil yield should not remain the focus of bio-oil production but rather bio-oil quality for specific end-use.

1.2 Research Objectives

The overall goal of this research is to develop methods for analyzing and recovering sugars and phenolic compounds from bio-oil. Specific objectives include (1) adapting analytical methods developed for sugar analysis in the food industry to measure total water-soluble sugars in the aqueous phase of bio-oil; (2) adapting analytical methods developed for total phenolic analysis of wine to measure total phenolic content of bio-oil; (3) separate the heavy fraction of bio-oil into a concentrated sugar solution and a phenolic oligomer-rich raffinate; and (4) determine the effect of pyrolysis temperature on the yield of sugars and phenolic compounds in bio-oil. These are described in more detail below.

1.2.1 Methodology Evaluation for Quantification of Total Water-Soluble Sugars in Bio-Oil

The first research objective is to adapt analytical methodology developed for food chemistry to measure water-soluble sugars in bio-oil. Bio-oil contains sugars originating from cellulose. Traditional quantification of sugars in bio-oil is accomplished by gas

chromatography/mass spectroscopy (GC/MS) via derivatization, high-performance liquid chromatography (HPLC), ion-exchange chromatography (IC), or nuclear magnetic resonance (NMR) methodologies. These techniques are highly specific for each sugar, tedious to perform, expensive, and involve the use of hazardous solvents. A standardized test method developed for food and agriculture applications, AOAC Method 988.12 (44.1.30), is evaluated to quantify total sugars in the water-soluble fraction of bio-oil.

Among the most commercially important components of bio-oil is sugar [12]. Sugar within bio-oil's complex matrix is difficult to analyze. A standardized test method that can be used to quantify total water-soluble sugars in bio-oil would enable meaningful comparisons and provide more consistent results. The approach to this research is to adapt a standardized chemical analyses method developed by food chemistry to quantify total water-soluble sugars in bio-oil.

1.2.2 Methodology Evaluation for Quantification of Total Phenols in Bio-Oil

The second research objective is to evaluate the Folin-Ciocalteu colorimetry method used for quantifying total phenols in wine to determine total phenols in bio-oil. Bio-oil contains phenolic compounds derived from the lignin portion of the biomass. Traditional testing for total phenolic compounds in bio-oil is based on either a rough estimate of the weight percent bio-oil water-insolubles or on tedious liquid-liquid extraction methods. The Folin-Ciocalteu colorimetry method used for quantifying total phenols in wine to determine total phenols in bio-oil is evaluated. This method, based on the oxidation of phenolic compounds by the FC reagent, is fast and easy to perform.

Liquid-liquid extraction is very time consuming and it is not a good alternative to a faster, easier and standardized test methods. Basing total phenolic compounds on the water-insoluble fraction content of bio-oil is merely a rough estimation. A standardized test method that can be

used to quantify total phenolic compounds would allow for meaningful comparisons and provide more consistent results. There is a need for a fast, easy, reliable standardized test method for quantifying total phenols in bio-oil.

1.2.3 Development of an Effective Separation Method for the Removal of Total Water-Soluble Sugars and Phenolic Oligomer-Rich Raffinates from Bio-Oil

The third objective of this research is to develop a method for recovering two value-added products from the fast pyrolysis of lignocellulosic biomass: a concentrated sugar-rich solution and phenolic oligomer-rich raffinate.

A vital aspect in the separation and recovery of chemicals from bio-oil is fractionation; where bio-oil is separated into less complex fractions or mixtures [3]. Fractionated bio-oil is pertinent for upgrading to transportation fuels. Distillation, as applied to crude petroleum oil, is not a valid route to bio-oil fractionation due to thermal and chemical instability [3] therefore, other fractionation methods must be utilized.

Significant progress has been achieved in the advancement of bio-oil collection, allowing for an effective bio-oil fractionation method. Iowa State University (ISU) has developed a bio-oil recovery system from fast pyrolysis of lignocellulosic biomass as stage fractions (SF) with distinct chemical and physical properties using a system of condensers with carefully controlled coolant temperatures and electrostatic precipitators (ESP) [1].

The separated phenolic oligomer-rich raffinate has potential for upgrading to transportation fuels, as well as other platform chemicals. The removal of pyrolytic sugars and their conversion into liquid drop-in fuels while using phenols to produce high value products (i.e. resins, adhesives) could be a promising approach to economic viability of bio-oil refineries [12-15]. The recovery of separate sugar-rich and phenolic oligomer-rich streams from the heavy-ends represents numerous opportunities for value-added products.

1.2.4 Effects of Pyrolysis Temperatures on Yields of Sugar and Phenolic Compounds

The fourth research objective is to consider bio-oil quality when recovered as SFs using different reactor temperatures, evaluation for the production of specific chemical constituents. Previously, fast pyrolysis has been carried out with the goal of maximizing the production of bio-oil. Minimum research has been conducted concerning the production of specific bio-oil constituents with little consideration given to process conditions that optimize higher quality bio-oil [9-11]. Emphasis should not remain on optimizing bio-oil production but rather bio-oil quality for specific end-use.

1.3 Guiding Hypothesis

Sugars and phenolic compounds can be quantified in bio-oil utilizing standardized testing methodology developed by food chemistry. Both the water-soluble sugars and water-insoluble phenol compounds can be successfully separated from bio-oil. Furthermore, the pyrolysis reactor can be operated at temperatures that optimize effective production of sugars, phenolic compounds and other valuable chemicals.

1.4 Hypothesis 1

Analytical methods developed by food chemistry to measure sugars can be adapted to the analyses of bio-oil.

The first research goal includes determination of the applicability of established methodology from food chemistry for sugars determination in bio-oil.

1.5 Hypothesis 2

Analytical methodology developed for the food industry to measure total phenolic compounds can be adapted to the analyses of bio-oil.

The second research goal includes determination of the applicability of established methodology from food chemistry for the quantification of total phenolic compounds in bio-oil.

1.6 Hypothesis 1 and 2 Discussion

Bio-oil properties are drastically different from that of petroleum-derived oils [8,16]. Methodology exists in the evaluation of natural products in both the agricultural and food chemistry sectors of industry.

This is a rational approach because many of the chemical constituents in bio-oil are remarkably similar to those evaluated in food chemistry including carbohydrates and phenolic compounds. Methodologies for food evaluation are well proven and readily available. Food application chemistry may offer faster, less complex methods targeting specific chemical groups within bio-oil. Potentially, these methods will offer easy, rapid, and repeatable results in comparison to the current approaches to bio-oil analyses.

1.7 Hypothesis 3 and Discussion

The water-soluble sugars and water-insoluble phenolic compounds can be separated from bio-oil produced using a fractionated condenser system.

The third research goal includes the separation of two commercially important bio-oil constituents, sugars and phenolic compounds. This offers the unique opportunity for use in specific value-added applications. The separated water-insoluble fraction exhibits potential for upgrading to transportation fuels, as well as other platform chemicals. The removal of pyrolytic sugars and their conversion into liquid drop-in fuels while using phenolic compounds to produce high value products (i.e. resins, adhesives) promotes the economic viability of the thermochemical conversion of biomass for use in direct upgrading to fuels and other important applications.

1.8 Hypothesis 4 and Discussion

Pyrolysis reactors can be operated at specific temperatures for optimum yields of sugars, phenolic compounds and other chemicals of interest.

The fourth research goal examines biomass pyrolysis temperature effects on lignin-derived oligomers, levoglucosan, and other important constituents, as recovered in SFs. This will offer insight on appropriate reactor operating conditions for specifically desired end-products.

Ultimately, providing economic vitality to a fledgling bio-oil industry.

CHAPTER 2. BACKGROUND

2.1 Demand for Liquid Transportation Fuel and Chemicals

Liquid transportation fuels are primarily derived from petroleum [17] and movement towards a sustainable bioeconomy has spurred development of renewable energy and chemical sources [3,18]. Biomass, a renewable energy source, has potential to become economically feasible and generate similar quality liquid fuels to those derived from petroleum. The utilization of lignocellulosic biomass will play a progressively important role in the future [19,20] because it offers a number of conversion technologies that can be used to produce liquid fuels [3,18,19]. Among these, fast pyrolysis of biomass has been undergoing rapid development because it has the potential to solve liquid fuel shortage problems [16,19-21] and provide a source from which energy, fuels, and chemicals can be co-produced in a biorefinery integrated system [3,18,19].

Fast pyrolysis of biomass produces solid bio-char, liquid bio-oil, and NCGs. A typical yield from woody biomass includes 60-75 wt% bio-oil, 15-25 wt% bio-char, and 10-20 wt% gas [16]. Agricultural feedstocks result in lower bio-oil yields (36-45 wt%) for straws and hay [22] and 12.5 to 18 wt% for sugarcane bagasse (*in natura*) bio-oil produced by fast pyrolysis at temperatures ranging from 350-450°C [23].

Bio-oil is increasingly being recognized as an important feedstock [24,25] for thermochemical-based biorefinery applications for transportation fuels, energy and chemicals [3,12] even though bio-oil exhibits negative characteristics. Bio-oil contains 42 to 48 wt% oxygen [16,22], which must be reduced by catalytic upgrading before co-feeding in a refinery unit to produce transportation fuels [24]. Several other problems to direct upgrading of bio-oil includes high water content (15-30%), limited stability, and high acidity [24,26,27]. To lower acidity and improve stability of bio-oil the removal of acids is important [28]. Because of bio-oil

detrimental qualities, separation of bio-oil constituents is necessary before utilization of bio-oil can be accomplished.

2.2 Current Testing Practices for Phenolic Compounds and Sugars Quantification and Identification in Bio-Oil

Fermentable sugars obtained from lignocellulosic material shows great potential as a cheap renewable feedstock for bio-fuels. The sugars are produced by the depolymerization of the cellulose and hemicellulose in plant biomass. This plant material represents an “untapped” source of fermentable sugars for significant industrial use [29].

Traditional quantification of sugars in bio-oil is complicated and difficult to accomplish. Consequently, complete analyses of sugars is lacking in bio-oil research. The current techniques for sugar identification and quantification are specific to each sugar and involve expensive analytical instrumentation as well as highly trained personnel. Bio-oil sugars are not well identified. Reported sugars include levoglucosan, glucose, arabinose, and cellobiose [30] fucose, galactose, mannose, fructose, and ribose [31]. It would be beneficial to quantify total sugars with a simple and fast test methodology suitable for biorefinery settings.

Bio-oil phenolic compounds also are problematic for individual identity and quantification. Wet chemistry methods are used to help identify specific functional groups within water-insoluble phenolic oligomers (pyrolytic lignin). These include carbonyl, methoxyl, and hydroxyl groups [4]. Although wet chemistry methods are time consuming and require the use of hazardous solvents, these methods are essential for basic understanding of pyrolytic lignin; revealing possible reactions, radical formation, and structural models. But in light of the complexity of the oligomeric phenols in bio-oil, it is not feasible to identify these individually. It is undoubtedly more important to estimate the total phenolic compounds content of the oil which

influences both the total acid number (TAN) for the oil as well as the aromatic content of fuels refined from the oil [30]. As to date, phenols are “estimated” by measuring the water-insoluble fraction of bio-oil. This fraction is derived from lignin and is considered to be comprised of mainly phenolic compounds.

The importance of reliable and accurate testing procedures for the measurement of sugars and phenols in bio-oil cannot be overstated. The future of thermochemical conversion processes are dependent on successful quantification of important chemical constituents in bio-oil by standardized test methods. Without accurate testing procedures, little headway or acceptance of the products will occur.

2.3 Bio-Oil Fractionation

It is vital that bio-oil be separated into less complex fractions/mixtures for successful upgrading to liquid drop-in transportation fuels and/or valuable chemicals. As discussed previously, distillation is not a valid route to bio-oil fractionation because of bio-oils thermal and chemical instability [3].

Iowa State University’s bio-oil recovery system consisting of condensers and electrostatic precipitators (ESPs) (Figure 1) has provided bio-oil fractionation during bio-oil production. This system offers prospects for separating commercially important compounds from pyrolysis vapor streams. For example, the heavy fraction contained in SF 1 and 2 contains both water-soluble sugars and water insoluble phenolic oligomers that if separated could provide substrate for fermentation and a bitumen-substitute for production of asphalt binder.

This bio-oil collection system was developed on the premise that both vapors and aerosols are generated during fast pyrolysis. Vapors were presumed to be predominantly decomposition products of carbohydrate polymers while aerosols were the non-volatile, lignin-derived

oligomers often described as “pyrolytic lignin” in bio-oil. The condenser stages were designed to recover vapors according to condensation temperatures. Electrostatic precipitators were used to effectively recover aerosols. Five stages of bio-oil recovery allows separate collection of both vapors and aerosols from the oligomeric rich “heavy-ends”, a middle portion of monomeric phenols and furans, and an aqueous phase containing most of the “light oxygenates” [1].

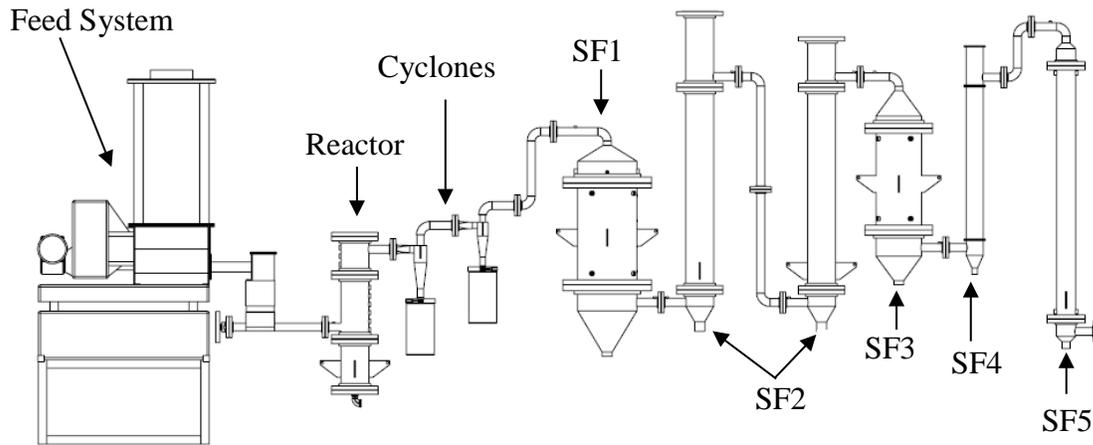


Figure 1. Schematic of the fast pyrolysis reactor and the five stage fractions (SF) of bio-oil recovery [1].

Stage 1 captures high boiling point compounds such as levoglucosan (1,6-anhydro- β -D-glucopyranose, $C_6H_{10}O_5$, Figure 2) and phenolic oligomers while Stage 2 captures aerosols formed from these compounds either during pyrolysis or cooling in the condenser of Stage 1 [1]. The highest weight percentage water-insolubles are captured in SFs 1 and 2, as well as the highest weight percentage levoglucosan. Stage 1, a condenser, consists of a shell-and tube heat exchanger consisting of thirty, 2.54 cm diameter stainless steel tubes and operates with gas inlet and outlet temperatures of 345°C and 102°C, respectively. The temperature of the coolant water is controlled to 85°C. Stage 2, an electrostatic precipitator (ESP) operated at 40 kVDC, is

constructed from 15.24 cm diameter stainless steel pipe with a 2.54 cm diameter electrode extending the length of the pipe along its axis. It is heat traced to 129°C to prevent condensation of vapors. Both Stages 1-2 bio-oil are black and extremely viscous even when hot. Upon cooling, they are resinous solids at room temperature [1]. Further detail of the reactor is described in detail by Pollard et al. [1].

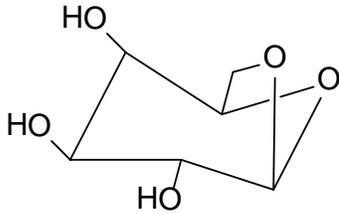


Figure 2. Structure of levoglucosan.

High molecular weight phenolic oligomers, which comprise the water-insoluble fraction of bio-oil, are condensed in stage 1 and 2. Pollard et al. [1] reported the highest weight percentage water-insolubles were captured in SF 1 and 2 as well as the highest weight percentage levoglucosan. In conclusion, the authors reported the concentration of water-soluble levoglucosan and water-insoluble “pyrolytic lignin” in the first two SFs suggest their separation by water washing, ultimately yielding an aqueous stream of fermentable sugar [1]. The separated water-insoluble portion exhibits potential for upgrading to transportation fuels, as well as other platform chemicals (Figure 3).

The removal of pyrolytic sugars and their conversion into liquid drop-in fuels while using phenols to produce high value products (i.e. green diesel, resins, adhesives) could be a promising approach to economic viability of bio-oil refineries [13-15]. Other opportunities exist for the phenolic oligomeric fraction (water-insolubles) from SF 1-2 as use in asphalt.

The determination of applicability and suitability of SF 1-2 in asphalt is current on-going

research at ISU [32,33]. Williams et al. [34] report the development of fractionated bio-oil during production is a source for beneficial asphalt additives, modifiers, and extenders.

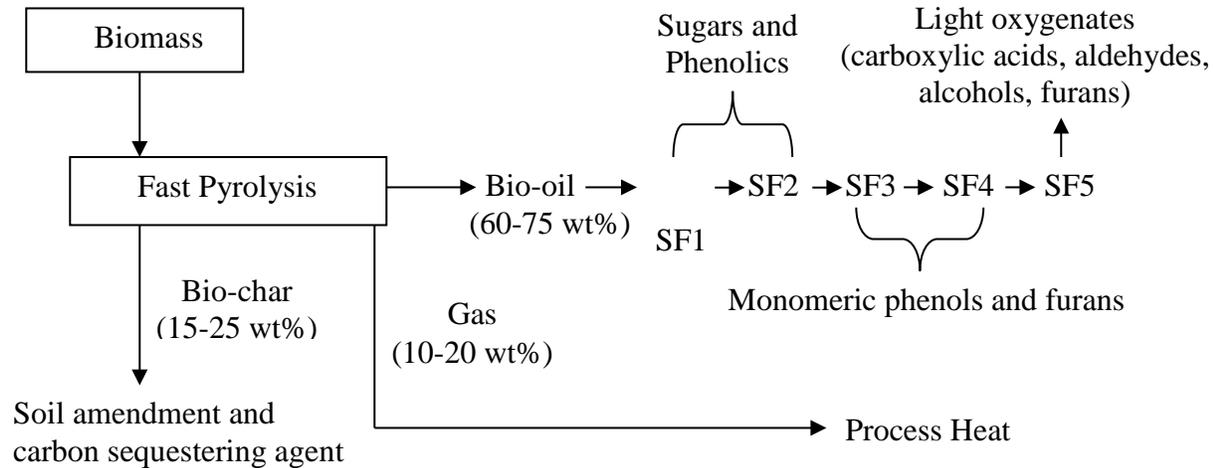


Figure 3. Biomass fast pyrolysis and 5 staged bio-oil upgrading diagram [1-3].

2.4 “Washing” to Separate the Water-Soluble and Water-Insoluble Phases of Bio-Oil

It has been shown that the bio-oil aqueous phase is an excellent source to isolate the water-soluble sugars [3,35]. Separation of bio-oil water-soluble from the bio-oil water-insoluble phases has been used to isolate specific groups of chemicals [3,4,16,26,31,35-39]. Bio-oils have high oxygen content due to carboxylic acids, aldehydes, ketones, carbohydrates, alcohols, esters, phenols, and lignin-derived material. These compounds cause the polarity of bio-oils which make them highly soluble in other polar solvents. With the addition of increasing amounts of water-to-bio-oil, phase separation can be forced to occur [38]. The aqueous phase contains the polar carbohydrate-derived compounds while the viscous layer is predominantly less polar lignin-derived compounds [16]. The presence of both the water-soluble and water-insoluble phases can complicate bio-oil applications. Both phases can be treated separately to produce different value-added products either as an intermediate or as the final product [3].

Bio-oil aqueous phase is a starting feedstock to isolate chemicals, such as water-soluble sugars and acetic acid [3]. Even though water addition is commonly applied to bio-oil characterization there is little research in this area and traditionally is only applied to bio-oil captured in one fraction as “whole” bio-oil. Optimization and separation of levoglucosan by varying total water content used to extract the water-soluble fraction in whole bio-oil produced from Scots Pine is shown by Bennett et al. [35] prior to hydrolysis and fermentation to produce bio-ethanol. Oasmaa and Kuoppala [40] also describe whole bio-oil sample preparation for acquiring the water-soluble fraction for sugar determination in bio-oil.

Separation and quantification of the water-insoluble fraction of bio-oil is important in advancing pyrolysis technologies, as well. Sometimes called “pyrolytic lignin,” it is mostly non-volatile organic compounds derived from the lignin fraction of the biomass feedstock [41]. This oligomeric fraction is considered to react with other oligomers within the pyrolytic lignin or with reactive monomers such as eugenol, isoeugenol, vinyl guaiacol, vinyl-syringol, and 3-hydroxy-5,6-(4H)-pyran-4-one [42] causing bio-oil stability issues.

The composition and structural features of the water-insoluble fraction is important in stabilizing bio-oil and upgrading it to transportation fuels and other chemical products [39]. According to Bayerback and Meier [4], the constituents within the water-insoluble fraction have detrimental effects on several bio-oil properties including viscosity, reactivity, and stability. The viscous water-insoluble fraction is largely responsible for the poor combustion performance of bio-oil in boilers and engines. On the other hand, the water-insoluble fraction is attractive for the production of extenders in resin formulations [16] or phenolic compounds [4]. Typical phenolic compounds in bio-oil are guaiacol, catechol, resorcinol, syringol, eugenol, vanillin, isoeugenol,

and syringaldehyde. These compounds have commercial value with their main applications in the food and fragrance industry [43].

Elder and Soltes [44] estimated that 75% of the phenolic fraction is nonvolatile and potentially polymeric. When the water-soluble material from the initial extraction of the bio-oil was evaluated for phenols, it was found to be very low in comparison with the phenols found in the water-insoluble fraction [44].

Bio-oil contains more than 300 compounds of different molecular sizes with around 35-50% of these constituents being non-volatile [45]. Bayerback and Meier [4] classified bio-oil from wood into four main fractions (Figure 4). These are (1) medium-polar monomers

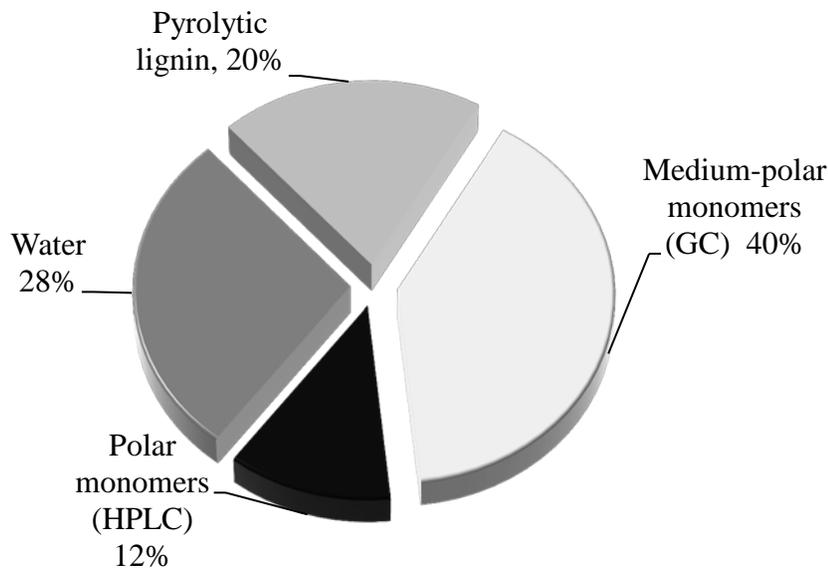


Figure 4. The main groups in whole bio-oil from the fast pyrolysis of wood [4].

detected by GC, 40 wt%, (2) polar monomers detectable directly by HPLC or GC after derivatization, 12 wt%, (3) water derived from reaction water and feedstock moisture, 28 wt%, and (4) oligomeric material (water-insoluble pyrolytic lignin) 20 wt%. The oligomeric portion is an important constituent of bio-oil, however, only scarce information on this fraction is available

in literature [4]. Pyrolytic lignin is normally reported as mass fraction of the whole bio-oil without additional chemical data.

2.5 Effects of Pyrolysis Temperature on Recovery of Bio-Oil as Distinctive

Stage Fractions

The fast pyrolysis process can be adjusted to favor specific product formation (i.e. bio-char, bio-oil, or NCGs). Maximum yields of bio-oil are obtained in the temperature range of 450-550°C [46] where approximately 70% of the biomass is converted to bio-oil [9]. Over this temperature range there is a bio-char yield decrease with a corresponding increase in NCG yield [46,47]. This increase in NCG yield is attributed to the secondary cracking of pyrolysis vapors and char into NCGs, depending on the temperature [48]. Often research on fast pyrolysis has been carried out with the goal of maximizing the production of bio-oil and bio-oil constituents without being aware of the process conditions that optimize higher quality bio-oil [9-11].

Bio-oil quality is essential for specific targeted applications [9]. Higher molecular weight oligomers are important if bio-oil end-use is asphalt, resins, polymers, etc. If sugar production is the goal; temperature control for the production of sugars is important. The yield and structure of chemical constituents would be essential in a biorefinery scenario utilizing direct upgrading to liquid fuels. Maximizing bio-oil yield should not remain the focus of bio-oil production but rather bio-oil quality for specific end-use.

2.6 Dissertation Format

This dissertation consists of a compilation of journal manuscripts, supplemented with a background section, literature review and overall conclusions. The first manuscript details analytical work and results of total water-soluble sugar quantification using AOAC Method

988.12 (44.1.30). Total water-soluble sugars quantification and comparison is provided for 3 separate methodologies: AOAC Method 988.12 (44.1.30), liquid-liquid extraction, and hydrolysis with quantification by HPLC. The second manuscript discusses the Folin-Ciocalteu method and provides a direct comparison of total phenolic concentrations in bio-oil using the Folin-Ciocalteu method and a liquid-liquid extraction method, obtained from literature, for total phenolic compound determination. The third manuscript details analytical work and results for the efficient removal of sugars and phenolic oligomers from SF 1 and SF 2 bio-oil. The fourth manuscript discusses bio-oil chemical constituent quantities and distribution. These bio-oils were produced and collected utilizing ISU process design unit fast pyrolysis reactor with the collection system at five different reactor temperatures: 350, 400, 450, 500, and 550°C. This manuscript discusses the importance of temperature in the optimization of the fast pyrolysis reactor for production of specific chemicals for desired end-use applications.

CHAPTER 3. LITERATURE REVIEW

3.1 Background

Bio-oil is the main product obtained from the thermal conversion of biomass and is considered a possible replacement for petroleum-based fuels and chemicals. However, bio-oil is compositionally distinct from petroleum. Many of the standard test methods used to characterize petroleum are ineffective for bio-oil characterization.

Firstly, this literature review explores the relevance of analytical methodologies developed for the food industry in the evaluation of bio-oil. Bio-oil constituents are similar to those evaluated by food chemistry including both sugars and phenols. Methodology for food evaluation is well proven and readily available.

Secondly, it includes information for effective removal of phenolic compounds and sugars from SF 1-2 bio-oil produced on the ISU process design unit fast pyrolysis reactor equipped with a unique condenser system. Once recovered as individual components, both sugars and phenolic compounds can be used in specific applications such as fermentation or direct up-grading to transportation fuels. Ultimately providing economic viability to thermochemical conversion processes.

Lastly, this review comprises temperature effects of fast pyrolysis on the produced bio-oil chemical constituent quantities and SF distribution. This knowledge offers the opportunity to target exact end-use applications and maximize the production of the required chemical constituents.

3.2 Introduction

Bio-oil is produced by the pyrolysis of biomass in a high-temperature, oxygen-free environment. It is comprised of multi-component mixtures of varying sized molecules derived

from cellulose, hemicellulose and lignin [49]. Bio-oil's complex physical and chemical makeup does not necessarily allow for accurate and reliable physicochemical characterization using current methodologies. This complexity makes bio-oil difficult to test and often leads to tedious testing procedures with high standard deviations.

A number of methodologies used in bio-oil characterization have been approved by the American Society for Testing and Materials International (ASTM) Committee D02 Petroleum Products and Lubricants. Standard methodologies are important for various reasons; they ensure that products conform to specifications, comparisons can be made on product performance for consumers as well as manufacturers and producers, and testing standards ensure that specific tests are being performed precisely the same at each laboratory.

Unfortunately, many of the standard methods developed for petroleum oil are used to characterize bio-oil even though these test methods may not be suitable nor reflect corresponding results [30]. For example, ASTM D664-09 is used to determine TAN of petroleum crudes [50]. This test method is also used to determine TAN of bio-oil. When used for the evaluation of bio-oil, this test method includes strong acids (i.e. mineral acids), weak acids (i.e. carboxylic acids) and very weak acids (i.e. phenols and substituted phenols). This does not necessarily reflect bio-oil corrosion properties or degradation, as intended.

Total acid number is defined as the quantity (mg) of potassium hydroxide needed to neutralize the acids contained in one gram of petroleum oil. According to ASTM D664-09 [51], it is applicable for acid determinations that have a dissociation constant (K_a) in water larger than 10^{-9} ; extremely weak acids whose dissociation constants are smaller than 10^{-9} do not interfere. Therefore very weak acids such as phenol with a K_a of 1.28×10^{-10} , *o*-cresol with a K_a of 6.3×10^{-11} , *m*-cresol with a K_a of 9.8×10^{-11} , and *p*-cresol with a

K_a of 6.7×10^{-11} would not be included [52]. However, bio-oil's high oxygen content and wide range of chemical functional groups results in ASTM D664-09 analyses to include strong acids, weak acids, and very weak acids. Consequently, bio-oil TAN values range as high as 400 mg KOH/ g sample. This is unfortunate because petroleum industry considers oils containing TAN values higher than 0.5 to be high acid crudes. Petroleum refinery processing of high acid crude oil causes major corrosion of equipment. Petroleum acids are primarily monocarboxylic acids which include aliphatic, naphthenic, and aromatic acids. It has been determined that naphthenic acids are currently considered the major source of this corrosion [53].

Total acid numbers may give an indication if a specific petroleum crude oil will be corrosive. However, TAN is a poor quantitative indicator of the expected corrosion severity. It is suspected that corrosion is not caused by total acid numbers but by a specific group of acids. Significant differences in corrosivity can be obtained from oils with the same TAN values, depending on the chemical structure of the naphthenic acids within the oils [54]. Crude petroleum oils are also complex mixtures. They contain sulfur and chlorides which may play a role in the nature and extent of the corrosion as well [55].

Further study is necessary before comparing TAN obtained from petroleum with those acquired with bio-oil. It is not known to what extent corrosion will occur from the bio-oil acids. It would be beneficial to compare corrosion with specific acids within bio-oil. Sulfur and chloride play a role in the corrosion process in regards to petroleum crude. Bio-oil contains very little sulfur thus its influence would be minimized. On the other hand, bio-oil does contain chlorides. Studies have not been performed to see the effect chloride plays in corrosion. Therefore, ASTM D664-09, is a poor indicator of corrosion characteristic of bio-oil and should not be used.

Problems occur when using petroleum ASTM standards and other analytical methodologies for bio-oil evaluation and characterization. As discussed, testing protocol for petroleum oil does not always reflect comparable parameters [16]. Round robin testing on bio-oil was organized in Europe in the 1980s to measure the accuracy of all physical and chemical analyses of bio-oil. Due to bio-oil's heterogeneity, erroneous results were obtained. More extensive round robin testing has been initiated due to problems of reproducibility between laboratories [30].

Bio-oil testing protocol has evolved over recent decades but problems remain because of the large variation in the physical and chemical nature of the oil. It differs from petroleum oils in chemical composition as well as physical properties [30]. Bio-oil is a complex mixture of chemicals consisting of several functional groups including carboxylic acids, aldehydes, furans, ketones, phenols, and carbohydrates. It is acidic, displays instability and phase separation in storage and contains large amounts of water and oxygen that contributes to its immiscibility in petroleum-based fuels. The heating value is approximately one half that of petroleum due to the large percentage of oxygen, approximately 40-50%, present in bio-oil [8,16,26]. It is essential for bio-oil's success as a renewable resource to provide standardized testing procedures that can be used world-wide.

3.3 Applicable Food Chemistry Methodologies for Bio-Oil Characterization

The present investigation considers the applicability of established methodology from food chemistry for bio-oil characterization. Methodology exists in the evaluation of natural products in both the agricultural and food chemistry sectors of industry. These testing protocols have been in continual development due to requirements for measuring food composition and characteristics. Many of these testing methods are well proven, have been used for decades, and are used internationally.

A few of the organizations that have official methods of analysis for food samples include AOAC, American Oil Chemist's Society, and American Association of Cereal Chemists. The choice of methodology is determined by the nature of the food sample and the specific reason for the analysis. This offers the unique opportunity to couple existing food chemistry methodology with analysis of bio-oil. Bio-oil chemical makeup is more similar to food products versus that of petroleum. It may be more beneficial to evaluate pyrolysis composition utilizing food product methodology opposed to petroleum oil methodology.

Oasmaa and Kuoppala [40] used the Brix method, developed to measure the sugar content of juice and wine, to characterize carbohydrates in the water-soluble fraction of bio-oil. The sugar content is determined via a hydrometer that indicates a liquid's specific gravity [40]. Brix is a measure of the mass ratio of soluble solids to water, which can be used as a proxy for the amount of sugar in solution [56]. Although Oasmaa and Kuoppala [40] reported the sugar fraction correlated well with the Brix determinations, it systematically gave 20 wt% higher values for fresh liquids. They compared sugar determinations of newly produced bio-oil to that of various stages of aging. The authors explained the higher values for fresh liquids was due to the fact that the Brix method is used in the sugar industry for samples that do not contain hydroxy aldehydes. Carbohydrates are polyhydroxy aldehydes or polyhydroxy ketones. When the Brix method is used, it not only gives the total amount of sugar but also includes hydroxyl aldehydes, and hydroxyl ketones. They concluded there is a need for a rapid method of analyzing the carbohydrate content of bio-oils because carbohydrates in bio-oil cause problems in fuel oil due to their reactivity and sticking tendency [40].

3.4 AOAC Method 988.12 (44.1.30) Phenol-Sulfuric Acid Assay for Total Carbohydrate Determination using Ultraviolet-Visible Range Spectroscopy (UV-Vis)

Both, qualitative and quantitative analyses of sugars are a complex and challenging areas for analytical chemistry [57]. Traditionally, sugar content in foods is estimated on the basis of refractive index measurements or volumetric procedures, which provide total sugar content information and the amount of reducing sugars. Commonly used procedures include various HPLC methodologies for sugar analysis. Other highly specific procedures for quantifying each sugar separately include enzymatic analysis, derivatization, and online dialysis. Precision is reasonable but the time required for analysis can be long. Also, these methods are chemical based, tedious, and often require reagents that are unfriendly to the environment or hazardous and generate chemical waste [57].

Total soluble carbohydrate concentration can be determined by AOAC Method 988.12 (44.1.30). It is simple, fast, accurate, and specific to carbohydrate methodology. Nearly all classes of sugars (i.e. sugar derivatives, oligosaccharides, polysaccharides) can be determined. When treated with phenol and concentrated sulfuric acid, the reducing groups give an orange-yellow color which shows absorbs light in the ultraviolet visible range. The reagent is inexpensive and stable. The color produced is permanent, thus, it is not necessary to pay special attention to controlling conditions [5].

In 1951, DuBois et al. [58], discovered that phenol in the presence of sulfuric acid provided a simple fast method for the quantitative colorimetric determination of ketoses and aldoses that was applicable to reducing carbohydrates. DuBois et al. [5] ran a series of standard curves for various monosaccharides, disaccharides, and trisaccharides during further development of this method in 1956. Absorption curves were obtained by plotting absorbance versus wavelength.

The absorption curve was characteristic for each sugar tested. Their wavelength determinations are shown in Table 1. They determined the pentoses have an absorption maximum at 480 nm, while the hexoses have an absorption maximum at 485-490 nm. The authors concluded AOAC Method 988.12 (44.1.30) can be applied to the analyses of mixtures of sugars and their methyl derivatives.

In the late 1980s, Rao and Pattabiraman [59,60] describe a modification that reduces the variability of the assay towards different sugars while increasing sensitivity. Their modification avoids sulfonation of the phenols by adding the phenols after the sugars have been converted to furfurals with strong sulfuric acid and lowering the heat of reaction. They reported that the color intensities for many hexoses and pentoses was decreased when phenol-sulfonic acid was formed.

Table 1. Wavelength data for certain carbohydrates determined by the phenol-sulfuric acid reagent [5].

Carbohydrate	Wavelength (nm)
D-Fructose	490
D-Glucose	485
Sucrose	490
Dextran	488
D-Galactose	487
D-Mannose	487
L-Arabinose	480
D-Xylose	480
Maltose	490

BeMiller [7] describes the phenol-sulfuric acid assay for total carbohydrate determination. He explains the method's principals and characteristics. Carbohydrates are destroyed by strong acids and/or high temperatures and under these conditions a series of reactions take place, beginning with a dehydration reaction (Figure 1). With continued heating in the presence of acid, various furan derivatives are produced (Figure 2) [5-7]. These can then condense with various phenolic compounds to produce colored compounds.

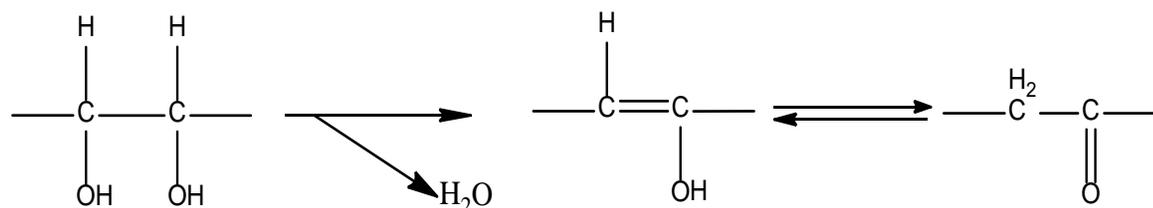


Figure 1. Dehydration reaction occurs when strong acid is added to carbohydrates [5-7].

Method validity and the type of sugars that fit within the scope of this method can be found in literature, as well. Fournier [61] states that with the exception of certain deoxy sugars, the method can be applied to reducing and non-reducing sugars and to many classes of carbohydrates including oligosaccharides. Fucose is a deoxy sugar reported in bio-oil [31] in low concentrations. Under proper conditions, the AOAC method is accurate to $\pm 2\%$.

In addition to the food industry, agricultural industries are also utilizing this method. Giannoccaro et al. [62] discuss AOAC Method 988.12 (44.1.30) which is widely used for the total sugar determination in soybeans. The procedure detects soluble sugars as well as oligomeric/polymeric sugars because the high sulfuric acid concentration can hydrolyze oligomeric/polymeric sugars into monomers. Sucrose and glucose are used for establishing the standard curve in the phenol-sulfuric acid method for determination of soybean sugars.

The color response of the AOAC method varies with different sugars due to their differences in molecular structure [63]. The quantification of total sugars present in a particular sample will be affected by the type of sugar used in preparing the standard curve [7]. Giannoccaro et al. [62] found that significant differences in total sugars were observed when extracts from the same soybean line were analyzed using standard curves prepared from different sugars. Giannoccaro et al. [62] demonstrated that the quantification of sugars using the AOAC method strongly depends

on the sugar used for the standard curve. Because different sugars have a distinct response to the phenol-sulfuric acid reaction, the final color is assumed to be a combination of the colors that resulted from the reaction of different sugars [7,63]. Giannoccaro et.al [62] proposed sucrose to be more suitable than any other sugar for standard curve preparation in quantifying total soluble sugars in soybeans because it represents the majority of soluble sugars present.

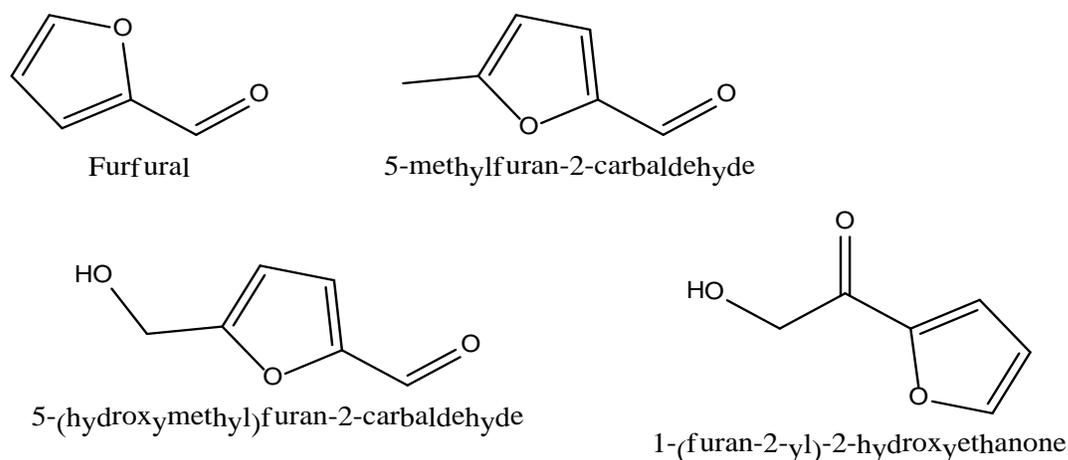


Figure 2. Furan products that may arise from carbohydrates upon addition of a strong acid [5-7].

Levoglucosan, an anhydrosugar, is one of the predominant sugars from the degradation of cellulose during fast pyrolysis [35] and has been reported in concentrations ranging from 3 to 6 wt% in bio-oil [64]. Yu and Zhang [65] report that anhydrosugars formed during pyrolysis (i.e. levoglucosan and cellobiosan) may be converted to glucose by dilute acid hydrolysis. Their work shows that the addition of sulfuric acid to pyrolysis oil generated more glucose than could be accounted for by the amount of levoglucosan present. While this extra glucose was attributed to unknown carbohydrate oligomers (i.e. cellobiosan and other sugars) there is little known about identity or relative proportions. Li and Zhang [66] also report levoglucosan can be readily hydrolyzed to glucose by acid hydrolysis.

3.5 Folin-Ciocalteu Colorimetry Method

The potential exists to evaluate bio-oil for phenolic compounds content, as well. Bio-oil contains numerous phenolic compounds. It is not feasible to identify these individually and probably more important to estimate the total phenolic compounds content of the oil, which influences both the TAN for the oil as well as the aromatic content of fuels refined from the oil.

Extraction work is time consuming, tedious, and involves the use of many different hazardous solvents. There is a need for a fast, easy, reliable standardized test method for quantification of total phenols in bio-oil.

The wine industry utilizes the Folin-Ciocalteu Colorimetry Method to determine total phenolic compounds in their products. Wine is not the only food containing phenols. Phenolic compounds are found in all foods, most of which contain very low levels. Prominent foods high in phenolic compounds include coffee, tea, chocolate, fruits, oils, spices, and some whole grains [67].

The Folin-Ciocalteu method is based on a chemical reduction of the reagent (mixture of tungsten and molybdenum oxides). The products of the metal oxide reduction have blue color that has broad light absorption with a maximum at 765 nm [67]. The chemistry of tungstates and molybdates is very complex. The isopolyphosphotungstates are colorless in the fully oxidized 6^+ valence state of the metal and the molybdenum compounds are yellow. They form mixed heteropolyphosphotungstates-molybdates and exist in acid solution as hydrated octahedral complexes of the metal oxides coordinated around a central phosphate. Sequences of reversible one or two electron reductions lead to blue species such as $(\text{PMoW}_{11}\text{O}_{40})^{4-}$. In principle, the addition of an electron to a formally nonbonding orbital reduces nominal MoO^{4+} units to

isostructural MoO^{3+} blue species [68]. The intensity of the color is proportional to the concentration of phenols. A disadvantage of the FC method is that it is nonspecific and can be affected by other nonphenolic reducing molecules. The reagent is commercially available but can be prepared in the laboratory [67].

Due to the color formation of the Folin-Ciocalteu reaction via the reduction of the reagent, this reaction is general enough to allow for interferences. The most problematic interference may be sugar. Proteins are also oxidized by the Folin-Ciocalteu reagent. In wine analysis the interference of sugar is corrected by subtraction of established correction factors [67].

3.6 Sugar and Phenols Recovery from the Heavy-Ends of Fractionated Bio-Oil

Bio-oils have high oxygen content because of the highly polar constituents: carboxylic acids, aldehydes, ketones, carbohydrates, alcohols, esters, phenols, and lignin-derived constituents. These compounds cause the polarity, thus making bio-oil highly soluble in polar solvents such as water, methanol, and acetone [38]. In its present condition, water is miscible with the oligomeric lignin derived components due to the solubilizing effect of other polar hydrophilic compounds [26]. During the separation of the water soluble compounds from the water insoluble fraction there is co-extraction between the two phases. It is well known that, in the presence of large amounts of water, the lignin oligomers spontaneously precipitate [69,70]. By adding increasing amounts of water to bio-oil, phase separation can be forced to occur [38].

Two separated distinct streams; a water-soluble sugar rich stream and a phenolic oligomer-rich raffinate offer numerous opportunities for end-product uses. This has the potential to provide economic viability to bio-oil refineries [13-15] as well as to thermochemical conversion processes as a means of production of biobased products as an alternative to petroleum.

3.7 Effects of Pyrolysis Temperature on Recovery of Bio-Oil as Distinctive Stage

Fractions

Research on fast pyrolysis continues to focus on maximizing production of bio-oil and bio-oil constituents. Little consideration has been put into the necessary process conditions that optimize higher quality bio-oil [9-11]. It is crucial to make headway and exploit endless opportunities that exist for producing quality bio-oil for specific targeted end-use.

Bio-oils are multicomponent mixtures containing 35-40 wt% oxygen which is present in most of the more than 300 identified compounds [8]. It is comprised of both volatile and non-volatile compounds and viscous oligomers including water, acids, alcohols, aldehydes, esters, ketones, sugars, phenols, quaiacols, syringols, vanillins, furans, and multifunctional compounds (i.e. hydroxyacetic acid, hydroxyacetaldehyde, hydroxy acetone, 3-hydroxy-3-methoxy benzaldehyde) [1,16,71,72].

The major chemical components of lignocellulosic biomass are cellulose, hemicellulose, and lignin [73]. Lignocellulosic biomass typically contains 85-90% cellulose, hemicellulose, and lignin while organic extractives and inorganic mineral matter constitutes the remaining mass. The cellulose, hemicellulose, and lignin constituents of wood, pyrolyze to mainly monomer and monomer-related fragments [74]. Hemicellulose breaks down first at approximately 197-320°C followed by cellulose in the temperature range of 310-390°C. Lignin is the last component to be pyrolyzed at 200-550°C [75,76]. These differences in reactivity of biomass due to the variations in chemical composition must be better understood in order to optimize the fast pyrolysis process to obtain bio-fuels and valuable chemicals with high selectivity and efficiency [73,77].

Bio-oil quality is essential for specific targeted applications [9]. Higher molecular weight oligomers are important if bio-oil end-use is asphalt, resins, polymers, etc. If sugar production is the goal; temperature control for the production of sugars is important. On the other hand, yield and the structure of chemical constituents would be essential in a biorefinery scenario utilizing direct upgrading to liquid fuels. Maximizing bio-oil yield should not remain the focus of bio-oil production but rather bio-oil quality for specific end-use.

3.8 Summary

Bio-oils are recognized for their potential as liquid drop-in fuels and chemicals. Firstly, problems hindering their advancement include standardized testing procedures that are applicable and provide correct, repeatable results. Food application chemistry may offer faster, less complex methodology targeting specific chemical groups within bio-oil. Potentially, the development of these methods will offer easy, rapid, and accurate results in comparison to the current test methods used for bio-oil analyses. Secondly, the separation of two key products from bio-oil is significant. Currently, sugars and phenolic compounds are being used in the production of biofuels and other important chemistries. Thirdly, temperature control of pyrolysis reactors to produce higher quantities of specific chemical families or individual constituents is central to economic viability of these processes. By continuing to maximize bio-oil quantity instead of optimizing quality, little progress will be made in advancing thermochemical technologies. My research goals include:

1. The evaluation of the total sugar content in the water-soluble fraction of bio-oil utilizing the AOAC Method 988.12 (44.1.30) Phenol-sulfuric Acid Assay for Total Carbohydrate Determination. Individual sugars in bio-oil have not been identified. Potentially, those sugars

that are water-soluble can be subjected to this test method that has been in existence and used for decades.

The potential of bio-oil is increasingly recognized even though most research is still at a fundamental scale [25]. It is important to bio-oil's future to implement standard test methods and gain understanding of specific "families" contributing to bio-oils complexity that make upgrading problematic. Looking to existing food methodology may allow for faster advancement of bio-oil in the pursuit of bio-fuels and chemicals.

2. The water-insoluble fraction of bio-oil is comprised of various phenolic compounds. Complete identification may not be feasible. Total quantification of phenols is a more realistic approach and can possibly be accomplished using methodology common in the wine industry: the Folin-Ciocalteu Colorimetry Method. Total phenolic compound quantification will allow for evaluations of pyrolysis condition changes and its effect on total phenolic compounds concentration. The phenolic fraction of bio-oil evolves from pyrolysis of the biomass lignin fraction and it shows potential for use as feedstock in the production of liquid fuels and chemicals.
3. The ability to separate two very important chemical families from bio-oil is important to its viability for use in fuel and chemical production. Besides the detrimental problems caused by both sugars and phenolic compounds when they are not separated, their influence on each other during upgrading processes cannot be overlooked. Sugars causes coking, stickiness, and other problems during upgrading processes when it is not removed from bio-oil. On the other hand, phenolics cause problems with fermentation of pyrolytic sugars as they are toxic to micro-organisms.

4. The ability to operate the pyrolysis reactor at temperatures to optimize the production of the chemical of interest is vital. If sugars is the main interest, both feedstock and reactor temperatures can be optimized for its production. If phenolic compounds are the key concern, optimization is also available.

CHAPTER 4: TOTAL WATER-SOLUBLE SUGARS QUANTIFICATION IN BIO-OIL USING THE PHENOL-SULFURIC ACID ASSAY

A paper submitted to the *Journal of Analytical and Applied Pyrolysis*

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Abstract

Bio-oil produced from fast pyrolysis of biomass contains sugars originating from cellulose. Traditional quantification of sugars in bio-oil is accomplished by gas chromatography/mass spectroscopy (GC/MS) via derivatization, high-performance liquid chromatography (HPLC), ion-exchange chromatography (IC), or nuclear magnetic resonance (NMR) methodologies. These techniques are highly specific for each sugar, tedious to perform, expensive, and involve the use of hazardous solvents. We have evaluated a standardized test method developed for food and agriculture applications, the Association of Analytical Communities, International, (AOAC) Method 988.12 (44.1.30) Phenol-Sulfuric Acid Assay for Total Carbohydrate Determination, to quantify total sugars in the water-soluble fraction of bio-oil. This study investigates accuracy relative to matrix effects caused by non-sugar compounds using positive and negative controls. Positive controls included levoglucosan, D-glucose, D-mannose, D-xylose, D-fructose, D-galactose, L-arabinose, L-fucose, and cellobiosan. Negative controls included phenol, acetic acid, formic acid, propionic acid, glycolic acid, acetol, furfural, 5-hydroxymethylfurfural (5HMF), furfuryl alcohol, 2-methylfuran and 2(5H)-furanone. Potential interference with the quantification of total water-soluble sugars by the AOAC Method 988.12 (44.1.30) was calculated for all positive and negative controls by using data obtained when adding the contributor (positive controls) and the interferent (negative controls) into the water-soluble fraction of bio-oil with typical concentrations found in bio-oil. It was found that furfural, 2(5H)-furanone, 5HMF, and furfuryl alcohol influenced results with a range of potential errors of 9.56-

29.7%, 9.52-29.8%, 2.91-24.8%, and 1.34-11.9%, respectively. A correction factor of 0.76 wt% was established to reduce or eliminate this influence. Total water-soluble sugars content in bio-oil detected by AOAC Method 988.12 (44.1.30) was comparable to the quantity of sugars detected using hydrolysis with quantification by HPLC. The uncertainty of measurement of water-soluble sugars in bio-oil at 95% confidence was $\pm 1.7\%$ using AOAC Method 988.12 (44.1.30) when the correction factor was employed.

Introduction

The goal of this research is to adapt standard analytical methodology developed for the food industry to measure water-soluble sugars in bio-oil. To accomplish this, research was performed using AOAC Method 988.12 (44.1.30) Phenol-Sulfuric Acid Assay for Total Carbohydrate Determination in quantifying total sugar content in the water-soluble fraction of bio-oil. Traditionally, sugar quantification in bio-oil is accomplished by liquid-liquid extraction, HPLC, IC, GC/MS utilizing derivatization, or NMR. Qualitative and quantitative analyses of sugars are complex and difficult areas for analytical chemistry. Precision is reasonable but the time required for analysis can be long. Also, these methods are chemically based, tedious, and often require reagents that are unfriendly to the environment or hazardous and generate chemical waste [57].

Bio-oil, the liquid product from the fast pyrolysis of biomass, is an exceedingly complex mixture of organic compounds with many oxygen-containing functional groups which include acids, aldehydes, ketones, phenols, furans, and sugars. It has a high water content (approximately 20%), high acidity (pH of 2-3), and low storage stability [78].

Bio-oil characterization is challenging and many analytical techniques must be used to obtain a detailed product distribution which is still incomplete. Only 40% of bio-oil compounds are volatile enough to be analyzed by GC while only 10-15% can be determined by HPLC [4,30,64].

For further development of bio-oil applications, simple and direct analytical methods are needed [64].

Although the application of analytical methods developed for food chemistry to analyze bio-oil is uncommon, there is precedence for doing so. Oasmaa and Kuoppala [40] used the Brix method, developed to measure the sugar content of juice and wine, to characterize carbohydrates in the water-soluble fraction of bio-oil. The sugar content is determined via a hydrometer that indicates a liquid's specific gravity [40]. Brix is a measure of the mass ratio of soluble solids to water, which can be used as a proxy for the amount of sugar in solution [56]. Although Oasmaa and Kuoppala [40] reported the sugar fraction correlated well with the Brix determinations, it systematically gave 20 wt% higher values for fresh liquids. They compared sugar determinations of newly produced bio-oil to that of various stages of aging. The authors explained the higher values for fresh liquids was due to the fact that the Brix method is used in the sugar industry for samples that do not contain hydroxy aldehydes. Carbohydrates are polyhydroxy aldehydes or polyhydroxy ketones. When the Brix method is used, it not only gives the total amount of sugar but also includes hydroxyl aldehydes, and hydroxyl ketones. They concluded there is a need for a rapid method of analyzing the carbohydrate content of bio-oils because carbohydrates in bio-oil cause problems in fuel oil due to their reactivity and sticking tendency [40].

Total soluble carbohydrate concentration can be determined by AOAC method 988.12 (44.1.30). It is simple, fast, accurate, and specific to carbohydrate methodology. Nearly all classes of sugars (i.e. sugar derivatives, oligosaccharides, polysaccharides) can be determined. When treated with phenol and concentrated sulfuric acid, the reducing groups give an orange-yellow color that absorbs light in the ultraviolet visible range. The chromophore electron spectral transitions shift the absorption of light to longer wavelengths (200-700 nm) which are convenient

for UV-Vis analyses [79]. The color produced by the AOAC method is stable for several hours and readings may be made later if necessary [5].

In 1951, DuBois et al. [58], discovered that phenol in the presence of sulfuric acid provided a simple, fast method for the quantitative UV-Vis determination of ketoses and aldoses that was applicable to reducing carbohydrates. DuBois et al. [5] employed a series of standard curves for various monosaccharides, disaccharides, and trisaccharides to further develop this method. Absorption curves were obtained by plotting absorbance versus wavelength. The absorption curve was characteristic for each sugar tested. They determined that pentoses have an absorption maximum at 480 nm, while hexoses have an absorption maximum at 485-490 nm. The authors concluded this method could be applied to the analyses of mixtures of sugars and their methyl derivatives.

BeMiller [7] provides an in-depth explanation of AOAC Method 988.12 (44.1.30), detailing the method's principals and characteristics. Carbohydrates are hydrolyzed by strong acids and/or high temperatures. Under these conditions a series of reactions take place, beginning with a dehydration reaction (Figure 1). With continued heating in the presence of acid, various furan derivatives are produced (Figure 2) [5-7]. These can then condense with various phenolic compounds to produce colored compounds.

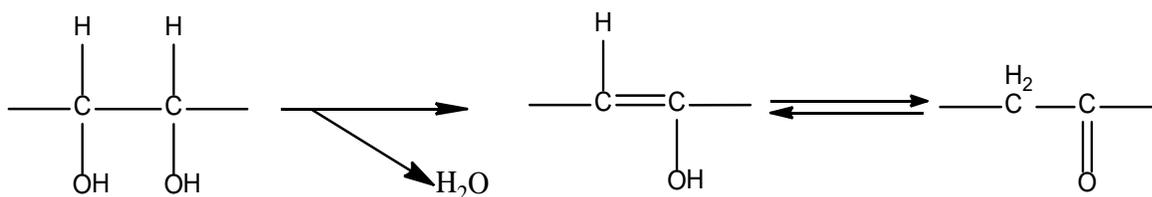


Figure 1. Dehydration reaction occurs when strong acid is added to carbohydrates [5-7].

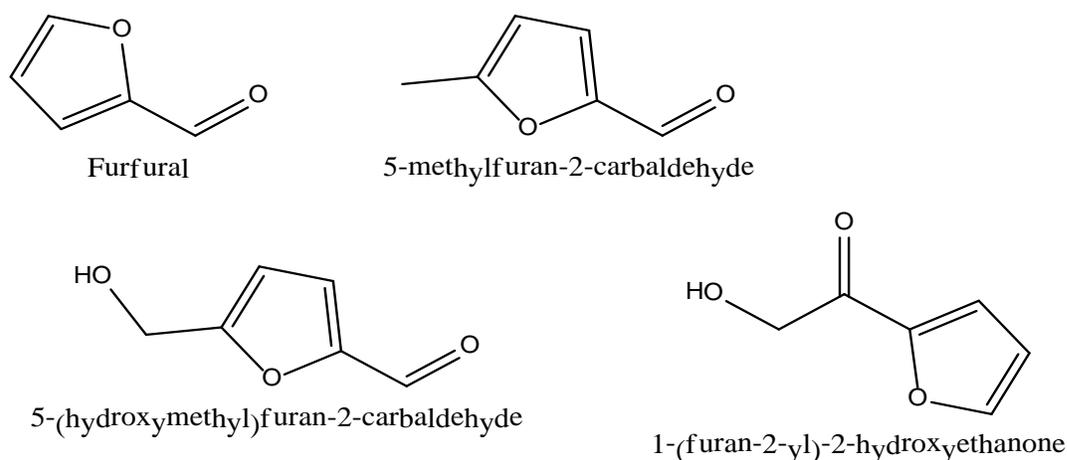


Figure 2. Furan products that can arise from carbohydrates upon addition of a strong acid [5-7].

Method validity and the type of sugars that fit within the scope of this method can also be found in literature. Fournier [61] states that with the exception of certain deoxy sugars, the method can be applied to reducing and non-reducing sugars and to many classes of carbohydrates including oligosaccharides. Under proper conditions, AOAC Method 988.12 (44.1.30) is accurate to $\pm 2\%$ [61].

In addition to the food industry, other agricultural sectors also utilize this method. Giannoccaro et al. [62] discuss AOAC Method 988.12 (44.1.30) which is also widely used for total sugar determination in soybeans. The procedure detects soluble sugars as well as oligomeric/polymeric sugars because the high sulfuric acid concentration can hydrolyze oligomeric/polymeric sugars into monomers.

The color response of AOAC Method 988.12 (44.1.30) varies with different sugars due to their differences in molecular structure [63]. Each sugar has an absorption maximum at a specific wavelength when absorption curves are obtained by plotting absorbance versus wavelength.

Therefore, the quantification of total sugars present in a particular sample will be affected by the

type of sugar used in preparing the standard curve [7]. It is important to use the sugar that is in highest abundance in the specific substrate when applying AOAC Method 988.12 (44.1.30).

Levoglucosan, an anhydrosugar, is one of the predominant sugars produced from cellulose during fast pyrolysis and is reported in literature at 3-6 wt% [64]. Yu and Zhang [65] report that anhydrosugars formed during pyrolysis can be converted to glucose by dilute acid hydrolysis. Their work shows the addition of sulfuric acid to pyrolysis oil generated more glucose than could be accounted for by the amount of levoglucosan present. This extra glucose was attributed to unknown carbohydrate oligomers (i.e. cellobiosan and other sugars). Li and Zhang [66] also report levoglucosan can be readily hydrolyzed to glucose by acid. Oasmaa and Kuoppala [40] report that the carbohydrate fraction of bio-oil contains monosaccharides, anhydrosugars (especially levoglucosan, cellobiosan), and anhydro-polysaccharides. They indicate that this fraction cannot be thoroughly identified using present analytical tools; one problem being the lack of standards. Other sugars reported to be found in bio-oil at low concentrations include xylose, arabinose [30] fucose, galactose, mannose, fructose, and ribose [31].

Among the most commercially important components of bio-oil is sugar [12]. Sugar within the bio-oil matrix is difficult to analyze because of the large number of variations that can occur and potential interferences with other bio-oil components. A standardized test method that can be used to quantify total water-soluble sugars in bio-oil would enable meaningful comparisons and provide more consistent results. The approach to this research is to adapt a chemical analysis method developed by the food industry to quantify total water-soluble sugars in bio-oil using a standardized test method.

Methods

Bio-oil was produced in a fast pyrolysis process development unit (PDU) consisting of a fluidized bed operated at 450-500°C and a bio-oil recovery system that recovers bio-oil in multiple stage fractions (SFs) having distinctive properties from one another, as described by Pollard et al. [1]. SF 1 was designed to capture levoglucosan and other high molecular weight compounds. SF 2 consists of an electrostatic precipitator (ESP) and was designed to collect aerosols while stage 3 was designed to capture monomeric compounds with condensation points close to that of phenol. Stage 4 was an insulated ESP and stage 5 was designed to remove water and light oxygenated compounds such as acetic acid [1]. Red oak (*Quercus rubra*) (Wood Residual Solutions of Montello, WI) was used as feedstock for production of the bio-oil. The bio-oil collected from the stages were combined immediately after recovery and referred to as whole bio-oil. Total sugars in SF 1-5 were also determined. All results acquired using AOAC Method 988.12 (44.1.30) were compared to results obtained by a bio-oil liquid-liquid extraction method to quantify water-soluble sugars described in literature [80,81] and a hydrolysis method used to quantify total bio-oil sugars. The bio-oil was stored at 5°C in polypropylene containers.

Carbohydrate analysis by AOAC Method 988.12 (44.1.30) from literature was followed [82]. Since each sugar has a unique absorption maximum at a specific wavelength, absorption curves were obtained by plotting absorbance versus wavelength for each of the positive control sugars. The absorption maximum for levoglucosan, the most prominent sugar in bio-oil, was 490 nm. All samples were treated using the same AOAC Method 988.12 (44.1.30) conditions. Firstly, the sample (1090µL) was placed in a glass test tube. Secondly, 3260µl concentrated sulfuric acid was added rapidly. Thirdly, a 5% phenol solution (650µL) was immediately added. This mixture was heated for 5 min in a 90°C static water bath. After cooling to room temperature for 5 min in

another water bath, the sample was transferred to a polystyrene cuvette. The sample absorbance was measured at 490 nm, with a 1 cm path length and 1.5 nm split width, using a Varian Cary 50 UV-Visible Spectrophotometer (Agilent Technologies, Inc. Santa Clara, CA) with Cary WinUV Simple Reads module software (Agilent Technologies, Inc. Santa Clara, CA). Concentrations were diluted to keep absorbance between 0.2 and 1.0. Determination of the maximum wavelength absorption for each sugar used as a positive control was accomplished by scanning with the Cary 50 scan application. Wavelengths were acquired from 300 to 800 nm. Averages of 5-6 trials with a minimum of 5 separate replications, for the positive and negative controls, were obtained using typical values for bio-oil as reported in the literature and our own laboratory. The color produced by AOAC Method 988.12 (44.1.30) is stable for several hours and readings may be made later if necessary [5].

Positive and negative controls were used for AOAC Method 988.12 (44.1.30) validation of total water-soluble sugars in bio-oil. Positive controls included sugars reported in bio-oil. These positive controls were added to the water-soluble fraction of bio-oil to determine whether AOAC Method 988.12 (44.1.30) could accurately measure their concentration in the presence of other bio-oil water-soluble components. Positive controls used with this method included levoglucosan, D-glucose, D-mannose, D-xylose, D-fructose, D-galactose, L-arabinose, L-fucose, and cellobiosan. This allowed appraisal of AOAC Method 988.12 (44.1.30) for its ability to detect the correct amounts of spiked sugars. Negative controls were other chemicals known to exist in the bio-oil water-soluble fraction that might cause errors in quantification of total water-soluble sugars. The negative controls included furfural, 5HMF, furfuryl alcohol, 2-methyl furan, 2(5H)-furanone, formic acid, glycolic acid, propionic acid, acetic acid, acetol, and phenol. Both positive and negative controls were purchased from Thermo Scientific® (Hanover Park, IL) with

the exception of levoglucosan and cellobiosan which were purchased through Carbosynth (Compton, Berkshire, UK). The range of concentrations tested for contributing compounds (positive controls) and potentially interfering compounds (negative controls) were based on typical values for bio-oil as reported in the literature and our own laboratory data. The positive and negative controls were added into a prepared water-soluble sample of bio-oil and subjected to AOAC Method 988.12 (44.1.30).

Total sugar concentrations (wt%) indicated by AOAC Method 988.12 (44.1.30) analyses were linearly correlated to concentrations (wt%) of positive and negative controls. The slope of the line represents responsivity of the positive or negative control to AOAC Method 988.12 (44.1.30). The extrapolated baseline concentration of sugar compounds in bio-oil (wt%) is indicated by the Y-intercept. The potential contribution to results (%) was determined by solving the linear equation Y-variable (concentration of sugar wt%) using typical values for bio-oil as reported in the literature and our own laboratory data as the X-variable.

The negative controls; furfural, 2(5H)-furanone, 5HMF, and furfuryl alcohol showed interference with sugar analyses using AOAC Method 988.12 (44.1.30). These components were each added separately in concentrations based on typical values for bio-oil as reported in the literature and our own laboratory data to deionized water and analyzed by AOAC Method 988.12 (44.1.30). The range of weight percent commonly found in bio-oil (X-variable) allowed for the calculation of the Y-variable. The average calculated weight percent influence (correction factor) from these furans was subtracted from total sugars quantified by AOAC Method 988.12 (44.1.30). The correction factor was determined to be 0.76 wt%.

The water-soluble fraction of bio-oil was obtained following protocol from Oasmaa and Kuoppala [40]. After thorough mixing, three grams of bio-oil were weighed and placed into a 45

mL centrifuge tube, spreading it thinly along the bottom and lower sides of the tube to facilitate the removal of the water-soluble fraction. Thirty mL of water was added to the centrifuge tube giving a ratio of 10:1 water to bio-oil. According to Oasmaa and Kuoppala [40], the water to bio-oil ratio must not exceed 11:1 or fall below 9:1. The centrifuge tube was placed in an ultrasonic bath (320W) for 30 min ensuring the temperature did not exceed 40°C. The tube was centrifuged (accuSpin™ 1R, Thermo Scientific®, Hanover Park, IL) at 1307g force for 30 min to fully separate the water-soluble fraction from the water-insoluble fraction. The water-soluble fraction was decanted into a clean centrifuge tube and stored at 5°C for no longer than 1 week prior to testing by AOAC Method 988.12 (44.1.30) and the liquid-liquid extraction method. All samples were done in triplicate and averaged.

A liquid-liquid extraction method [80,81] was used to remove sugars from the water-soluble fraction of bio-oil. This liquid-liquid extraction was used as a control for comparison of results obtained by AOAC Method 988.12 (44.1.30). In this liquid-liquid extraction scheme, the water-soluble fraction of bio-oil is extracted with DEE and DCM to remove the sugars. The DEE is used at a 1:1 ratio by sample weight. Dichloromethane is used at a 1:1 ratio by weight of the DEE-insoluble. The DEE/DCM-insolubles are then evaporated at $\leq 40^\circ\text{C}$ in a rotary evaporator (Büchi Rotavapor RII, Thermo Scientific®, Hanover Park, IL) using 25 inches of mercury reduced pressure. The dried residues are weighed [80,81].

Hydrolysis of bio-oil was also used as a control for comparison of total water-soluble sugar results obtained by AOAC Method 988.12 (44.1.30) and the liquid-liquid extraction. In the hydrolysis method the sugars in bio-oil were acid hydrolyzed with 400 mM H_2SO_4 at 125°C for 44 min to glucose following Bennett et al. [35]. Aliquots of 6 mL of 400 mM H_2SO_4 and 60 mg of bio-oil were added to sealed glass vials. Pure compounds of levoglucosan and cellobiosan

were hydrolyzed under the same conditions as the samples to establish complete hydrolysis conditions and were used as reference standards. All samples and standards solutions were prepared using ultrapure 18.2 mega-ohm deionized water from a Barnstead E-Pure system (part of Thermo Fisher Scientific, Waltham, MA). The sulfuric acid used was certified 10N with an assay of (9.95-10.05) from Thermo Fisher Scientific. The HPLC system used to quantify the hydrolyzed sugars was a Dionex Ultimate 3000 LC system (Sunnyvale, CA) with a quaternary analytical pump and a Shodex Refractive Index (RI) Detector (New York, NY). The analytical column used was 300 mm X 7.7 mm 8 μ m particle size HyperRez XP Carbohydrate (p/n 69008-307780). The guard column used for the HyperRez was a Carbohydrate H+ cartridge (p/n 69008-903027) with the guard holder (p/n 69208-90327). The instrument parameters for the HyperRez were as follows: The mobile phase was ultrapure 18.2 mega-ohm deionized water with a flow rate of 0.2 mL min⁻¹ and a column temperature was set at 55°C [83].

The positive and negative controls were evaluated for relative absorbance using levoglucosan =100. Equal weights of the controls were subjected to AOAC Method 988.12 (44.1.30) and their absorbance compared to that of levoglucosan. These analyses allowed for a comparison of reactivity of the controls to AOAC Method 988.12 (44.1.30).

Moisture content of the bio-oil was determined by a MKS 500 Karl Fischer Moisture Titrator (Kyoto Electronics Manufacturing Co., LTD, Kyoto, Japan) using ASTM E203 Standard Test Method for Water Using Karl Fischer Reagent. The reagent used was Hydranal Composite 5K and the solvent was Hydranal Working Medium K purchased from Thermo Scientific® (Hanover Park, IL). The percent moisture of the bio-oil samples was determined in a minimum of four trials.

Acid content was determined by IC. The IC system used was a Dionex ICS3000 (Thermo Scientific®, Sunnyvale, CA) equipped with a conductivity detector and an Anion Micromembrane Suppressor AMMS-ICE300. The suppressor regenerant used was 5mM tetrabutylammonia hydroxide (TBAOH) at a flow rate of 4-5 mL min⁻¹. The eluent used was 1.0 mM heptafluorobutyric acid with an IonPac® ICE-AS1 4X50 mm guard column and IonPac® ICE-AS1 4X250 mm analytical column with a flow rate of 0.120 mL min⁻¹ at 19°C. The software used to control the instrument and evaluate the samples was Dionex Chromeleon (Thermo Scientific®, Sunnyvale, CA) version 6.8. The bio-oil samples were prepared using 6 mL deionized water and 1.5 mL of methanol. If concentrations of organic acids fell outside of the calibration curve (extrapolation) a second sample was diluted with more water while keeping the methanol concentration at 1.5 mL. All samples were filtered with a Whatman 0.45µL Glass Microfiber (Thermo Scientific® Hanover Park, IL) syringe filter prior to IC analysis. Samples were analyzed in duplicate.

Other water-soluble bio-oil constituents were evaluated and quantified using GC with a flame ionization detector (GC/FID). All chemical analyses were performed on a 430 GC/FID (Bruker Corporation, Bruker Daltonics, Inc., Fremont, CA) fitted with a Zebron ZB-WAXplus capillary column 30 meters in length, 0.25 mm inner diameter with a 0.25 mm film thickness (Phenomenex, Inc. Torrance, CA). The operating system used was Galaxie Chromatography Data System version 1.9.302.530 (Bruker Corporation, Bruker Daltonics, Inc., Fremont, CA). The carrier gas was helium (99.9995%) with a constant flow rate of 1.0 mL min⁻¹. The helium make-up was 25 mL min⁻¹, hydrogen flow at 30 mL min⁻¹ with an air flow of 300 mL min⁻¹. The oven was programmed to be held for 6 min at 35°C ramped at 5°C per min to 60°C and held for 2 min and ramped again at 10°C per min to 210°C and held for 1 min for a total of 29 min. A

sample volume of 1 μ L was injected utilizing a Varian CP 8400 (Bruker Corporation, Bruker Daltonics, Inc., Fremont, CA) auto sampler with a split ratio of 1:25. Peak identification was based on calibration standards purchased from Fisher Scientific (Thermo Scientific® Hanover Park, IL). For each of the calibration standards, calibration lines were made by injecting a minimum of five standard solutions on the GC/FID run in triplicate. The concentration range was determined by injection of the standard solutions until a range was determined that comprised the quantified value [84]. Each of the triplicate samples obtained in the water wash was run and analyzed on the GC/FID in triplicate or greater.

To determine if any chemicals removed using the bio-oil liquid-liquid extraction remained with the DEE/DCM-insoluble after evaporation, water was added to the DEE/DCM-insoluble residue in a 1:1 ratio by weight. These prepared samples were analyzed for remaining acids by IC and other chemicals by GC/FID. All samples were analyzed in duplicate (IC) or triplicate (GC/FID).

To determine the reliability of AOAC Method 988.12 (44.1.30) for quantifying total water-soluble sugars in bio-oil, the mean, standard deviation, and 95% confidence intervals were calculated. Quantification of total water-soluble sugars for each bio-oil utilizing AOAC Method 988.12 (44.1.30) was included in this calculation.

To determine the reliability of AOAC Method 988.12 (44.1.30) for quantifying total water-soluble sugars in bio-oil, the uncertainty interval of measurement was determined by the average of the repeated measurements (Equation 1) and sample standard deviation (Equation 2). The coverage factor in the uncertainty interval representing the t-distribution is $t(n-1)$ where n is the number of repeated trials. The confidence limits μ (Equation 3) is equal to the mean \pm Student t values acquired from a statistical table [79] multiplied by the standard deviation s and divided by

the square root of the number of sample trials n [79,85]. This was done to estimate the probability that the population mean lied within the region centered at the experimental mean (\bar{x}) of our measurements [79].

Equation 1.

$$\text{Mean} = \bar{x} = \sum_{i=1}^{i=n} xi$$

Equation 2.

$$\text{Standard Deviation} = s = \sqrt{\frac{\sum_{i=1}^{i=n} (x_i - \bar{x})^2}{(n - 1)}}$$

Equation 3.

$$\text{Confidence Limits} = \mu = \bar{x} \pm \frac{ts}{\sqrt{n}}$$

Results and Discussion

The AOAC Method 988.12 (44.1.30) allows for a variable absorbance response and resulting absorbance maximum for individual sugars because of the different furan products that arise from the addition of a strong acid. Consequently, applicability of AOAC Method 988.12 (44.1.30) was examined for the color formation of the resulting chromophores formed by the phenol-sulfuric acid reaction. Relative absorbance numbers were compared between the sugars and were based on the absorbance obtained with levoglucosan as 100. As shown in Table 1, the relative absorbance was largest for xylose, fructose, and cellobiosan. Sugars having absorbance maximum at 490 nm were expected to show higher relative absorption because levoglucosan, the standard, has an absorbance maximum at 490 nm. It is interesting that the absorption observed for D-xylose (480 nm) was higher than other pentoses resulting in a higher relative absorbance.

According to Dubois et al. [5], the spectral curves for D-xylose and furfural are very similar and under the assumption that the amount of color is proportional to the amount of furfural present or produced, the conversion of D-xylose to furfural under the conditions of AOAC Method 988.12 (44.1.30) is 93% of theory. Our relative absorbance results for D-xylose were lower (81). Other sugars showing lower relative absorption have absorption maximum at 470 to 485 nm except for mannose which showed an absorption maximum at 487 nm.

Table 1. Absorbance response (reactivity) of sugars when utilizing AOAC Method 988.12 (44.1.30) Phenol-Sulfuric Acid Assay for Total Carbohydrate Determination relative to levoglucosan=100.

Contributor (Positive Control)	Maximum Wavelength Absorption (nm)	Relative Absorbance to Levoglucosan=100
Levoglucosan	490	100
D-Fructose	490	84
D-Xylose	480	81
Cellobiosan	490	80
D-Glucose	485	65
D-Galactose	485	63
L-Arabinose	480	62
D-Mannose	487	49
L-Fucose	475	31

Literature reports that some deoxy-sugars are not detected by AOAC Method 988.12 (44.1.30) [61]. We did detect fucose, a deoxy-sugar found in bio-oil at low concentration. Fucose, reported in bio-oil at 0.058 wt% [31], gave the lowest relative absorbance among the contributors. According to Feather and Harris [86], there are ultraviolet-absorbing products formed from specific deoxy sugars when subjected to concentrated sulfuric acid. The formation of furfural from the acid treatment of these deoxy sugars is in the order of 30%. Our results agree with these findings with a relative absorbance of fucose to levoglucosan of 31.

The regression line for the positive controls showed a strong linear relationship between X and Y-variables (Table 2) with r^2 values of ≥ 0.92 except for fucose. The Y-intercept was the

baseline concentration of water-soluble sugars while the X-variable was the wt% positive control added to the water-soluble fraction of bio-oil. The total sugar acquired by hydrolysis was 13.0 ± 0.1 wt% wet basis (wb). This indicated an 18% difference between the hydrolysis value and the average Y-intercept baseline value.

The contributor response factors (slope) show levoglucosan, fructose, xylose, and cellobiosan were the highest for the sugars tested. This was in agreement with relative absorbance values. Levoglucosan and fructose reacted similarly to the AOAC Method 988.12 (44.1.30) reagents while xylose and cellobiosan showed approximately 50% less response versus levoglucosan. Both the response factor and the relative absorbance values follow a similar trend.

The applicability of AOAC Method 988.12 (44.1.30) was also examined for the color formation reaction with the interferents. As shown in Figure 3, the relative absorbance was high for furfural, 2(5H)-furanone, 5-HMF, and furfuryl alcohol based on the absorbance obtained with levoglucosan as 100. The high relative absorbance for these furans indicated interference from them when utilizing AOAC Method 988.12 (44.1.30) for total water-soluble sugar quantification. On the other hand, formic acid, glycolic acid, propionic acid, acetic acid, acetol, 2-methylfuran and phenol showed very small relative absorbance, therefore not influencing the total sugar results with AOAC Method 988.12 (44.1.30).

The response factor and potential contribution of interferents to quantification of total sugars using AOAC Method 988.12 (44.1.30) were examined. The results, shown in Table 3, indicate formic acid, glycolic acid, propionic acid, acetic acid, acetol, 2-methylfuran and phenol do not interfere with the application of AOAC Method 988.12 (44.1.30) to the bio-oil water-soluble fraction. The r^2 values were also very low. These results correspond with the relative absorbance obtained for these compounds, as well. As stated, the presence of furfural, 2(5H)-furanone, 5-

HMF, and furfuryl alcohol cause an over reporting of total water-soluble sugars when using AOAC Method 988.12 (44.1.30); contributing to errors in the range of 1-30%. To account for this influence, a correction factor was determined by adding known amounts of each of the listed furans to deionized water and subjecting these samples to AOAC Method 988.12 (44.1.30). The resulting graphs were linear (not shown). The range of weight percent commonly found in bio-oil (X-variable) allowed for the calculation of the Y-variable. The average calculated weight percent influence for the furans listed were subtracted from the total sugars determined by AOAC Method 988.12 (44.1.30). For the samples used in this research, the correction factor was determined to be 0.76 wt%.

Table 2. The effect of contributors (positive controls) on quantification of total water-soluble sugars in whole bio-oil when analyzed by AOAC Method 988.12 (44.1.30) Phenol-Sulfuric Acid Assay for Total Carbohydrate Determination.

Contributor (Positive Control)	Typical Concentration in bio-oil (wt%) X-variable	Response Factor (slope)	Indicated Concentration (wt% wb) Y-variable	R ² Value	Potential Contribution to Results (%)
Levoglucosan ¹	4.0-6.0	16.1	10.8	1.00	85.0-90.0
D-Fructose ³	0.115	14.5	10.8	0.99	13.4
D-Xylose ¹	0.1-1.4	8.54	10.4	0.92	7.59-46.5
Cellobiosan ¹	1.4-1.5	8.10	10.4	0.97	52.2-53.9
L-Arabinose ²	0.1	7.96	10.7	0.95	6.92
D-Glucose ²	0.4-1.3	7.15	10.1	0.97	22.1-47.9
D-Galactose ³	0.20	6.91	10.8	0.93	11.3
D-Mannose ¹	0.02-0.03	3.26	10.5	1.00	0.617-0.923
L-Fucose ³	0.115	0.97	10.8	0.58	1.02

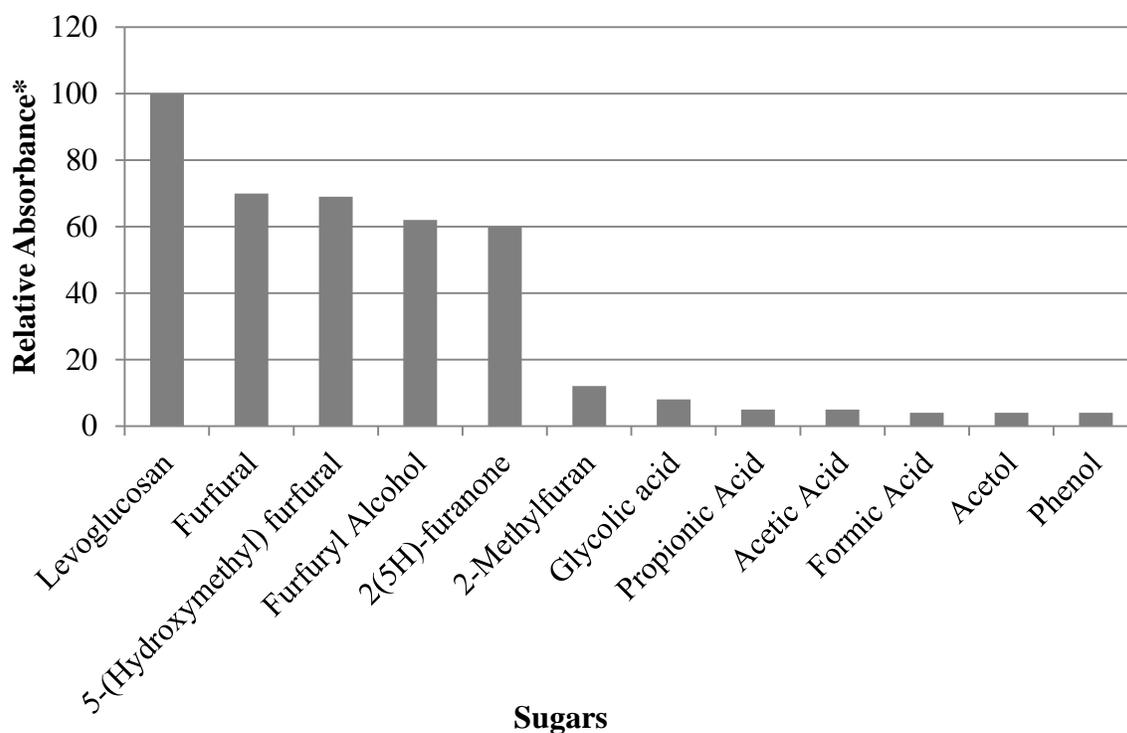
¹as measured in our laboratory (wt% wb)

²as measured in literature [87]

³as measured in literature [31]

A carbohydrate-rich solution is obtained by washing bio-oil with water. Unfortunately, some water-insoluble compounds are also washed out into solution. In addition to the water-soluble

sugars, there are various other compounds in the water-soluble fraction (11.3 wt% wb quantified) (Table 4). These GC/FID and IC calibrated compounds are both water-soluble and slightly



*Relative absorbance number is based on absorbance obtained with levoglucosan as 100.

Figure 3. Effect of bio-oil water-soluble compounds on the color response (reactivity) of interferents when utilizing AOAC Method 988.12 (44.1.30) Phenol-Sulfuric Acid Assay for Total Carbohydrate Determination for quantification of total sugars relative to levoglucosan =100.

water-soluble. For example, phenol is appreciably soluble in water (8.3 g per 100 mL) while 2,6-dimethoxyphenol is somewhat soluble at 2 g per 100 mL water. This phenomenon may well lead to overestimation when utilizing the liquid-liquid extraction for total water-soluble sugars in bio-oil.

With the intention of quantifying compounds remaining behind with the DEE/DCM-insoluble residues after liquid-liquid extraction of the water-soluble fraction of bio-oil, water was added to the remaining residue in a 1:1 by weight ratio and analyzed by GC/FID and IC. Nearly 3.7 wt% of calibrated constituents remained behind in the DEE/DCM extraction residue

(Table 5). This is approximately 33 wt% of the calibrated compounds remaining with the sugars.

Undesirable ramifications arising from this include gross over-estimation of the amount of DEE/DCM-insoluble which is often considered and reported as “sugars” in bio-oil.

Table 3. The effect of interferents (negative controls) on quantification of total water-soluble sugars in whole bio-oil when analyzed by AOAC Method 988.12 (44.1.30) Phenol-Sulfuric Acid Assay for Total Carbohydrate Determination.

Interferent (Negative Control)	Typical Concentration in Bio-Oil (wt%) X-variable	Response Factor (slope)	Indicated Concentration (wt% wb) Y-variable	R ² Value	Potential Contribution to Results (%)
Furfural ¹	0.1-0.4	12.9	12.2	0.97	9.56-29.7
5-(hydroxymethyl) furfural ¹	0.1-0.4	12.2	11.5	0.99	9.52-29.8
2(5H)-furanone ¹	0.1-1.1	3.72	12.4	0.91	2.91-24.8
Furfuryl Alcohol ¹	0.1-1.0	1.60	11.8	0.96	1.34-11.9
Glycolic Acid ¹	0.4-0.5	0.73	11.7	0.26	2.43-3.02
Formic Acid ¹	0.6-1.0	0.25	11.6	0.18	1.28-2.11
Propionic Acid ¹	0.1-0.6	0.19	10.3	0.15	0.184-1.09
Acetic Acid ¹	0.5-5.5	0.18	11.9	0.17	0.751-7.68
Phenol ¹	0.03-0.2	0.01	11.0	0.0002	0.00273-0.0182
2-Methylfuran ¹	0.0-0.1	0.03	11.0	0.0003	≤0.0273

¹as measured in our laboratory (wt% wb)

²as measured in literature [87]

Literature values state bio-oil contains from 30-35 wt% DEE/DCM-insoluble (anhydrosugars, anhydro-oligomers, monosaccharides and hydroxy acids ≤C10) [40,80,81]. Table 6 summarizes the results from the liquid-liquid extraction of the bio-oil water-soluble fraction. Our results were lower than stated literature values, showing 23.1±1.1 wt% wb DEE/DCM-insoluble. When the total calibrated constituents and percent moisture were subtracted; 17 wt% wb DEE/DCM-insoluble remained. In comparison, AOAC Method 988.12 (44.1.30) gave 11.9±0.9 wt% wb sugars after subtraction of the furan correction factor while hydrolysis revealed 13.0±0.10 wt% wb total sugars. As expected, the DEE/DCM insoluble results seriously overestimated sugars. There was 78% difference between the hydrolysis and

Table 4. A comparison of total water-soluble sugars in whole bio-oil by three different methods; DEE/DCM-insoluble, AOAC Method 988.12 (44.1.30) Phenol-Sulfuric Acid Assay for Total Carbohydrate Determination, and hydrolysis with GC/FID and IC analyses of quantified compounds (wt% wb) in the water-soluble fraction of bio-oil.

Sugar Determination Method	Sugars in Whole Bio-Oil (wt% wb)
DEE/DCM-insoluble (anhydrosugars, anhydro-oligomers, monosaccharides and hydroxy acids $\leq C_{10}$)	23.1 \pm 1.1
AOAC Method 988.12 (44.1.30) (total water-soluble sugars)	11.9 \pm 0.9
Hydrolysis (total bio-oil sugars)	13.0 \pm 0.1
Chemicals in Water-Soluble Bio-Oil	Whole Bio-Oil (wt% wb)
Methanol	0.54 \pm 0.05
Acetic Acid	4.66 \pm 0.01
Formic Acid	0.61 \pm 0.002
Furfural	0.37 \pm 0.01
Propionic Acid	0.08 \pm 0.001
Glycolic Acid	0.50 \pm 0.01
1,2-Ethanediol	1.54 \pm 0.18
Furfuryl Alcohol	0.06 \pm 0.02
Phenol	0.42 \pm 0.05
Acetol	1.63 \pm 0.09
2,6-Dimethoxyphenol	0.09 \pm 0.0001
2(5H)-Furanone	0.24 \pm 0.02
3-Methyl-1,2-cyclopentanedione	0.22 \pm 0.01
Guaiacol	0.12 \pm 0.001
Vanillin	0.12 \pm 0.06
5-(Hydroxymethyl) furfural	0.12 \pm 0.001
Total Chemicals in Water-Soluble Bio-Oil	11.3\pm0.03

the DEE/DCM-insoluble, signifying this was not a reliable approach to quantification of water-soluble sugars in bio-oil. On the other hand, only an 8.5% difference was observed between the hydrolysis results and those acquired with AOAC Method 988.12 (44.1.30) : demonstrating the reliability of this method as a quick easy method for use during bio-oil production.

Table 5. GC/FID and IC quantified bio-oil compounds remaining with the water-soluble sugars in the evaporated DEE/DCM-insoluble residue.

Chemicals Remaining with the Evaporated DEE/DCM-Insoluble	Remaining Chemical in the Evaporated DEE/DCM-Insoluble (wt% wb)
Methanol	0.01±0.001
Acetic Acid	0.39±0.01
Formic Acid	0.25±0.003
Furfural	0.07±0.002
Glycolic Acid	0.46±0.01
1,2-Ethanediol	1.2±0.02
Furfuryl Alcohol	0.06±0.01
Phenol	0.26±0.01
Acetol	0.25±0.01
2,6-Dimethoxyphenol	0.09±0.01
2(5H)-Furanone	0.16±0.004
3-Methyl-1,2-cyclopentanedione	0.22±0.01
Guaiacol	0.01±0.001
Vanillin	0.12±0.01
5-(hydroxymethyl) furfural	0.12±0.003
Total	3.7±0.01

Table 6. The effect on results from the remaining moisture and GC/FID and IC quantified compounds in the evaporated diethyl ether and dichloromethane residue obtained using whole bio-oil.

Components	Total (wt% wb)
DEE/DCM-Insoluble	23.1±1.1
Calibrated compounds remaining after evaporation	3.69±0.010
Moisture (%)	2.38±0.020
Total DEE/DCM-insoluble	17.03±0.38

The fluidized bed fast pyrolysis reactor with the staged condenser system used for production of the research bio-oil was designed to collect levoglucosan in stage SFs 1 and 2, monomeric phenols and furans in SFs 3 and 4, and an aqueous phase containing acids and other “light oxygenates” (SF 5) [1]. The SF bio-oil yield distribution was 17.6% in SF 1, 31.0% in SF 2, 6.06% in SF 3, 1.1% in SF 4, and 43.6% bio-oil was captured in SF 5.

SF analyses demonstrated a noteworthy trend when comparing the amount of the water-soluble constituents found in each SF. Due to the fractionated condenser system [1] levoglucosan was condensed in SFs 1 and 2 (Table 7). Levoglucosan is hydrolyzed into glucose. Due to the collection system design, levoglucosan was present in the first two SFs only, with no glucose present in SFs 3-5 after hydrolysis. Furthermore, the majority of furans were not condensed in SFs 1 and 2 but were condensed in SFs 3-5.

The SF analyses were performed on constituents removed with the water wash. Calculated on a bio-oil yield distribution, the GC/FID and IC analyses revealed 13.8 wt% other constituents were in the water-soluble fraction of bio-oil (Table 7). The DEE/ DCM insoluble residue, after additional water was added in a 1:1 ratio by weight, was analyzed by GC/FID and IC. It showed 7.11 wt% wb still remained behind in the DEE/DCM residue “sugar” sample (Table 8). This was 52 wt% wb of other quantified water- soluble or partially soluble constituents remaining with the sugars resulting in an overestimation of sugars by the liquid-liquid extraction.

Table 9 shows the bio-oil weighted average yield and sugars calculated on a biomass feedstock basis for the three methods used in this research for quantification of sugars in bio-oil. The hydrolysis sugars yielded 9.63 wt% wb, while AOAC Method 988.12 (44.1.30) with furfural correction was 9.57 wt% wb and the DEE/DCM-insoluble was 22.5 wt% wb on a bio-oil yield distribution. Calculated on a biomass feedstock basis, 6.22 wt% wb, 6.17 wt% wb and 14.5 wt% wb, respectively was sugar.

To determine the reliability of AOAC Method 988.12 (44.1.30) for quantifying total water-soluble sugars in bio-oil, the mean, standard deviation, and 95% confidence intervals were calculated for all bio-oil analyses using AOAC Method 988.12 (44.1.30). The uncertainty of

Table 7. A comparison of total water-soluble sugars by three different methods; DEE/DCM-insoluble, AOAC Method 988.12 (44.1.30) Phenol-Sulfuric Acid Assay for Total Carbohydrate Determination, and hydrolysis in fractionated bio-oil with other constituents in the water-soluble fraction of bio-oil quantified by GC/FID and IC.

Sugar Determination Method	SF 1	SF 2	SF 3	SF 4	SF 5
DEE/DCM-insoluble (anhydrosugars, anhydro-oligomers, monosaccharides and hydroxy acids \leq C10) (wt% wb)	15.7 \pm 1.3	24.2 \pm 1.3	37.1 \pm 1.8	41.1 \pm 0.83	21.3 \pm 1.6
AOAC Method 988.12 (44.1.30) (total water-soluble sugars) (wt% wb)	15.1 \pm 0.96	15.6 \pm 0.14	9.88 \pm 0.38	9.44 \pm 0.75	3.06 \pm 0.37
Hydrolysis (total bio-oil sugars) (wt% wb)	16.3 \pm 0.19	22.0 \pm 0.23	0	0	0
Chemicals in Water-Soluble Bio-Oil					
Methanol (wt% wb)	0.03 \pm 0.01	0.02 \pm 0.001	0.26 \pm 0.09	0.33 \pm 0.01	1.71 \pm 0.08
Acetic Acid (wt% wb)	1.33 \pm 0.001	0.46 \pm 0.01	5.13 \pm 0.01	6.18 \pm 0.08	9.74 \pm 0.01
Formic Acid (wt% wb)	0.93 \pm 0.01	0.26 \pm 0.001	1.74 \pm 0.02	1.63 \pm 0.02	0.78 \pm 0.001
Furfural (wt% wb)	0.34 \pm 0.07	0.10 \pm 0.02	1.17 \pm 0.4	1.09 \pm 0.06	1.05 \pm 0.02
Propionic Acid (wt% wb)	0.26 \pm 0.001	0.22 \pm 0.02	1.03 \pm 0.04	1.13 \pm 0.02	1.40 \pm 0.04
Glycolic Acid (wt% wb)	1.12 \pm 0.002	0.40 \pm 0.004	0.91 \pm 0.05	0.95 \pm 0.04	0.70 \pm 0.3
1,2-Ethanediol (wt% wb)	0.58 \pm 0.05	0.11 \pm 0.02	2.92 \pm 0.6	1.77 \pm 0.7	0.37 \pm 0.04
Furfuryl Alcohol (wt% wb)	0.16 \pm 0.03	0.04 \pm 0.01	0.44 \pm 0.09	0.33 \pm 0.2	0.09 \pm 0.03
Phenol (wt% wb)	0.74 \pm 0.04	1.01 \pm 0.2	0.98 \pm 0.2	0.84 \pm 0.1	0.41 \pm 0.06
Acetol (wt% wb)	0.69 \pm 0.07	0.26 \pm 0.06	2.03 \pm 0.09	2.65 \pm 0.1	2.85 \pm 0.2
2,6-Dimethoxyphenol (wt% wb)	0.05 \pm 0.002	0.30 \pm 0.03	0.33 \pm 0.2	0.22 \pm 0.04	0.23 \pm 0.09
2(5H)-furanone (wt% wb)	1.03 \pm 0.2	0.26 \pm 0.03	2.58 \pm 0.6	1.87 \pm 0.1	0.56 \pm 0.06
3-Methyl-1,2-cyclopentanedione (wt% wb)	0.60 \pm 0.1	0.11 \pm 0.03	1.49 \pm 0.4	1.10 \pm 0.03	0.18 \pm 0.02
Guaiacol (wt% wb)	0.18 \pm 0.06	0.15 \pm 0.06	0.78 \pm 0.2	0.54 \pm 0.02	0.28 \pm 0.08
Vanillin (wt% wb)	0.19 \pm 0.06	0.50 \pm 0.08	0.14 \pm 0.02	0.09 \pm 0.01	0.00 \pm 0.00
5-(Hydroxymethyl) furfural (wt% wb)	0.67 \pm 0.1	0.16 \pm 0.02	1.69 \pm 0.4	1.23 \pm 0.1	0.37 \pm 0.04
Total Chemicals in Water-Soluble Bio-Oil (wt% wb)	8.90\pm0.1	4.36\pm0.03	23.62\pm0.2	21.95\pm0.1	20.72\pm0.1

measurement of water-soluble sugars in bio-oil at 95% confidence was \pm 1.7% using AOAC Method 988.12 (44.1.30) when the correction factor was employed.

Table 8. GC/FID quantified compounds remaining with the water-soluble sugars in the DEE/DCM-insoluble residue after liquid-liquid extraction of bio-oil water-soluble sugars.

Chemical	SF 1	SF 2	SF 3	SF 4	SF 5
Methanol (wt% wb)	0.00±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.02±0.01
Acetic Acid (wt% wb)	0.24±0.02	0.10±0.01	0.80±0.09	0.66±0.2	0.50±0.1
Formic Acid (wt% wb)	0.52±0.04	0.25±0.03	0.80±0.1	0.73±0.1	0.27±0.05
Furfural (wt% wb)	0.04±0.01	0.11±0.00	0.14±0.08	0.14±0.04	0.20±0.07
Propionic Acid (wt% wb)	0.23±0.05	0.10±0.07	0.70±0.05	0.64±0.08	0.73±0.02
Glycolic Acid (wt% wb)	0.93±0.05	0.56±0.03	0.90±0.2	1.00±0.06	0.39±0.02
1,2-Ethanediol (wt% wb)	1.29±0.1	0.45±0.07	4.15±0.7	3.07±0.2	2.28±0.4
Furfuryl Alcohol (wt% wb)	0.25±0.02	0.12±0.03	0.78±0.1	0.56±0.06	0.54±0.2
Phenol (wt% wb)	0.11±0.02	0.07±0.03	0.05±0.03	0.16±0.07	0.11±0.03
Acetol (wt% wb)	0.18±0.01	0.09±0.01	0.80±0.1	0.87±0.27	2.67 ±0.2
2(5H)-furanone (wt% wb)	0.25±0.03	0.09±0.001	0.64±0.08	0.56±0.06	0.38±0.02
2,6-Dimethoxyphenol (wt% wb)	0.00±0.00	0.02±0.001	1.41±0.4	0.61±0.07	2.05±0.7
3-Methyl-1,2-cyclopentanedione (wt% wb)	0.15±0.01	0.04±0.001	0.24±0.03	0.25±0.02	0.09±0.0001
Guaiacol (wt% wb)	0.01±0.00	0.02±0.001	0.03±0.00001	0.03±0.0001	0.03±0.0001
Vanillin (wt% wb)	0.11±0.03	0.09±0.02	0.07±0.01	0.07±0.01	0.23±0.1
5-(Hydroxymethyl) furfural (wt% wb)	0.40±0.03	0.16±0.01	0.41±0.05	0.36±0.04	0.22±0.04
Total (wt% wb)	4.71±0.04	2.28±0.03	11.9±0.2	9.72±0.1	10.7±0.2

Table 9. A comparison of total water-soluble sugars captured in the stage fractions reported on bio-oil yield distribution (wt% wb) and biomass basis (wt% wb) utilizing a liquid-liquid extraction method, the AOAC Method 988.12 (44.1.30) Phenol-Sulfuric Acid Assay for Total Carbohydrate Determination, and hydrolysis with quantification by HPLC.

Sugar Determination Method	Stage Fraction Weighted Average Yield (wt% wb)	Biomass Basis (wt% wb)
Liquid-Liquid Extraction	22.5±1.4	14.5
AOAC	9.57±0.52	6.17
Hydrolysis	9.63±0.21	6.22

Conclusions

We successfully demonstrated AOAC Method 988.12 (44.1.30) Phenol-Sulfuric Acid Assay for Total Carbohydrate Determination, originally designed to evaluate sugars in food products, can be used to the measure water-soluble sugars in bio-oil with ±1.7% uncertainty of

measurement. Both whole bio-oil and SFs 1-5 bio-oil were examined and all show similar results in comparison to a hydrolysis method utilizing HPLC for quantification. This research has shown that the typical liquid-liquid extraction bio-oil method greatly overestimates the total water-soluble sugars and should not be used for these analyses. Interferents include furans, therefore a correction factor of 0.76 wt% has been established for use with bio-oil. This research has demonstrated AOAC Method 988.12 (44.1.30) gave highly reproducible results while providing a reliable standardized test method for quantification of total water-soluble sugars in bio-oil.

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CHAPTER 5. QUANTIFICATION OF TOTAL PHENOLS IN BIO-OIL USING THE FOLIN-CIOCALTEU METHOD

A paper submitted to the *Journal of Analytical and Applied Pyrolysis*

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Abstract

Bio-oil from fast pyrolysis of biomass contains phenolic compounds derived from the lignin portion of the biomass. Traditional testing for total phenolic compounds in bio-oil is based on either a rough estimate of the weight percent water-insolubles in bio-oil or on tedious liquid-liquid extraction methods. We have evaluated the Folin-Ciocalteu (FC) colorimetry method used for quantifying total phenols in wine to determine total phenols in bio-oil. This method, based on the oxidation of phenolic compounds by the FC reagent, is fast and easy to perform. This study evaluated its accuracy relative to interferences by the use of positive and negative controls. Positive controls included phenol, 4-methylphenol, 3-ethylphenol, guaiacol, 2,6-dimethoxyphenol and eugenol. The negative controls included sugars, furfural, and acids. Potential interferences with the quantification of total phenols by the FC method was calculated for all positive and negative controls by using data obtained when adding the contributor (positive controls) and the interferent (negative controls) into bio-oil using typical concentrations found in bio-oil. The positive and several of the negative controls produced strongly correlated linear relationships between the indicated phenolic content of the bio-oil and the amount of contributor or interferent added. However, the slopes of these relationships for the negative controls were much smaller than those for the positive controls, indicating that the error in the prediction of phenolic content was small even for large concentrations of interferent compounds. For typical concentrations of non-phenolic compounds in bio-oil, the error in predicted phenolic

content as a result of their presence was $\leq 5.8\%$. Total phenolic content in bio-oil detected by the FC method was comparable to the quantity of total phenolic compounds obtained by liquid-liquid extraction. All results fell within the margin of error and the uncertainty of the measurement by the FC method indicating there was no significant difference in the results between the two methods. The FC method uncertainty of measurement was $\pm 1.1\%$ at the 95% confidence level.

Introduction

The goal of this research is to determine if a fast and easy standardized test method used in the food industry to quantify total phenols in wine will provide reliable results for quantifying total phenolic compounds in bio-oil. Traditionally, quantification of phenols is done either by liquid-liquid extraction processes and/or estimated as the amount of water-insoluble fraction (WIF) in the bio-oil, which consists mostly of phenolic oligomers.

Bio-oil arises from the depolymerization and fragmentation of cellulose, hemicelluloses, and lignin in plant materials [1,8,88]. Little deoxygenation occurs during fast pyrolysis, producing bio-oil with an elemental composition closely resembling the original biomass [1,26]. Bio-oil is considered a possible alternative to petroleum as a source of liquid fuels [26] and chemicals [89].

Bio-oil is a complex mixture of water (15-30%), ketones, acids, aldehydes, sugars, phenolic compounds and other oligomeric lignin derivatives. Approximately 35-50% of bio-oil is comprised of constituents that are nonvolatile [8,90]. Softwoods have the highest lignin content (25-35%), mainly the guaiacyl type, while hardwoods contain from 16-25% lignin comprised of the guaiacyl-syringyl type [16,90]. Bio-oil characteristics, which include extreme complexity, instability, heterogeneity, and low pH [91], necessitate refining or upgrading to enable utilization.

Lignin has attracted attention because of the wide variety of phenolic compounds that can be produced from it (i.e. methyl, ethyl, methoxy, dimethoxy, and other alkylated derivatives).

Phenol, derived from lignin during fast pyrolysis, is a commodity chemical manufactured from increasingly expensive crude petroleum oil [92]. The high content of oxygenated compounds in bio-oil makes it a potential source for these organic compounds [8], either from whole bio-oil or major fractions of bio-oil [90]. One important product from the lignin-derived fraction of bio-oil is phenolic replacement in phenol-formaldehyde resins [90], which is utilized as a raw material for laminate industries and specialty chemical manufacturing [93].

Quantifying phenols in bio-oil is important because phenols influence reactivity and stability. Upon thermal degradation of biomass, lignin breaks down into a complex bio-oil with the major fraction consisting of phenolic compounds [93], which comprise the WIF of bio-oil. The phenol concentration in bio-oil is typically very low, on the order of 0.1 wt%, while monomeric phenols analyzed by gas chromatography (GC) range from 1 to 4 wt% [30]. Many phenolic compounds are present in bio-oil as oligomers containing varying numbers of acidic, phenolic, and carboxylic acid hydroxyl groups as well as aldehyde, alcohol, and ether functions. These oligomers typically have molecular weight distributions of several hundred to 5000 g mol^{-1} depending on the pyrolysis process severity (i.e. temperatures, residence time, heating rates) [92], which is adequately high enough that they cannot be analyzed by GC.

The WIF of bio-oil is often referred to as “pyrolytic lignin” [39] although this is not a particularly accurate description of the phenolic oligomers making up the WIF. These oligomers consist of aromatic rings substituted with various methoxy groups and linked by various types of aliphatic linkers [94]. Water extraction precipitates the pyrolytic lignin and removes the water-soluble carbonyl compounds, sugars, etc. that are derived from cellulose and hemicellulose

during pyrolysis. The WIF can be recovered by centrifuging or filtering. Upon further washing and drying the WIF gives a light brown powder product. Yields of pyrolytic lignin are approximately 22 to 28% of the crude bio-oil [95]. Literature states the method for the determination of pyrolytic lignin requires improvement for better reliability [30,41]. This statement indicates that estimation of total phenolic compounds by weight of the WIF is not reliable.

The wine industry utilizes the Folin-Ciocalteu (FC) colorimetry method to determine total phenolic compounds in their products. A major advantage of the FC method is that it has an equivalent response to different phenolic substances in wine, making it suitable for measuring accurate mass levels of total phenolic compounds [67]. Slinkard and Singleton [96] stated that the FC method is the best method for determining the total content of phenols of all types in dry wines, plant extracts, brandies, and similar products. Yu and Dahlgren [97] could not recommend a single optimal protocol for the quantification of total phenols and condensed tannins (i.e. polyphenolic compounds) in conifer foliage. However, they stated that the FC method, which takes into account all hydroxyl aromatic compounds, is one of two methods that is superior for quantification of condensed tannins [97]. Derkyi [98] reported that different types of polyphenols react similarly with the FC reagent, making them more easily quantifiable. Chapuis-Lardy et al. [99] utilized the FC method to determine the water-soluble phenolic compounds in leaf litter of *Eucalyptus* and reported that the FC method provides a rapid test for a large number of samples and allows the characterization of phenolic compounds. High-performance liquid chromatography was used for semi-quantitative analyses of components in water extracts of the *Eucalyptus* leaf litter and the sum of the identified phenolic compounds was only about 10% of the water-soluble phenolic fraction estimated with the FC reagent [99].

The FC method is based on chemical reduction of the reagent (mixture of tungsten and molybdenum oxides). The products of the metal oxide reduction have a blue color that has broad light absorption with a maximum at 765 nm [67]. The chemistries of tungstates and molybdates are very complex. The isopolyphosphotungstates are colorless in the fully oxidized 6^+ valence state of the metal and the molybdenum compounds are yellow. They form mixed heteropolyphosphotungstates-molybdates and exist in an acid solution as hydrated octahedral complexes of the metal oxides coordinated around a central phosphate. Sequences of reversible one or two electron reductions lead to blue species such as $(\text{PMoW}_{11}\text{O}_{40})^{4-}$. In principle, the addition of an electron to a nonbonding orbital reduces nominal MoO^{4+} units to isostructural MoO^{3+} blue species [68]. The intensity of the light is proportional to the concentration of phenols.

A disadvantage of the FC method is that it is nonspecific and can be affected by other nonphenolic reducing molecules. This method depends on the selective oxidation of similar easily-oxidized substances that when present contribute to the apparent total phenol content. Other easily-oxidized substances besides phenols include aromatic amines, sulfur dioxide, ascorbic acid plus endiols. Sugars break down in alkali to give endiols, which are readily oxidized [96]. The FC reagent also oxidizes proteins. Due to the color formation of the FC reaction via the reduction of the reagent, this reaction is general enough to allow for these types of interferences, the most problematic of which may be sugar. Waterhouse [67] explains that sugars create a complex issue because different sugars yield different interferences when using the FC method for total phenolic compounds determinations in wine. Levoglucosan is the main sugar reported in literature at 3-6 wt% [64] while other sugars reported at low concentrations

include xylose, arabinose, fucose, galactose, mannose, fructose, and ribose [30,64]. The FC reagent is commercially available but can be prepared in the laboratory [67].

Liquid-liquid extraction is time consuming, tedious, and can involve the use of many different hazardous solvents. Basing total phenolic compounds on the WIF content of bio-oil is merely a rough estimation. A standardized test method that can be used to quantify total phenolic compounds would allow for meaningful comparisons and provide more consistent results. There is a need for a fast, easy, reliable standardized test method for quantifying total phenols in bio-oil.

Methods

Red oak (*Quercus rubra*) from Wood Residual Solutions, LLC of Montello, WI was used as feedstock for production of bio-oil. Bio-oil was produced in a fast pyrolysis process development unit (PDU) consisting of a fluidized-bed operated at 450 to 500°C and a bio-oil recovery system that collects bio-oil in multiple stage fractions (SF) having distinct properties from one another, as described by Pollard et al. [1]. Stage 1 was designed to capture levoglucosan and phenolic oligomers with high condensation points and was operated with gas inlet and outlet temperatures of 345°C and 102°C, respectively. Coolant water temperature was controlled to 85°C. Stage 2 consists of an electrostatic precipitator (ESP) operated at 40 kVDC and heat traced to 129°C to prevent condensation of vapors. Sugars and phenolic oligomers are the main constituents of stage 2, as well. Stage 3 was designed to capture compounds with condensation points close to that of phenol and other phenolic monomers. It was operated with gas inlet and outlet temperatures of 129°C and 77°C, respectively. The coolant water was controlled to 65°C. Stage 4, an insulated ESP, utilizes an operating temperature of about 77°C. Larger molecular weight oligomers that escape stage 2 are also collected in SF 4. Stage 5 was designed to remove water and light

oxygenated compounds such as acetic acid. Its coolant was water entering at 18°C. Residence times in the individual stages of the bio-oil collection system ranged from 1 s to 10 s [1]. The bio-oil collected in each stage was recombined immediately after recovery and referred to as whole bio-oil. The WIF was separated from the water-soluble components of SF 2 by mixing equal weights of bio-oil and water. The solution was manually stirred by hand to blend the bio-oil and water. The sample was placed on a shaker table (MaxQ 2506, Thermo Scientific®, Hanover Park, IL) for 30 min at 250 motions per min and centrifuged (accuSpin™ 1R, Thermo Scientific®, Hanover Park, IL) at 1307g force for 30 min. The water-soluble portion was decanted leaving behind the WIF.

A liquid-liquid extraction technique for the removal of phenols from bio-oil was utilized [100]. A bio-oil sample in the range of 4-20 g was dissolved in an equal quantity of ethyl acetate. The mixture was filtered with a Büchner funnel using Whatman #42 filter paper. The filtered oil-ethyl acetate layer was placed in a separatory funnel and sodium bicarbonate (5 wt %) solution at a 1:1 ratio of oil-ethyl acetate to 5% sodium bicarbonate solution. The resulting aqueous bicarbonate layer contained the strong (mineral) and weak (organic) acids and highly polar compounds. This layer was retained and concentrated hydrochloric acid added to make the strong acids and highly polar compounds water insoluble. These insolubles were filtered using Whatman #42 filter paper; the residue was dried and weighed. The ethyl acetate soluble fraction contained phenolic monomers and oligomers as well as the neutral fraction (aromatics, ethers, esters, aldehydes, and ketones) and very weak bases [101]. This layer was concentrated using a rotary evaporator (Büchi Rotavapor RII, Thermo Scientific®, Hanover Park, IL) at 60°C with reduced pressure of 25 inches of mercury. The residue remaining was mixed with sodium hydroxide solution (5 wt %) in a 1:1 ratio with the residue. This mixture was kept at pH 12-13

and the alkaline aqueous phase separated out. This was decanted and acidified with a solution of sulfuric acid (50 wt %) to pH 6. Ethyl acetate was added to the neutralized aqueous solution in a 1:1 ratio by weight to extract the phenolic compounds. The insolubles were filtered with Whatman #42 filter paper, dried and weighed. The ethyl acetate layer was separated and concentrated as described and the remaining residue consisted of phenolic compounds. A ratio of 4:1 solvent-to-bio-oil was used for each step with the SF 2 WIF, as described previously. This ratio was used to help visually see the separations of the very dark WIF in SF 2. A minimum of three extractions were performed for each sample, with results averaged and standard deviation calculated.

Micro-scale methodology for the FC colorimetry method was utilized [67]. A 20 μ l bio-oil sample, a blank (deionized water), and a gallic acid calibration standard were each placed in 2 ml polystyrene cuvettes. The bio-oil sample was dissolved in ethanol and filtered with a 0.45 μ m Corning syringe filter. Deionized water (1.58 ml) was added, followed by 100 μ l FC reagent. The solutions in each cuvette was mixed thoroughly by pipetting and incubated for 1-8 min. Sodium carbonate solution was added (300 μ l), mixed, and incubated for 2 h at room temperature. The sample absorbance was measured at 765 nm with 1 cm cells and a 1.5 nm bandwidth with a Varian Cary 50 UV-Visible Spectrophotometer (Agilent Technologies, Inc. Santa Clara, CA) using Cary WinUV (Agilent Technologies, Inc. Santa Clara, CA) Simple Reads module software. Samples were diluted to keep absorption between 0.06 and 0.6. The gallic acid calibration standards were made by dissolving 0.5 g gallic acid in 10 ml ethanol and then diluted to 100 ml with water (5 g l⁻¹ final). Standards with 50, 100, 250, and 500 mg l⁻¹ concentrations were created by diluting 1, 2, 5, and 10 ml to 100 ml with water, respectively. These can be stored up to 2 weeks at 4°C. The sodium carbonate solution was made by dissolving 200 g

anhydrous sodium carbonate in 800 ml water and bringing it to a boil. After cooling, a few crystals of sodium carbonate were added. The solution was stored for 24 h at room temperature. It was then filtered with Whatman #42 filter paper and water added to make 1 liter. This solution can be stored indefinitely at room temperature. A minimum of five trials were performed for each sample, with results averaged and standard deviation calculated. All results are given in wt% gallic acid equivalents (GAE) as expressed by the FC method [67]. The FC reagent was purchased from Thermo Scientific® (Hanover Park, IL).

Positive and negative controls were used to validate the method. Positive controls were phenolic compounds added to bio-oil to determine whether the FC method could accurately measure their concentration in the presence of other bio-oil constituents. Positive controls utilized with this method included phenol, guaiacol, 3-ethylphenol, 4-methylphenol, 2,6-dimethoxyphenol, and eugenol. Negative controls were other chemical constituents in bio-oil added to make known the error in total phenolic determination as a result of their presence. Negative controls included furfural, levoglucosan, D-fructose, D-glucose, D-mannose, D-xylose, L-arabinose, cellobiosan, D-fucose, D-galactose, acetic acid, glycolic acid, propionic acid, and formic acid. Both positive and negative controls were purchased from Thermo Scientific® (Hanover Park, IL). The positive and negative controls were added into a prepared sample of bio-oil utilizing the FC methodology [67]. The negative control sugars were dissolved in water prior to adding to the prepared bio-oil. Sugars such as levoglucosan are not completely miscible in ethanol, therefore the use of water as solvent ensured the samples were completely dissolved.

Total phenolic concentrations (wt% GAE) indicated by FC analyses were linearly correlated to concentrations (wt %) of positive and negative controls. Typical concentrations for contributing compounds (positive controls) and potentially interfering compounds (negative

controls) in bio-oil were estimated from the literature and our own laboratory data. The slope of the line represents responsivity of the positive or negative control to the FC reagent (i.e. its ability to be oxidized by the FC reagent). The y-intercept corresponds to the baseline concentration (wt% GAE) of phenolic compounds in the bio-oil sample.

The Beer-Lambert-Bouguer Law (Beer's Law) [102] plots using the FC method results were linear. Molar absorptivity ($l \text{ mol}^{-1} \text{ cm}^{-1}$), a measure of the electronic absorption at a chosen wavelength of analyte chromophores, was calculated for each of the controls. The Beer's law relationship was applied to relate absorbance (A) with molar concentration (c) and the path length through the sample (b) using the well-known equation: $A = \epsilon bc$ to determine the molar absorptivity (ϵ) [102,103].

Moisture content of the bio-oil was determined by a MKS 500 Karl Fischer Moisture Titrator using ASTM E203 Standard Test Method for Water Using Karl Fischer Reagent. The reagent used was Hydranal Composite 5K and the solvent was Hydranal Working Medium K. The percent moisture of the bio-oil samples was determined in a minimum of four trials.

To determine the reliability of the FC method for total phenolic determination in bio-oil, the mean, standard deviation, and 95% confidence intervals were calculated. Quantifications of total phenolic compounds for each bio-oil utilizing the FC method were included in this calculation.

Results and Discussion

The absorbance of a series of dilutions of both positive and negative controls added to bio-oil were recorded and a plot prepared using the concentration (wt% GAE) of total phenolic compounds (y-axis) versus concentration (wt%) of the added positive or negative control (x-axis) yielding a straight line. Both positive and negative control samples were added into the bio-oil sample in concentrations normally found in bio-oil and quantified by the FC method.

Positive controls showed a strong linear relationship between x and y variables (Table 1) with r^2 values ≥ 98 . The y-intercept was the baseline concentration of phenolic compounds in the bio-oil sample. Total phenolic quantification in the bio-oil (wt%) by liquid-liquid extraction was measured and found to be 24.2 ± 0.40 wt% dry basis (db). The y-intercept baseline values average 22.1 wt% GAE. This indicates an 8.7% difference between the liquid-liquid extraction value and the y-intercept baseline average value.

The contributor response factors (ability of the contributor to be oxidized by the FC reagent) are the highest for guaiacol, 2,6-dimethoxyphenol, and phenol. This is in agreement with molar absorptivity values obtained for the contributors (Figure 1). Molar absorptivity ($\epsilon_{765 \text{ nm}} \times 10^{-3} \text{ l mol}^{-1} \text{ cm}^{-1}$) values show these three phenols responded similarly to the FC reagent. The response of a particular phenolic to the FC reagent is due to the number of phenolic groups it contains [67]. Both the response factor and molar absorptivity display comparable trends for 4-methyl and 3-ethylphenol. Percent difference between the phenol response factor and that of 4-methyl and 3-ethylphenol was 50% and 55%, respectively. Molar absorptivity percent difference between phenol and 4-methyl and 3-ethyl phenol was 51% and 56%, respectively. Singleton [104] reports phenols with substituent groups such as 3-ethyl or 4-methylphenol show a lower response to the FC reagent, approximately 40% less than phenol, while methoxyl groups can convert to or behave as phenolic hydroxyls in assay. As previously stated, our results demonstrate similar behavior. Singleton [104] reported the average molar absorptivity ($\epsilon_{765 \text{ nm}} \times 10^{-3}$) for the gallic acid standard was approximately 25.0. Our research resulted in 24.4 ($\epsilon_{765 \text{ nm}} \times 10^{-3} \text{ l mol}^{-1} \text{ cm}^{-1}$) for the gallic acid standard which was in agreement with the literature value.

Among the interferents (Table 2), fructose and glucose gave the highest response factor (ability of the interferent to be oxidized by the FC reagent) and the highest correlation

coefficients. Singleton [104] reported no interferents from reducing sugars except for fructose and glucose with fructose giving a higher response to the FC method. As predicted, the FC method did show a much higher response to fructose and glucose, with fructose

Table 1. Effect of contributors (positive controls) on total phenolic compounds (wt% GAE) quantified in whole bio-oil when analyzed by the Folin-Ciocalteu method.

Contributor (Positive Control)	Typical Contributor Content in Bio-oil ¹ (wt% wb)	Response Factor (slope)	Indicated Phenolic Content in Bio-oil (wt% GAE)	R ² Value	Potential Contribution to Results (%)
Guaiacol	0.1-0.7	5.8	21.0	0.98	2.8-19.0
2,6-dimethoxyphenol	0.1-0.7	5.7	22.9	0.99	2.5-17.0
Phenol	0.03-0.2	5.6	22.9	1.00	0.73-4.9
4-methylphenol	0.1-0.5	3.3	21.8	0.99	1.5-7.6
Eugenol	0.04-0.1	2.9	21.9	0.99	0.53-1.3
3-ethylphenol	0.01-0.03	2.3	22.2	0.98	0.10-0.31

¹as measured in our laboratory (wt% wb)

showing higher responsivity. As evident in Figure 2, both fructose and glucose had higher molar absorptivity values with fructose being the most responsive to the FC reagent. This was anticipated because the wine industry provides correction factors to the FC results for wines containing invert sugars [67]. Invert sugars are glucose and fructose which are broken down (invert) from sucrose by a small amount of acid [105]. Glucose and fructose have not been detected in our PDU bio-oils; therefore they would not interfere. The other sugar interferents show lower responsivity and molar absorptivity, ≤ 0.71 and ≤ 1.7 molar absorptivity ($\epsilon_{765\text{ nm}} \times 10^{-3} \text{ l mol}^{-1} \text{ cm}^{-1}$), respectively. The potential contribution from the sugar interferents was $\leq 2.7\%$. Singleton [104] reported reducing sugars were not reactive to the FC method reagent. The responsivity and molar absorptivity agree with this finding.

Acetic and formic acids are reported to be the main acids in bio-oil [30,72,81]. These acids show small response factors in comparison to the phenolic contributors. Therefore, the FC

method would have a smaller response to these prominent acids, generally 5.8% or less. The negative control acid molar absorptivities were low, ≤ 1.7 ($\epsilon_{765 \text{ nm}} \times 10^{-3} \text{ l mol}^{-1} \text{ cm}^{-1}$).

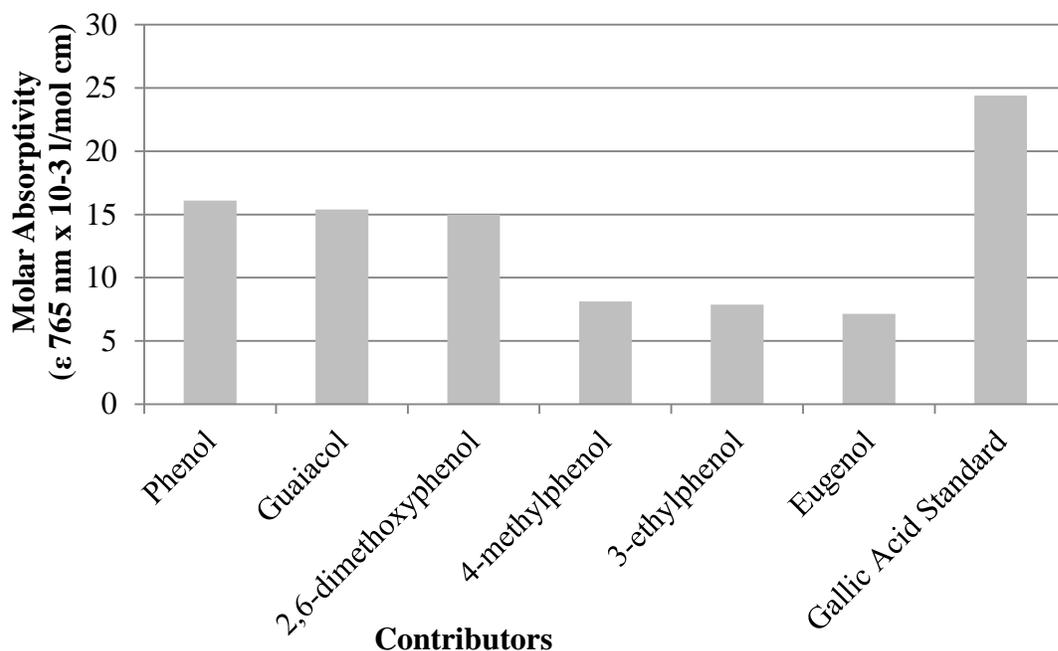


Figure 1. Comparison of contributor (positive controls) molar absorptivity ($\epsilon_{765 \text{ nm}} \times 10^{-3} \text{ l/mol cm}$) when spiked into bio-oil.

Furfural also showed a small response factor with lower correlation and a low molar absorptivity. Its concentrations in bio-oil are low making its influence on the FC method very minimal. Possible interferent from furfural was $\leq 0.01\%$.

The same bio-oil used for the positive control spiking was also used for the negative control spiking, and as stated earlier, the results were 24.2 ± 0.40 wt% db by liquid-liquid extraction. As measured by the y-intercept, average phenolic concentration was 22.4 wt% GAE. This was 7.4% less than the results obtained by liquid-liquid extraction.

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measured by the y-intercept, average phenolic concentration was 22.4 wt% GAE. This was 7.4% less than the results obtained by liquid-liquid extraction.

Table 2. Effect of interferents (negative controls) on total phenolic compounds (wt% GAE) quantified in whole bio-oil when analyzed by the Folin-Ciocalteu method.

Interferent (negative Control)	Typical Interferent Content in Bio-oil ¹ (wt%)	Response Factor (slope)	Indicated Phenolic Content in Bio-oil (wt% GAE)	R ² Values	Potential Contribution to Results (%)
D-fructose ³	0.115	2.2	22.5	0.96	1.1
D-glucose ²	0.4-1.3	1.1	22.2	0.95	1.98-6.4
L-arabinose ²	0.1	0.71	21.5	0.76	0.33
Formic Acid ¹	0.6-1.0	0.59	21.7	0.41	1.6-2.7
Glycolic Acid ¹	0.4-0.5	0.59	20.1	0.19	1.2-1.5
Propionic acid ¹	0.1-0.6	0.56	23.5	0.29	0.24-1.4
Furfural ¹	0.1-0.4	0.55	22.7	0.61	0.24-0.97
D-xylose ¹	0.1-1.4	0.45	23.0	0.52	0.20-2.7
Galactose ³	0.20	0.33	22.3	0.71	0.30
L-fucose ³	0.058	0.32	23.0	0.01	0.081
Levogluconan ¹	4.0-6.0	0.30	22.5	0.89	0.45-0.68
Acetic Acid ¹	0.5-5.5	0.27	23.7	0.15	0.56-5.8
D-mannose ¹	0.02-0.03	0.25	22.6	0.0003	0.022-0.033
Cellobiosan ¹	1.4-1.5	0.07	22.5	0.15	0.44-0.47

¹as measured in our laboratory (wt% wb)

²as measured in literature [87]

³as measured in literature [31]

To verify if the FC method results corresponded to traditional bio-oil testing procedures, a comparison of total phenolic compounds in whole bio-oil quantification by both the FC method and liquid-liquid extraction was performed (Table 3). The FC method results were very similar to those acquired by the liquid-liquid extraction of phenols for bio-oils #1-3. All results lie within the margin of error and the uncertainty of the measurement by the FC method, indicating no significant differences in the results between the two methods.

The WIF is expected to be an estimate of phenolic content on a gravimetric basis. The WIF was 28.1 wt% for bio-oil #1-2 and 21.1 wt% for bio-oil #3. Using the WIF to estimate total phenolic compounds in the whole bio-oil would give a fairly accurate assessment of bio-oil #1-2,

however bio-oil #3 shows an approximate 13% difference between this estimation and the FC method results.

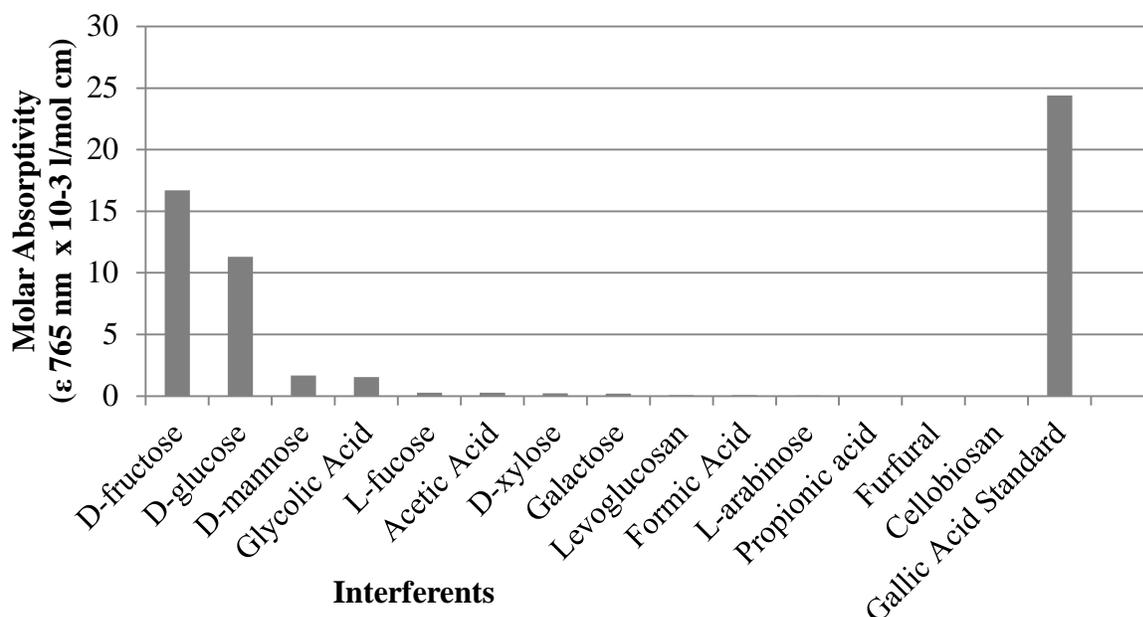


Figure 2. Comparison of interferent (negative controls) molar absorptivity ($\epsilon_{765 \text{ nm}} \times 10^{-3} \text{ l/mol cm}$) when spiked into bio-oil.

Table 3. Comparison of total phenols quantified by the Folin-Ciocalteu method and liquid-liquid extraction of phenols for three whole bio-oils (bio-oil #1-3).

Bio-Oil	Total Phenols by FC (wt% GAE)	Total Phenols by Liquid-Liquid Extraction (wt% db)
#1	27.8 \pm 1.5	28.1 \pm 0.50
#2	29.5 \pm 0.30	29.6 \pm 0.20
#3	25.8 \pm 0.80	24.2 \pm 0.40

By design, most of the pyrolytic lignin was recovered in the first two SFs of the bio-oil collection system [1]. SF 2 contained 63.5 \pm 3.0 wt% WIF. The WIF from SF 2 was analyzed using both the FC method and liquid-liquid extraction (Table 4). Four trials were performed on each sample and averaged. The total phenols by liquid-liquid extraction and the FC method gave similar results with differences falling within the standard deviation of the trials. It was

determined that there were no significant differences between the results for total phenolic determination using the FC method and the liquid-liquid extraction.

Table 4. Comparison of total phenolic determinations from the stage fraction 2 water-insoluble fraction of bio-oil analyzed by the Folin-Ciocalteu method and liquid-liquid extraction.

Component (wt% wb)	Total Phenols by Folin-Ciocalteu Method (wt% GAE)	Total Phenols by Liquid-Liquid Extraction (wt% wb)
Phenols	64.7±1.3	66.7±1.8
Neutrals and very weak bases	---	3.30±1.1
Strong and weak acids	---	1.50±0.20
Solids	---	3.20±0.20
Moisture (%)	21.6±0.10	21.6±0.10
Total	86.3	96.3

The mass balance for the liquid-liquid extraction was 96.3 wt% wet basis (wb). The FC method gave a mass balance of 86.3 wt% wb. The neutrals and very weak bases, acids, and solids were not included in the mass balance for the FC method; therefore, the mass balance was expected to be lower. The acids would include both the strong (i.e. mineral) and weak (i.e. carboxylic) acids. Other possible weak acids include phenolic compounds with carboxylic acid functionality. Potential weak bases could be aromatic structures with nitrogen attached.

Conclusions

We were successful in quantifying total phenolic content in bio-oil using an established food methodology, the Folin-Ciocalteu (FC) colorimetry method, with a ±1.1 wt% uncertainty of measurement. Three whole bio-oils and SF 2 WIF were examined and all show virtually the same result by the FC method in comparison to liquid-liquid extractions. This research has shown the FC method gave highly reproducible results and provided a reliable standardized test method for quantification of total phenolic compounds in bio-oil.

Acknowledgements

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CHAPTER 6. SUGARS AND PHENOLS RECOVERY FROM THE HEAVY-ENDS OF FRACTIONATED BIO-OIL

A paper to be submitted to *Bioresource Technology*

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Abstract

The present study explores the separate recovery of sugars and phenolic oligomers produced during the fast pyrolysis of lignocellulosic biomass. The experiments were conducted in an 8 kg/h fluidized bed pyrolysis process development unit. Bio-oil fractionation was accomplished with a five-stage system that recovers bio-oil according to “condensation points” of the constituent compounds. The first two stages capture “heavy- ends” consisting mostly of water soluble sugars derived from polysaccharides and water-insoluble phenolic oligomers derived from lignin. Exploiting differences in water solubility, we are able to recover a sugar-rich aqueous phase and a phenolic-rich raffinate. The soluble sugars are effectively washed from the phenolic oligomers allowing the production of “pyrolytic sugars” and a carbohydrate-free raffinate comprised of phenolic oligomers that readily flows at room temperature. Over 93 wt% of the sugars in stage fractions (SF) 1 and 2 are removed with two washes. The separated sugars from SF 1 and 2 are suitable for either fermentation or catalytic upgrading to biofuels. The phenolic oligomer-rich raffinate, which represents 44-47 wt% dry basis (db) of both SF 1 and 2, is less sticky and viscous than the unwashed SFs. The raffinate has potential for production of fuels, aromatic chemicals, unique polymers, resins, binders, coatings, adhesives, asphalt, and preservatives.

Introduction

The goal of this research is to investigate a method for recovering two value-added products from the fast pyrolysis of lignocellulosic biomass: a concentrated sugar-rich solution and phenolic oligomer-rich raffinate. Fractionation is accomplished by using a unique five-stage bio-oil recovery system [1] in combination with a washing procedure applied to the heavy-ends of bio-oil recovered by this system. We explore the effects of operating conditions on the effectiveness of the washing process including water-to-heavy-ends ratio, heavy-ends/water extraction temperatures, and the number of washes applied to the heavy-ends.

Bio-oil is recognized as an important feedstock for thermochemical-based production of transportation fuels, chemicals, and power [3,12,16,24,69,106]. However, bio-oil has several undesirable characteristics that must be overcome before wider commercial exploitation. These problems include high oxygen content, high acidity, and low thermal stability [16,22], which negatively impact its use as a co-feed at refineries for the production of transportation fuels [24].

Many of the problems with bio-oil are the direct result of it being a mixture of hundreds of different compounds representing a wide range of functionalities [106]. Thus, the ability to separate the compounds either through pretreatment of the biomass, staged pyrolysis, vapor phase fractionation, or separation of the condensed phase would significantly advance the field of pyrolysis [5]. Distillation, as applied to crude petroleum oil, is not a viable route to bio-oil fractionation due to thermal and chemical instabilities [106] therefore, other fractionation methods must be utilized. A bio-oil recovery system from fast pyrolysis of lignocellulosic biomass as SFs with distinct chemical and physical properties using a system of condensers with carefully controlled coolant temperatures and electrostatic precipitators has been developed [1].

This bio-oil collection system was developed on the premise that both vapors and aerosols are generated as primary or secondary products of fast pyrolysis. Vapors were presumed to be predominantly decomposition products of carbohydrate polymers while aerosols consisted of non-volatile, water-insoluble, lignin-derived oligomers often described as “pyrolytic lignin” in bio-oil. Stage 1 captures high boiling point compounds, such as levoglucosan (1,6-anhydro- β -D-glucopyranose, $C_6H_{10}O_5$) and phenolic oligomers, while Stage 2 captures aerosols formed from these compounds either during pyrolysis or cooling in the condenser of Stage 1 [1]. The highest weight percentage water-insolubles were captured in stage fractions (SFs) 1 and 2, as well as the highest weight percentage of levoglucosan. Stage 1, a condenser, consisted of a shell-and tube heat exchanger consisting of thirty, 2.54cm diameter stainless steel tubes and operated with gas inlet and outlet temperatures of 345°C and 102°C, respectively. The temperature of the coolant water is controlled to 85°C. Stage 2, an electrostatic precipitator (ESP) operated at 40 kVDC, was constructed from 15.24cm diameter stainless steel pipe with a 2.54cm diameter electrode extending the length of the pipe along its axis. It was heat traced to 129°C to prevent condensation of vapors. Both Stages 1-2 bio-oil are black and extremely viscous even when hot. Upon cooling, they were resinous solids at room temperature [1].

The separated phenolic oligomer-rich raffinate exhibited potential for upgrading to transportation fuels, as well as other platform chemicals. The removal of pyrolytic sugars and their conversion into liquid drop-in fuels while using phenols to produce high-value products (i.e. resins, adhesives) could be a promising approach to economic viability of bio-oil refineries [12-15]. The recovery of separate sugar-rich and phenolic oligomer-rich streams from the heavy-ends represents numerous opportunities for value-added products.

Methods

Red oak (*Quercus rubra*) from Wood Residual Solutions of Montello, WI, was used as feedstock for producing bio-oil. Bio-oil was produced in an 8kg/h fast pyrolysis development unit consisting of a fluidized bed operated at 450-500°C and a bio-oil recovery system that collects bio-oil in multiple SFs, having distinctive properties from one another. Stages 1, 3, and 5 were water-cooled condensers operated at progressively lower temperatures to collect SFs of bio-oil according to condensation points of the different compounds in the vapor stream. Stages 2 and 4 were ESPs designed to collect aerosols generated downstream from these stages. Stages 1 and 2 collect viscous, high-boiling point compounds collectively referred to as “heavy-ends” of the bio-oil. Stages 3 and 4 capture the compounds of intermediate molecular weight while stage 5 recovered an aqueous phase containing “light oxygenates,” including acids and aldehydes. The present study focused on separating sugars and phenolic oligomers from SF 1 and 2, which were stored in polypropylene containers at 5°C in the dark prior to conducting washing trials. Details of this system and a complete characterization of the SFs are found in Pollard et al. [1].

Specific analyses of the bio-oil were from methodologies described in detail in literature. The Association of Analytical Communities, International (AOAC) method 988.12 (44.1.30) Phenol-Sulfuric Acid Assay for Total Carbohydrate Determination was used to quantify the water-soluble sugar fraction in SF 1 and 2 [82,107]. Levoglucosan was used as the standard with a ultraviolet/visible range spectrophotometer wavelength set at 490 nm. The water-soluble fraction was evaluated by gas chromatography (GC) with a flame ionization detector (FID) following previously described methodology [107]. The acids analyzed in SFs 1 and 2 included acetic, formic, glycolic, and propionic by ion-exchange chromatography (IC) [107]. The ultimate analysis was performed using LECO TruSpec carbon, hydrogen, and nitrogen (CHN) (LECO

Corp., St. Joseph, MI) analyzer with the determination of oxygen by difference that has been previously described [1]. Ethylenediamine-tetraacetic acid (EDTA) was used as a standard for CHN determinations. Moisture determinations were performed using a MKS 500 Karl Fischer Moisture Titrator (Kyoto Electronics Manufacturing Co., LTD, Kyoto, Japan) that has been previously reported [1]. The percentages of moisture in the bio-oil samples were determined in a minimum of three trials. Water-insolubles were determined by using an in-house method described by Pollard et al. [1].

Gel permeation chromatography (GPC) was used to determine the molecular weight distribution of the phenolic oligomer-rich raffinates separated from the heavy-ends of the bio-oil. The high-performance liquid chromatography (HPLC) system used was a Dionex Ultimate 3000 (Sunnyvale, CA) equipped with a Shodex Refractive Index (RI) and Diode Array detector (DAD). The software used to control the instrument and evaluate the samples was Dionex Chromeleon version 6.8. For the GPC analyses, the eluent for the phenolic oligomers was tetrahydrofuran (THF) with two Agilent PLgel 3 μ m 100 \AA 300 x 7.5mm and one Mesopore 300 x 7.5mm. The column flow rate and temperature was 1.0 mL min⁻¹ at 25°C. The phenolic oligomers samples were prepared using 10 mL of THF and 0.02 g of heavy-ends from the bio-oil. All samples were filtered with a Whatman 0.45 μ Glass Microfiber (GMF) syringe filter before analysis. The GPC standards were purchased from Agilent (Agilent Technologies, Inc. Santa Clara, CA). These standards contained polystyrene ranging from 162 – 38,640 g mol⁻¹. The polystyrene standards were diluted with JT Baker HPLC-grade Stabilized THF.

The minimum amount of water required for the phase separation of bio-oil water-soluble constituents from the water-insoluble constituents was determined by the drop-wise addition of

deionized water into SFs 1 and 2 while stirring thoroughly by hand after each addition. This forced phase separation to occur. Water was dispensed utilizing an electronic repeater pipette.

The phenolic oligomer-rich raffinate was separated from the water-soluble components using a known amount of oil mixed at different ratios by weight with deionized water. The resulting solution was manually stirred to blend the bio-oil and water. The sample was placed on a shaker table for 30 min at 250 motions min^{-1} and centrifuged (accuSpin™ 1R, Thermo Scientific®, Hanover Park, IL) at 2561g force for 30 min. The water-soluble portion (sugar-rich solution) was decanted.

Results and Discussion

To establish when phase separation occurred, water was added drop-wise into SF 1 and 2. The mixtures were stirred after each addition to force phase separation. The moisture in SF 1 prior to the addition of water was $3.35 \pm 0.99\%$ and $3.34 \pm 1.20\%$ for SF 2. The results of this experiment gave the minimum water-to-heavy-ends ratio that could be used to extract water-soluble sugars from the heavy-ends. The minimum water-to-heavy-ends ratio for SF 1 was 0.5:1 whereas, the lowest water-to-heavy-ends ratio for SF 2 was 0.4:1. The total weight percent of extracted water-soluble sugars was determined by AOAC Method 988.12 (44.1.30) and plotted for both SF 1 and 2 (Figure 1). As indicated, a 0.5-5:1 water-to-heavy-ends ratio could be used to extract the water-soluble sugars from SF 1. The total quantified sugars resulted in similar weight percentages and fall within experimental error. On the other hand, SF 2 indicated a water- to-heavy-ends ratio of 1:1 to 2:1 was optimal for extracting water-soluble sugars.

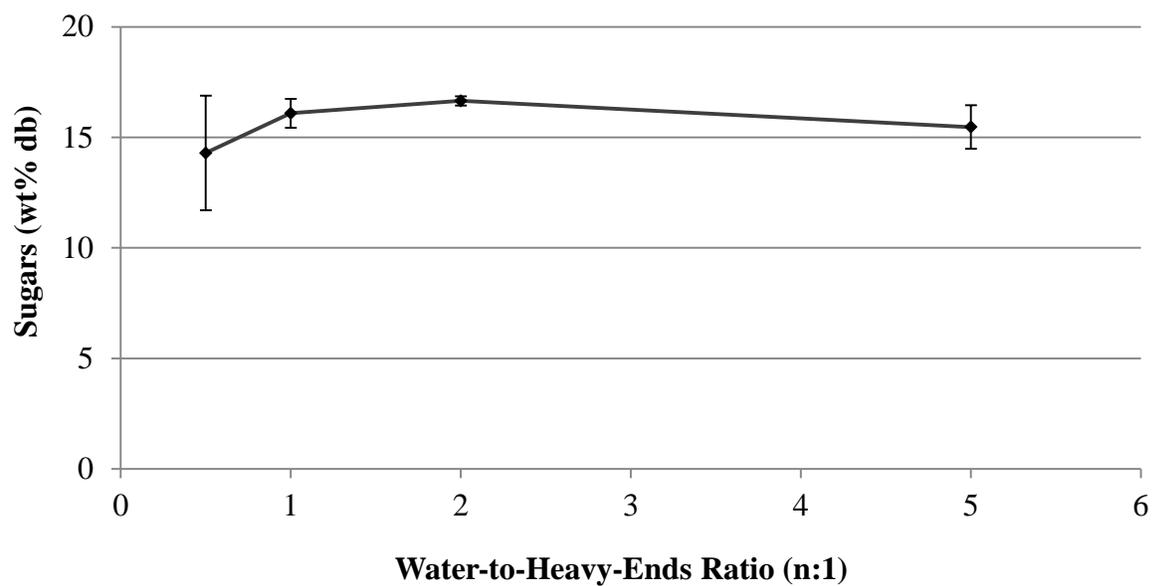
The optimal number of washes needed for removal of the water-soluble sugars in SF 1 and 2 were determined (Figure 2). A single wash removed 77.1 wt% sugars db (1.37 wt%

biomass db) from SF 1 and 80.1 wt% db sugars (3.11 wt% db in biomass) for SF 2. A second wash removed an additional 15.9 wt% db for the phenolic oligomer-rich raffinate of SF 1 and an additional 14.3 wt% sugars db for SF 2. Two consecutive washes removed approximately 93% of the sugars from SF 1 and 94% from SF 2. Sugar extraction could be improved by optimization of the washing system. An in-line counter current flow extraction system may remove the majority of the sugars.

The moisture content of the phenolic oligomer-rich raffinate was determined for SF 1 and 2 (Table 1) for each water-to-heavy-ends ratio. The moisture in the original heavy-ends bio-oil was $3.34 \pm 1.20\%$ for SF 1 and $3.35 \pm 0.99\%$ for SF 2. The percent moisture increased with the number of washes. This added moisture varied in the phenolic oligomer-rich raffinate between different water-to-heavy-ends ratios. The optimum water-to-heavy-ends ratio appears to be 1:1 when considering the amount of water left behind in the phenolic oligomer-rich raffinate. The moisture percent was lower at this ratio for both SF 1 and SF 2.

The effects of extraction temperature on removal of water-soluble sugars in SFs 1 and 2 were determined. As shown, in Figure 3, bio-oil temperature did not influence the extraction of the water-soluble sugars from SF 1, with all results falling within experimental error. However, extraction temperature did influence recovery of water-soluble sugars from SF 2. As temperature increased, extraction efficacy increased. Maximum sugar recovery from SF 2 occurred in the temperature range of 80 to 120°C. Coincidentally, bio-oil was recovered from Stage 1 (condenser) at 80-90°C and from Stage 2 (ESP) at 80-120°C [1], which suggested that on-line separation of sugars and phenolic oligomers from freshly produced heavy-ends could be readily implemented.

(a)



(b)

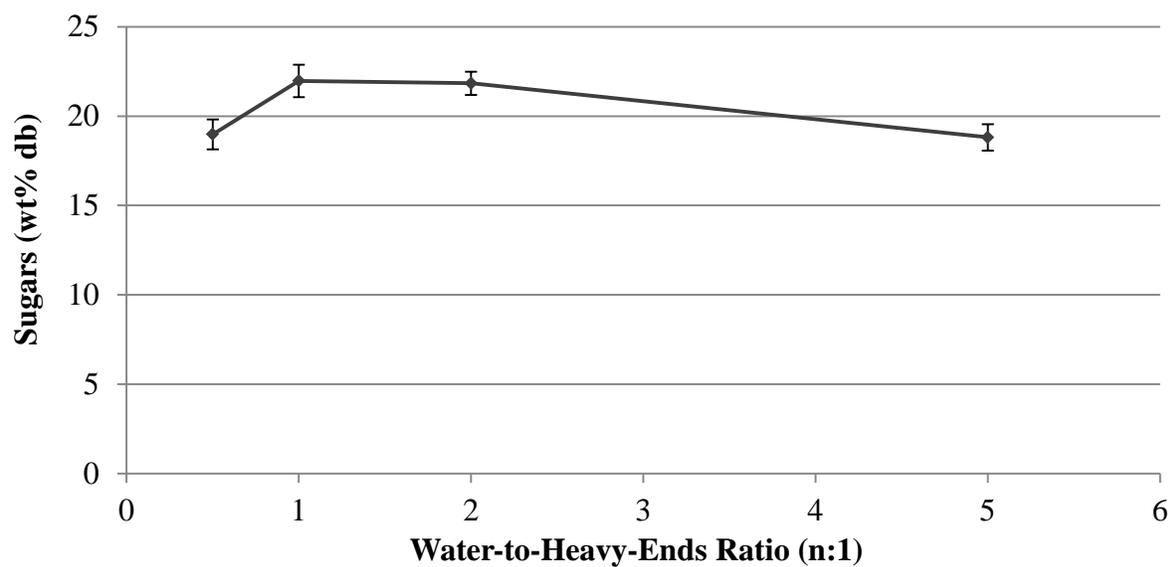


Figure 1. Total water-soluble sugars extracted from SF 1 (a) and SF 2 (b) as a function of water-to-heavy-ends ratio with total water-soluble sugar determination by using AOAC Method 988.12 (44.1.30).

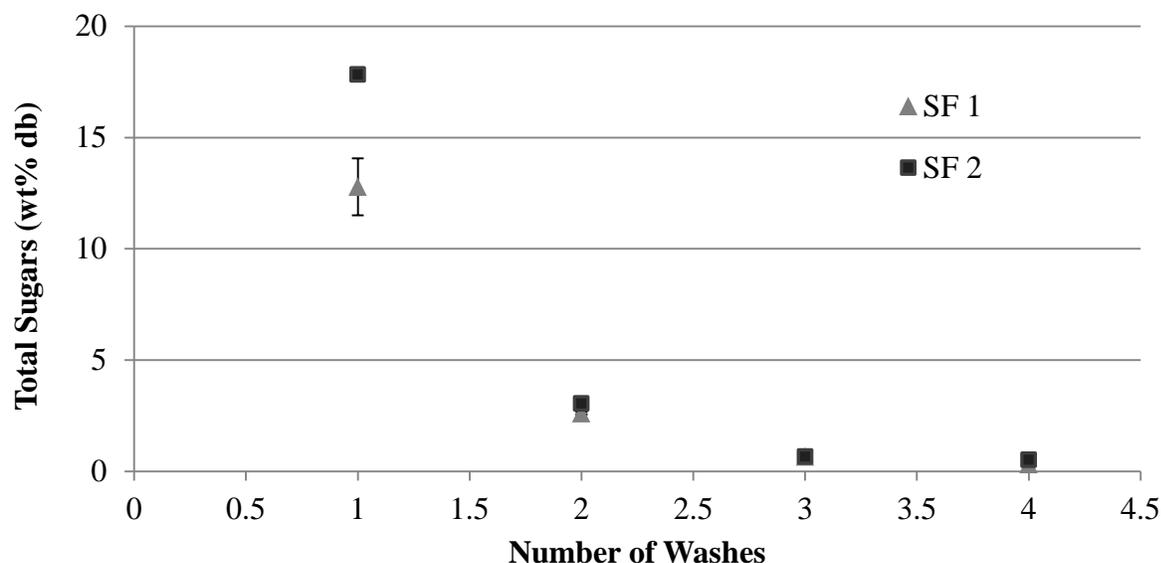


Figure 2. Total water-soluble sugars removed from SF 1 and 2 phenolic oligomer-rich raffinates (at approximately 40°C) with multiple washes with total water-soluble sugars determined using AOAC Method 988.12 (44.1.30).

Table 1. Moisture (%) in phenolic oligomer-rich raffinate in stage fraction SF 1 and 2 heavy-ends after water-soluble sugars extraction using different water-to-heavy-ends ratios.

Water-to-Heavy-ends Ratio	Phenolic Oligomer-Rich Raffinate SF 1 Heavy-Ends Moisture (%)	Phenolic Oligomer-Rich Raffinate SF 2 Heavy-Ends Moisture (%)
0.5:1	27.02±1.74	22.6±0.70
1:1	18.3±0.17	17.9±0.49
2:1	22.6±0.42	22.02±0.59
5:1	21.9±0.62	19.7±0.26

As shown in Figure 4, the molecular weight distribution (relative to polystyrene standards) for the phenolic oligomers obtained as raffinate from SF 1 had four peaks. The first and second peaks at 97 and 185 Da were consistent with the molecular weights for phenolic monomers [94]. Examples of possible monomers expected for red oak include phenol (94 g mol⁻¹) syringol (154 g mol⁻¹) and 4-propenyl syringol (194 g mol⁻¹) [108]. The third peak at 285 Da was likely a dimer while the fourth peak at 437 Da was believed to be comprised of trimers [94]. The

maximum molecular weight is 5770 Da for SF 1 phenolic oligomer-rich raffinate. SF 2 phenolic oligomer-rich raffinate indicates a slightly different profile versus SF 1 heavy-ends raffinate. There were only two prominent peaks, the first at 182 Da and the second at 429 Da with a maximum molecular weight of 5655 Da. These peaks were consistent with monomers and trimers. SF 1 and SF 2 GPC profiles were thought to have influenced higher extraction temperature requirements for SF 2 effective sugar removal. As reflected in the GPC profiles, a larger majority of trimers comprised SF 2 phenolic oligomers, whereas SF 1 majority consisted of monomers. The maximum molecular weight for phenolic oligomers obtained as raffinate from SF 2 was slightly lower than for raffinate from SF 1.

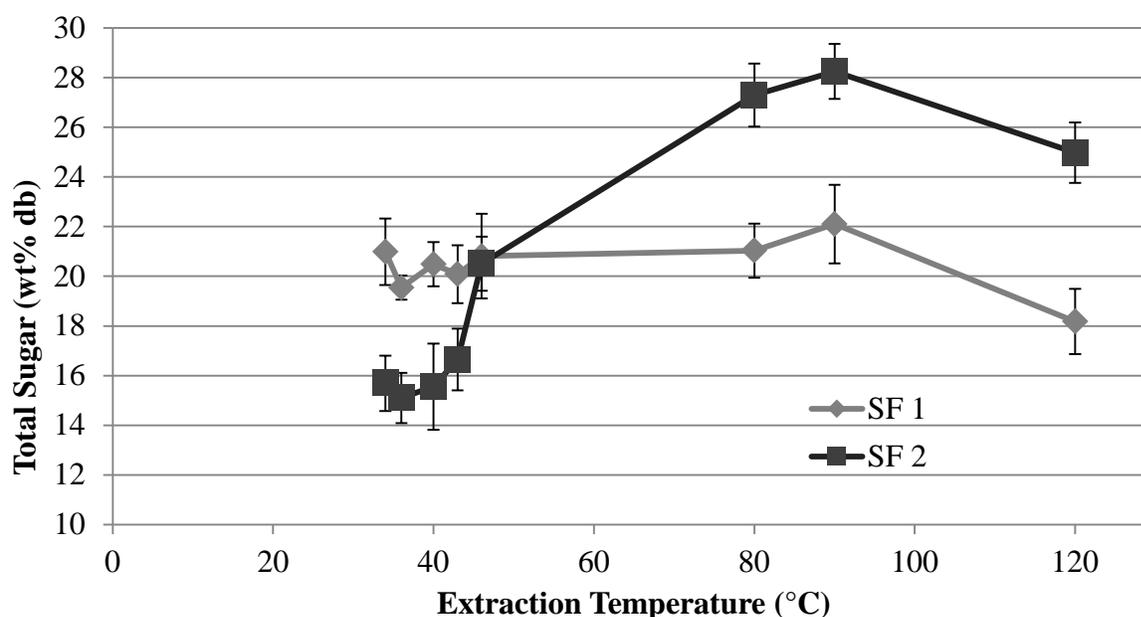


Figure 3. Effect of the extraction temperature on removal of water-soluble sugars from SF 1 and 2.

The empirical formulas for the phenolic oligomer-rich raffinate (db) after three washes were $\text{CH}_{1.03}\text{O}_{0.26}$ and $\text{CH}_{1.1}\text{O}_{0.25}$ for SF 1 and 2, respectively. For comparison, empirical formulas for possible phenolic monomers are shown in Table 2. As indicated, the empirical formula of the phenolic oligomer-rich raffinates for SF 1 and 2 fell within the various phenolic monomers

identified in hardwood lignin [4]. The phenolic oligomer-rich raffinate appeared to have less hydrogen and oxygen versus many of the indicated probable monomers showing possible deoxygenation by loss of hydroxyl groups in the raffinate.

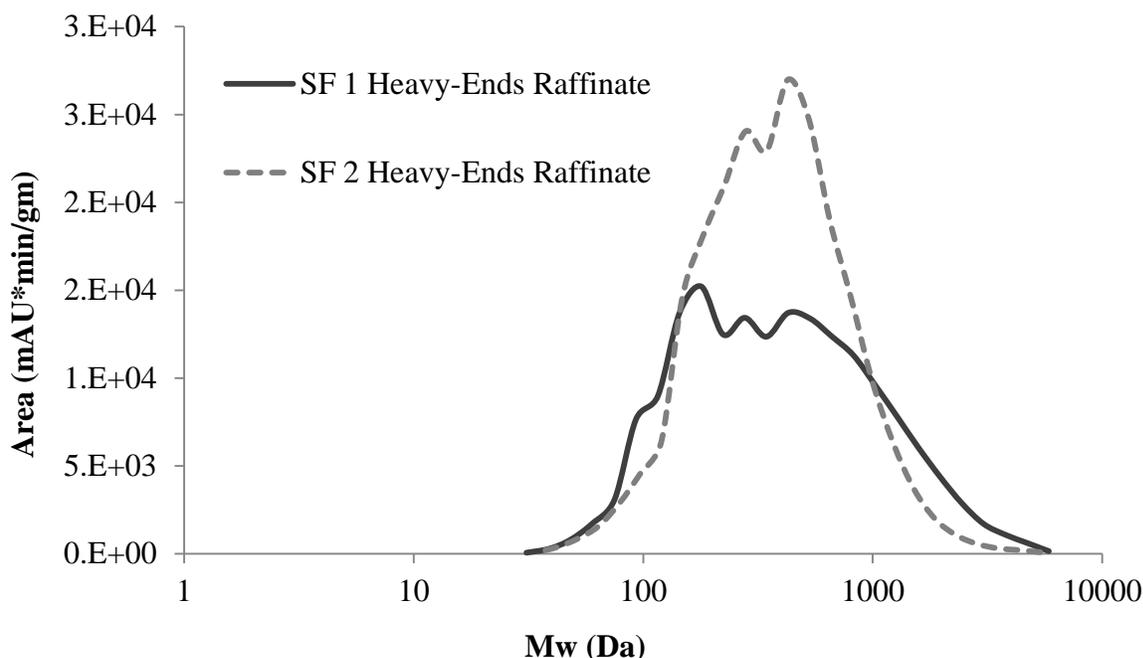


Figure 4. Molecular weight (relative to polystyrene standards) comparison of phenolic oligomer-rich raffinate for SF 1 and 2 after one water wash.

Table 2. Empirical formulas of phenolic monomers obtained from hardwood lignin [4].

Phenolic	Empirical Formula
Phenol	$\text{CHO}_{0.17}$
Vanillin	$\text{CHO}_{0.38}$
Guaiacol	$\text{CH}_{1.1}\text{O}_{0.29}$
Acetosyringone	$\text{CH}_{1.2}\text{O}_{0.40}$
4-Propenyl syringol	$\text{CH}_{1.3}\text{O}_{0.27}$
2,6-Dimethoxy-4-methylphenol	$\text{CH}_{1.3}\text{O}_{0.33}$
Syringyl acetone	$\text{CH}_{1.3}\text{O}_{0.36}$
Syringol	$\text{CH}_{1.3}\text{O}_{0.38}$

The empirical formula for sugar in the sugar-rich solution from a single wash of SF 1 was $\text{CH}_{1.5}\text{O}_{0.65}$. This indicated other compounds were present in the sugar-rich solution in addition to levoglucosan (empirical formula of $\text{CH}_{1.7}\text{O}_{0.83}$). Therefore, sugar-rich solutions obtained after

each wash for four washes of SF 1 and 2 were evaluated by using GC/FID and IC to quantify other water-soluble or partially water-soluble compounds (Tables 3-4). As indicated, approximately 6.5 wt% wet basis (wb) and 3.2 wt% wb constituents other than sugars were present in the sugar-rich solutions after the first washes of SF 1 and SF 2, respectively. SF 2, an electrostatic precipitator designed to capture aerosols, contained approximately 50% less “other constituents” in the solution from each wash in comparison to SF 1. As the phenolic oligomer-rich raffinates were washed additional times this percentage declined, as expected. The results indicate the water-soluble acids, ethylene glycol, furans, and acetol were extracted along with the water-soluble sugars. Usage of the sugar-rich solution for fermentation may require additional steps to remove any water-soluble compounds that are toxic to fermentation microbes.

Table 3. Comparison of water-soluble and partially soluble constituents other than sugars found in the sugar-rich solution from SF 1 after consecutive washes.

Chemical	First Wash (wt% wb)	Second Wash (wt% wb)	Third Wash (wt% wb)	Fourth Wash (wt% wb)
Methanol	0.04±0.01	0.03±0.001	0.01±0.002	0
Acetol	0.55±0.07	0.23±0.01	0.14±0.01	0.05±0.01
Furfural	0.25±0.06	0.13±0.02	0.10±0.01	0.05±0.004
2,6-Methoxyphenol	0.04±0.02	0.03±0.0009	0.02±0.004	0
Ethylene glycol	1.30±0.23	0.24±0.01	0.08±0.01	0.09±0.01
Furfuryl Alcohol	0.20±0.13	0.07±0.02	0.06±0.01	0.05±0.002
2(5H)-furanone	0.09±0.01	0.20±0.01	0.15±0.004	0.01±0.004
3-Methyl-1,2-cyclopentanedione	0.48±0.10	0.38±0.02	0.26±0.04	0.15±0.01
Guaiacol	0.10±0.04	0.13±0.004	0.09±0.03	0.03±0.0009
Vanillin	0.06±0.01	0.04±0.01	0.04±0.02	0.01±0.003
Phenol	0	0.29±0.09	0.41±0.04	0.03±0.003
5-(Hydroxymethyl)furfural	0.32±0.07	0.48±0.02	0.31±0.05	0.13±0.01
Acetic acid	0.89±0.01	0.40±0.005	0.20±0.004	0.04±0.0006
Formic acid	0.57±0.002	0.25±0.001	0.19±0.003	0.03±0.0009
Glycolic acid	0.84±0.01	0.32±0.001	0.18±0.0003	0.03±0.0003
Propionic acid	0.08±0.003	0.04±0.001	0.03±0.0005	0.006±0.0001
Total (wt% wb)	6.5±0.03	3.4±0.01	2.3±0.01	0.71±0.004

SFs 1 and 2 were evaluated for total water-insoluble constituents. The percentage of phenolic oligomer-rich raffinate from SF 1 after a single wash was 54.5 wt% db whereas, 46.4 wt% db remained after a second wash. Raffinate from SF 2 showed a similar trend with each additional wash. The insoluble content for the phenolic oligomer-rich raffinate from SF 1 after four washes was 40.5 wt% db and 44.7 wt% db was obtained for raffinate from SF 2. The present work suggests the phenolic oligomer-rich raffinate may require 2-3 washes to remove “other non-phenolic” water-soluble constituents.

Table 4. Comparison of water-soluble and partially water-soluble constituents other than sugars found in the sugar-rich solution from SF 2 after consecutive washes.

Chemical	First Wash (wt% wb)	Second Wash (wt% wb)	Third Wash (wt% wb)	Fourth Wash (wt% wb)
Methanol	0.03±0.002	0.01±0.003	0.01±0.0004	0
Acetol	0.31±0.01	0.18±0.01	0.09±0.01	0.02±0.0004
Furfural	0.08±0.01	0.06±0.01	0.05±0.01	0.02±0.001
2,6-Methoxyphenol	0	0.01±0.002	0.01±0.001	0
Ethylene glycol	0.42±0.05	0.10±0.02	0.03±0.01	0.03±0.003
Furfuryl Alcohol	0.09±0.04	0.03±0.01	0.03±0.01	0.03±0.002
2(5H)-Furanone	0.29±0.02	0.19±0.09	0	0
3-Methyl-1,2-cyclopentanedione	0.15±0.003	0.11±0.02	0.09±0.02	0.10±0.02
Guaiacol	0.05±0.01	0	0.02±0.002	0.02±0.0009
Vanillin	0	0	0.09±0.02	0
Phenol	0.06±0.01	0.25±0.05	0.28±0.02	0.02±0.0004
5-(Hydroxymethyl)furfural	0.33±0.02	0.26±0.11	0.12±0.02	0.05±0.002
Acetic acid	0.56±0.02	0.23±0.003	0.11±0.0002	0.03±0.0002
Formic acid	0.34±0.03	0.15±0.004	0.11±0.0008	0.03±0.0004
Glycolic acid	0.43±0.04	0.14±0.03	0.18±0.00005	0.03±0.00003
Propionic acid	0.07±0.01	0.03±0.001	0.02±0.0001	0.004±0.0001
Total (wt% wb)	3.2±0.02	1.9±0.02	1.4±0.01	0.38±0.003

Conclusions

We successfully demonstrated the ability to separate sugars and lignin-derived phenolic oligomers from the heavy fractions of bio-oil produced pyrolyzing lignocellulosic biomass. This

was made possible by the large difference in water solubility of the sugars and phenolic oligomers. The sugars were effectively extracted at over 93 wt% with two water washes. Approximately 3-7 wt% of other water-soluble or partially soluble constituents were removed with the water-soluble sugars. Sugars and phenolic oligomers can be separated, providing two separate streams for fermentation, catalytic upgrading, or other types of conversions to value-added products.

Acknowledgements

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CHAPTER 7. EFFECTS OF PYROLYSIS TEMPERATURE ON RECOVERY OF BIO-OIL AS DISTINCTIVE STAGE FRACTIONS

A paper to be submitted to *The Journal of Analytical and Applied Pyrolysis*

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Abstract

The goal of the present study was to investigate the effects of pyrolysis temperature on the recovery of various products from the pyrolysis of red oak by a fractionating bio-oil recovery system. Within the temperature range investigated, the maximum bio-char yield of 31.1 wt% occurred at 350°C, the maximum bio-oil yield of 66.7 wt% occurred at 400°C and the maximum non-condensable gas (NCG) yield of 26.3 wt% occurred at 550°C. The maximum production of sugar from biomass cellulose and hemicellulose, 13.5 wt%, occurred at 450°C. The sugars, in the form of anhydrosugars, were condensed in stage fractions (SF) 1-2. Production of phenolic compounds was highest at 400°C yielding 29.4 wt% dry basis (db) with the majority collected as oligomers in SF 1-2. Bio-oil moisture content was highest at 550°C, which is 91% of the total moisture. Gel permeation chromatography (GPC) indicated that the majority of compounds found in the heavy-ends of bio-oil (SF 1-2) ranged from oligomeric monomers to tetramers.

Introduction

Fast pyrolysis is the thermal decomposition of biomass into liquid (bio-oil), solids (bio-char), and non-condensable gases (syngas). Pyrolysis temperature affects the yield of these products; the goal of most pyrolysis research is to maximize the yield of liquids, which can be upgraded to transportation fuels. Little research has been directed toward the effect of temperature on the individual organic components of the bio-oil, possibly because they are usually recovered together as “whole bio-oil” with limited prospects for separating them after recovery.

Bio-oil is a multicomponent mixture containing 35-40 wt% oxygen and more than 300 identified compounds in bio-oil [8]. It is comprised of both volatile compounds, including water, acids, alcohols, aldehydes, esters, ketones, sugars, phenols, quaiacols, syringols, vanillins, and furans, and non-volatile compounds, especially viscous phenolic oligomers [1,16,71,72].

The complex chemical composition of bio-oil causes many problems including poor stability, both in storage and when heated for upgrading. Bio-oil constituents have a very wide range of boiling temperatures. Bio-oil starts to boil below 100°C but distillation ceases at 250-280°C leaving 35-50% of the starting material as residue [1,8]. Typical methods of separation and purification include liquid chromatography, extraction, centrifugation, and distillation, which are high cost and difficult to scale up [109].

These problems prompted us to develop a bio-oil recovery system that separates bio-oil into stage fractions (SFs) with distinctive chemical and physical characteristics [1]. This separation is accomplished by a combination of condensers with carefully controlled coolant temperatures to recover vapors and electrostatic precipitators (ESP) to recover aerosols.

The ability to fractionate bio-oil enables the possibility of optimizing pyrolysis operating conditions to produce desired products [7]. For example, for the purpose of producing asphalt or resins, pyrolysis conditions that increase the yield of higher molecular weight phenolic oligomers should be optimized. If monomeric sugar production is the goal, conditions that encourage depolymerization of polysaccharides should be favored. The present study investigates the effects of pyrolysis temperature on the recovery of various products from the pyrolysis of red oak using the fractionating bio-oil recovery system. Of particular focus is cellulose-derived levoglucosan and lignin-derived phenolic oligomers.

Methods

Biomass used for the pyrolysis experiments was red oak (*Quercus rubra*) procured from Wood Residual Solutions of Montello, WS. As-received biomass was passed through a 60 hp hammer mill equipped with a 3mm screen, resulting in approximately 200-500 μ m average particle size. The moisture content of the red oak was approximately 10%.

Experiments were performed in a fluidized bed pyrolyzer and bio-oil recovery system that separates the bio-oil into distinctive SFs, as previously described by Pollard et al. [1]. Stage 1, a condenser, captures high boiling point compounds such as levoglucosan and phenolic oligomers. It was a shell-and tube heat exchanger operated with gas inlet and outlet temperatures of 345°C and 102°C, respectively. The coolant water was controlled to 85°C. Stage 2 uses an electrostatic precipitator (ESP) to collect aerosols formed either during pyrolysis or during cooling in Stage 1. The ESP was operated at 40 kVDC and heat traced to 129°C to prevent premature condensation of vapors. Stage 3, another shell-and-tube heat exchanger, collected compounds with condensation points near that of phenol. It operated at gas inlet and outlet temperatures of 129°C and 77°C, respectively. The coolant water was controlled to 65°C. Stage 4, an ESP, was insulated and had an operating temperature of approximately 77°C. Stage 5 was a shell-in-tube heat exchanger using water at 18°C as coolant for the purpose of removing water, furans, and light oxygenated compounds (i.e. acids) [5].

Several improvements have been made to the system since first described by Pollard et al. [5]. The hopper in the feed system was expanded to allow extended test runs. Four Omega Type K thermocouple sensors (Omega Engineering, Inc., Stamford, CT) were inserted along the central axis of the fluidized bed spaced every 50 mm to improve monitoring and control of pyrolysis. The thermocouple located at the top of the fluidized bed region controlled power to the

external bed heaters and thus set the reaction temperature of the bed. Enhanced preheating of nitrogen entering the plenum of the reactor further improved temperature control of the reactor. The bio-char disengagement section was heat traced to improve its operational reliability. Better temperature control was added to the inlet of SF 1, which was previously prone to fouling. The collection efficiency of the ESPs was improved with changes to its geometry to improve corona discharge. These changes to the pyrolysis system enabled significant improvements in bio-oil yield and allow more sustained operation compared to previously work [5].

Five pyrolysis tests were performed at 350, 400 450, 500, and 550°C and 6 kg/h biomass feed rate. Bio-oil was collected and weighed to determine yield for each SF. Bio-oil was stored in polypropylene containers at 5°C in the dark until analyzed. Non-condensable gas flow rate was determined by injecting helium at a known mass flow rate as determined by an Alicat Mass Flow Controller (Alicat Scientific, Tucson, AZ) located at the gas exit of the bio-oil collection system. An Agilent Varian® CP-4900 Micro-GC (Gas Chromatograph) (Agilent Technologies, Inc. Santa Clara, CA) interfaced with Galaxie® Chromatography 1.9 software (Bruker Corporation, Bruker Daltonics, Inc., Fremont, CA) was used for NCG analysis. The micro-GC was programmed to sample for 30 s followed by 120 s of run time. The average composition over steady-state operation was then used to calculate NCG yields. In principle, bio-char could be determined directly from the cyclone catch, but sand often elutriated from the fluidized bed along with the bio-char, making problematic the accurate measurement of bio-char yield from the cyclone catch. Accordingly, bio-char yield was determined by difference rather than direct gravimetric measurement.

Specific analyses of the bio-oil were methodologies described in detail in the literature. The Association of Analytical Communities, International (AOAC) Method 988.12 (44.1.30)

(Phenol-Sulfuric Acid Assay for Total Carbohydrate Determination) was used to quantify the water-soluble sugar fraction in SF 1-5 [82]. Levoglucosan was used as the standard with the ultraviolet visible range spectrophotometer wavelength set to 490 nm. Oasmaa and Kuoppala [40] described bio-oil sample preparation for extracting the water-soluble fraction of bio-oil for sugar determination. The acids analyzed in each bio-oil SF included acetic, formic, glycolic, and propionic by ion-exchange chromatography (IC) [107]. These were done in triplicate with the standard deviation $\leq \pm 0.9$ wt% wet basis (wb). Gas chromatography/mass spectroscopy was used for bio-oil chemical analyses. The samples were done in triplicate. The standard deviations for phenol and alkylated phenols were $\leq \pm 0.10$ wt% wb, monomethoxyphenols $\leq \pm 0.41$ wt% wb, and dimethoxyphenols $\leq \pm 0.60$ wt% wb. The standard deviation for furans was $\leq \pm 0.01$ wt% wb. A complete description of the methodology is found in Pollard et al. [1]. The Folin-Ciocalteu colorimetry method [67] was used to determine total phenols in bio-oil [110]. The sampling was done in triplicate. The standard deviation was $\leq \pm 2.16$ wt% db. The water-insoluble content was determined by using an in-house method previously described in the literature [1]. The standard deviation for the water-insoluble content evaluation was $\leq \pm 2.34$ wt% db. Gel permeation chromatography (GPC) was used to determine the molecular weight distribution (relative to polystyrene standards) of the bio-oil constituents at 254 nm by utilizing a diode array detector. The GPC methodology was described in detail by Rover et al. [107].

Moisture content of the bio-oil was determined by using a MKS 500 Karl Fischer Moisture Titrator (Kyoto Electronics Manufacturing Co., LTD, Kyoto, Japan) and ASTM E203 Standard Test Method for Water Using Karl Fischer Reagent. The reagent used was Hydranal Composite 5K and the solvent was Hydranal Working Medium K purchased from Thermo Scientific®

(Hanover Park, IL). The percentage moisture of the bio-oil samples was determined for a minimum of four trials with a standard deviation of $\leq \pm 3.08\%$.

The ultimate analysis was performed using LECO TruSpec (LECO Corp., St. Joseph, MI) carbon, hydrogen, and nitrogen (CHN) analyzer with oxygen determined by difference. Ethylene diamine tetra acetic acid (EDTA) was used as a standard for carbon and hydrogen determinations. Calibration lines were prepared using four different concentrations of EDTA. The standard was purchased from LECO Corporation, St. Joseph, MI. A minimum of 3 trials were run for each sample. The standard deviation for the ultimate analyses was $\leq \pm 0.42$ wt% carbon and $\leq \pm 0.17$ wt% hydrogen.

Results and Discussion

The yields (weight of product/weight of feedstock) of bio-char, NCG, and bio-oil SFs were determined at five different temperatures: 350, 400, 450, 500, and 550°C by using red oak as feedstock (Table 1). Maximum yield of whole bio-oil was obtained in the temperature range of 400-450°C where approximately 67 wt% of the biomass was converted to bio-oil. However, there was some variation in the optimal temperature for production of bio-oil as SFs: 400°C for SF 1; 450°C for SF 2-3; 400°C for SF 4; and >550°C for SF 5. The reasons for these differences became apparent during subsequent analysis of SF composition. As expected, the NCG yield increased as temperature increased; with an 11.9 wt% product yield at 350°C and 26.3 wt% at 550°C. Higher NCG formation was attributed to cracking of pyrolysis vapors and chars at higher temperatures [48,111]. As anticipated, increasing NCG yield corresponded to decreasing bio-char yield. Bio-char yield decreased with increasing temperature. The bio-char product yield at 350°C was 31.1 wt% with 11.4 wt% at 550°C.

Changing reactor temperature during fast pyrolysis dramatically influenced NCG composition (Figure 1). The distribution of NCG (reported as g/g NCG), showed methane to increase from 1.26% to 7.00% and carbon dioxide to increase from 26.5 to 46.5% in the temperature range of 350-550°C. In contrast, carbon dioxide decreased from 73.2% at 350°C to 40.9% at 550°C. Ethane was not observed below 400°C while ethylene and propane appeared at 450°C. These gases increased as reactor temperature increased, although the yields were low. Hydrogen yield was low as well and did not appear until 550°C.

Table 1. Temperature effect on yields (wt%) of bio-char, bio-oil stage fractions (SF), and non-condensable gas (NCG) for fast pyrolysis of red oak.

Temperature (°C)	SF 1 (wt%)	SF 2 (wt%)	SF 3 (wt%)	SF 4 (wt%)	SF 5 (wt%)	Total Bio-Oil	NCG (wt%)	Bio-Char*
350	12.5	16.6	2.97	1.10	23.9	57.0	11.9	31.1
400	14.8	21.1	4.10	2.00	24.7	66.7	12.6	20.7
450	13.2	21.3	4.60	1.70	25.0	65.8	16.0	18.2
500	12.5	19.3	4.32	1.40	25.6	63.2	18.3	18.6
550	12.1	18.3	3.77	1.41	26.7	62.3	26.3	11.4

* Bio-char by difference

Analyses of the bio-oil compounds detected by GC/MS were categorized by groups and shown in Table 2. These compounds represented specific functional groups and were only a small fraction of those compounds volatile enough to be analyzed by GC/MS.

Phenol and alkylated phenols increased with pyrolysis temperatures in the range of 400-550°C in SF 2-5 (Figure 2). The majority of phenol and alkylated phenols were condensed in SF 3-5. The reactor collection system was designed to collect monomeric phenols (phenol and alkylated phenols) in SF 3-4. Because of the range of boiling points (182-227°C) it was not surprising they were also collected in SF 2. At 500°C pyrolysis temperature, approximately 47% wb of phenol and alkylated phenols were collected in SF 3 and 4, whereas SF 1-2 collected 22%

wb and SF 5 collected 31% wb. At 550°C pyrolysis temperature, approximately 54% wb phenol and alkylated phenols were captured in stages 3 and 4, while 15% wb were captured in stages 1

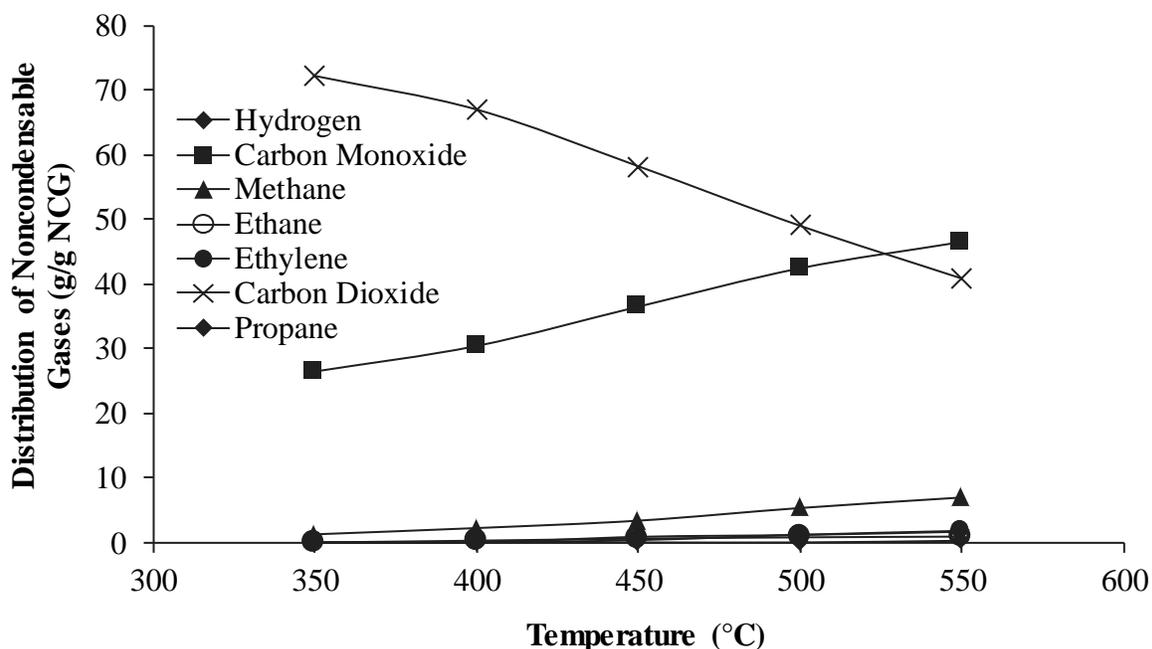


Figure 1. Effect of temperature on distribution of noncondensable gases (NCG) for fast pyrolysis of red oak.

Table 2. Categorized chemical compounds detected in bio-oil stage fractions by GC/MS.

<p>Levogluconan</p> <p>Phenol and alkylated phenols</p> <p>Phenol</p> <p>2-Methylphenol</p> <p>3-Methylphenol</p> <p>3-Ethylphenol</p> <p>2,4-Dimethylphenol</p> <p>3,4-Dimethylphenol</p> <p>Monomethoxy phenols</p> <p>2-Methoxyphenol</p> <p>2-Methoxy-4-methylphenol</p> <p>2-Methoxy-4-vinylphenol</p>	<p>Eugenol</p> <p>Isoeugenol</p> <p>Vanillin</p> <p>Dimethoxy phenols</p> <p>2,6-Dimethoxyphenol</p> <p>4-Allyl-2,6-dimethoxyphenol</p> <p>3',5'-Dimethoxy-4'-hydroxyacetone</p> <p>3,5-Dimethoxy-hydroxybenzaldehyde</p> <p>Furans</p> <p>Furfural</p> <p>Furfuryl Alcohol</p> <p>5-Methylfurfural</p>
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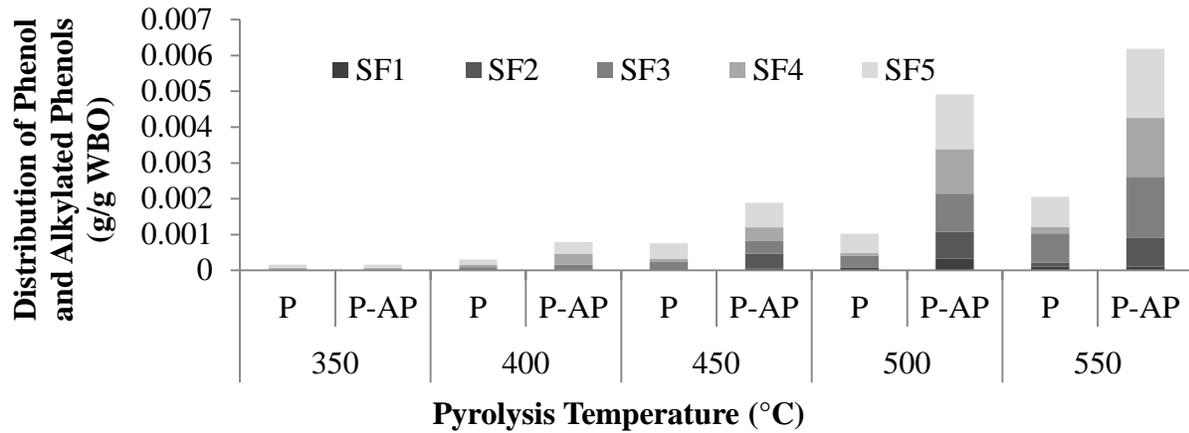
and 2. Stage 5 captured 31% wb. The amount of phenol and alkylated phenols did not increase in SF 5 as temperatures increased from 500-550°C. The amount collected in SF 1-2 decreased with

the resulting increase seen in SF 3-4. Phenol increased 88% as pyrolysis temperatures increased from 400 to 550°C. The increase in phenol as a function of temperature is important because this offers the opportunity to utilize phenol as an alternative to petroleum-based phenol for use as a valuable chemical.

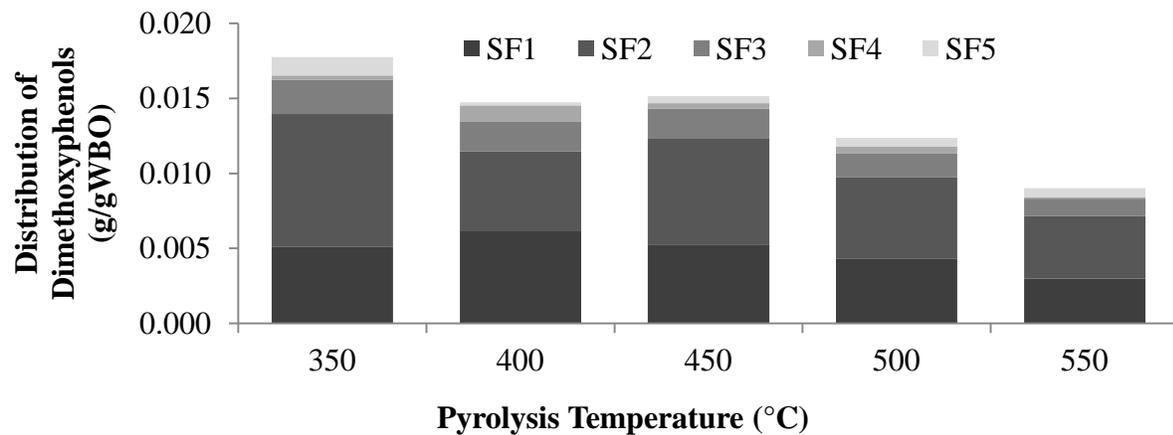
The monomethoxyphenols decreased in SF 1 as pyrolysis temperature increased from 400 to 550°C but the overall collection in SF 1 and 2 increased from 51% to 75% wb over this same temperature range. Pollard et al. [1] collected approximately 73% wb monomethoxyphenols at a pyrolysis temperature of 500°C in SF 1 and 2. At this same temperature 53% wb monomethoxyphenols were collected in SF 1-2. The percentage difference of monomethoxyphenols from their maximum production temperature of 450°C to the minimum collected at 550°C was 61% wb. At pyrolysis temperature of 450°C approximately 64% wb of the monomethoxyphenols were collected in SFs 1 and 2.

As seen with the monomethoxyphenols, the dimethoxyphenols also decreased in SF 1 as pyrolysis temperatures increased from 400°C to 550°C. At 400°C, SFs 1 and 2 collected approximately 78% wb of the dimethoxyphenols, which increased to at 81% wb at 450°C. At 500°C approximately 79% wb were collected in SFs 1-2 with SF 1 collecting 20% less than SF 2. At the same temperature, Pollard et al. [1] reported a similar percentage (78%) wb of dimethoxyphenols collected in SFs 1 and 2, although it was equally distributed in the two SFs. We postulated that a large fraction of the dimethoxyphenols entered the bio-oil recovery system as vapors [1]. These results suggested that a large fraction of dimethoxyphenols left the reactor as aerosols rather than vapor. At 550°C, approximately 80% wb were collected in SFs 1 and 2.

(a)



(b)



(c)

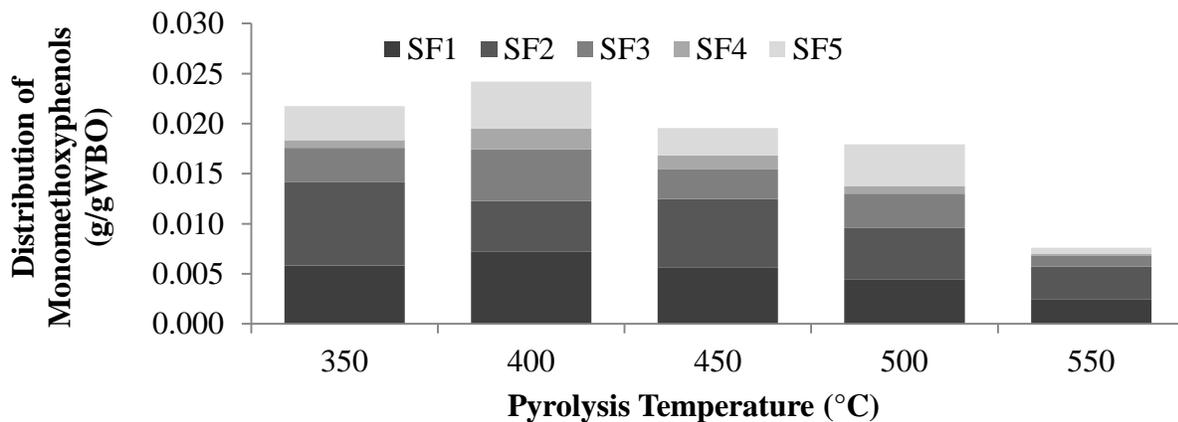


Figure 2. Distribution of phenol (P) and phenol and alkylated phenols (P-AP) (a), mono-methoxyphenols (b), and dimethoxyphenols (c) among bio-oil stage fractions (SF) on the basis of whole bio-oil (WBO) as a function of pyrolysis temperatures (wb).

The loss of monomethoxy and dimethoxyphenols while phenol and alkylated phenol increase with temperature was attributed to two types of processes that occurred during pyrolysis (a) primary reactions where the products are formed from biomass decomposition and (b) secondary reactions where the volatiles evolved from the biomass underwent further reactions [112]. The present work suggested the monomethoxy and dimethoxyphenols originated from primary reactions associated with the depolymerization of lignin, whereas phenol and alkylated phenols were products of continued degradation of lignin-derived oligomers and therefore continued to increase in concentration as temperatures increased.

Total phenolic compounds quantified (Figure 3) were collected in the highest concentrations, approximately 85% db, in SFs 1 and 2 for pyrolysis at 350-550°C. Researchers often use the water-insoluble content of bio-oil as an “estimate” of total phenolic compounds in bio-oil. As shown in Figure 4, the majority of water-insoluble content was collected in SFs 1 and 2 (97% db). Total phenols contents were higher than the water-insoluble contents. This indicated the total water-insoluble content was not a good proxy for the total phenols contents in bio-oil. Because SF 1 and 2 collected the heavy-ends, it was not surprising that significant quantities of water-insoluble content were present in the first two SFs compared to SF 3-5. The majority of water-insoluble content was collected in SF 2, indicating that the majority was leaving the reactor as aerosol. Approximately 14.5 wt% of the lignin in the original biomass was collected as lignin oligomers (water-insoluble content) at 500°C. This percentage decreased at 550°C, indicating that secondary reactions were likely cracking these oligomers.

Figure 5 shows the yield of levoglucosan (the major anhydrosugar product) and total water-soluble sugars (WSS) (which included anhydrosugars) as a function of pyrolysis temperature. The majority of sugars were captured in SFs 1 and 2. At 450°C approximately 87% wb total

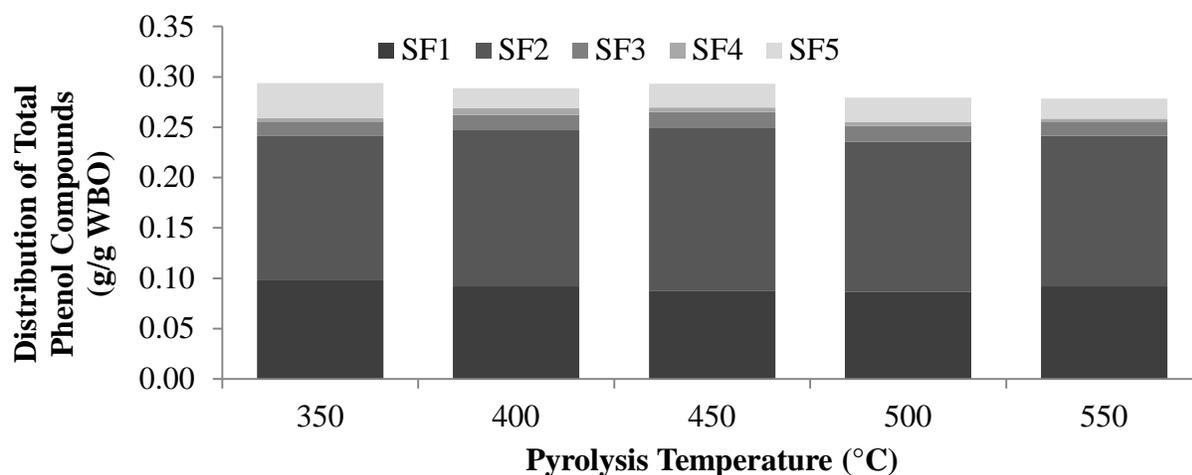


Figure 3. Distribution of total phenolic compounds across the stage fractions (SF) on the basis of whole bio-oil (WBO) as a function of pyrolysis temperature (db).

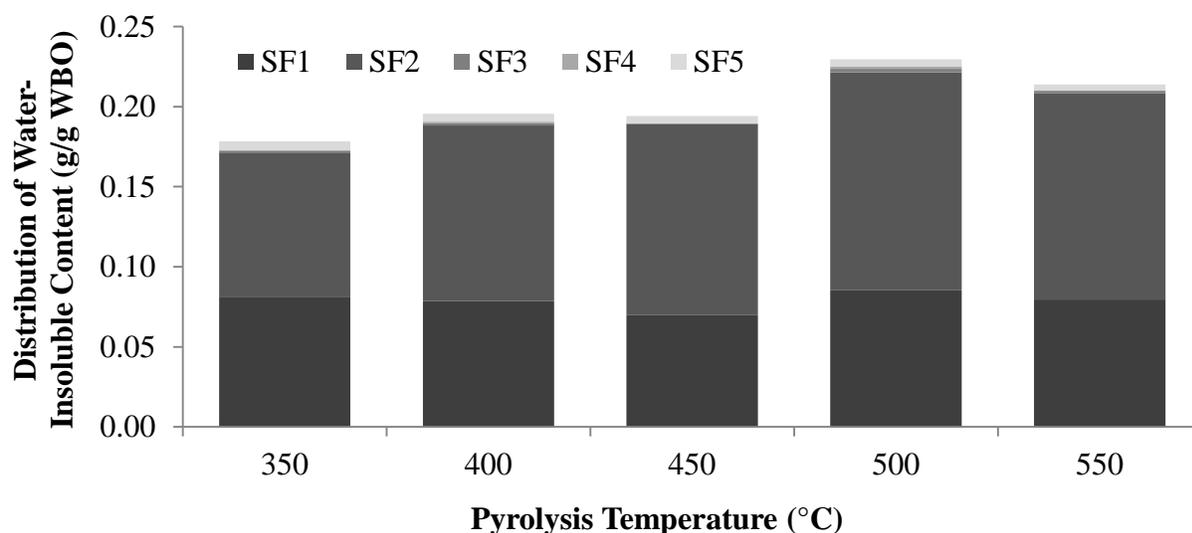


Figure 4. Distribution of water-insoluble content across stage fractions (SF) on the basis of whole bio-oil (WBO) as a function of pyrolysis temperature (db).

WSS was collected in SFs 1 and 2. In general, levoglucosan represented 40-50% of the total WSS in the bio-oil in the temperature range investigated. At lower pyrolysis temperatures cellulose was believed to dehydrate rather than depolymerize, as evidenced by the high bio-char yields at low temperatures shown in Table 1. At temperatures above the optimum range,

decreasing levoglucosan yields has been postulated to be the result of secondary reactions of levoglucosan in the vapor phase [113].

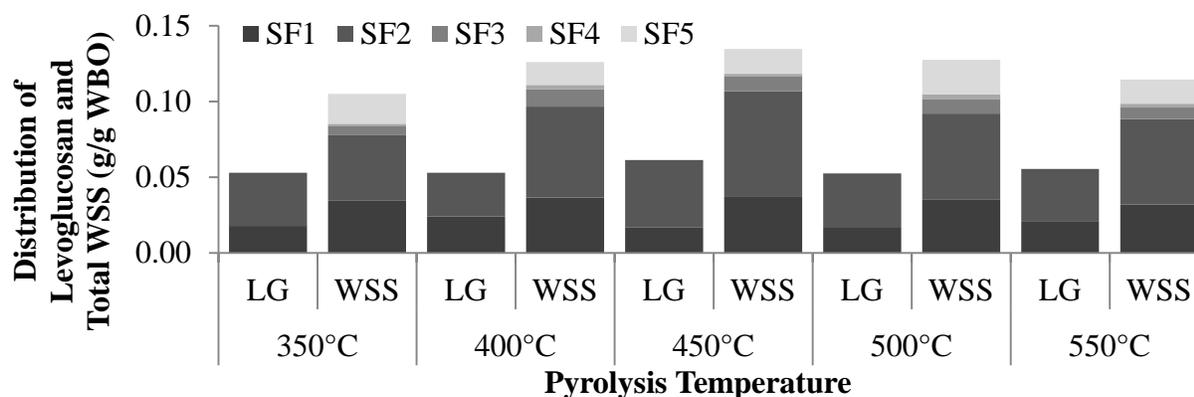


Figure 5. Levoglucosan (LG) and total water-soluble sugars (WSS) in stage fractions (SF) on the basis of whole bio-oil (WBO) as a function of pyrolysis temperatures (wb).

Figure 6 illustrates the effect of pyrolysis temperature on furan compounds. The vast majority of furans appeared in SF 5 for all temperatures evaluated. The highest yields of furans (furfural, furfuryl alcohol and 5-methylfurfural) occurred at 350°C. Furans decreased with increasing temperature. Lu et al. [114] and Dong et al. [115] discuss possible pyrolytic pathways for various furan formation. Furfural, derived from both cellulose and hemicellulose is influenced by pyrolysis temperature. 5-methylfurfural is produced from secondary reactions of 5-hydroxymethylfurfural (HMF) while furfural is formed concurrently with HMF [114,115].

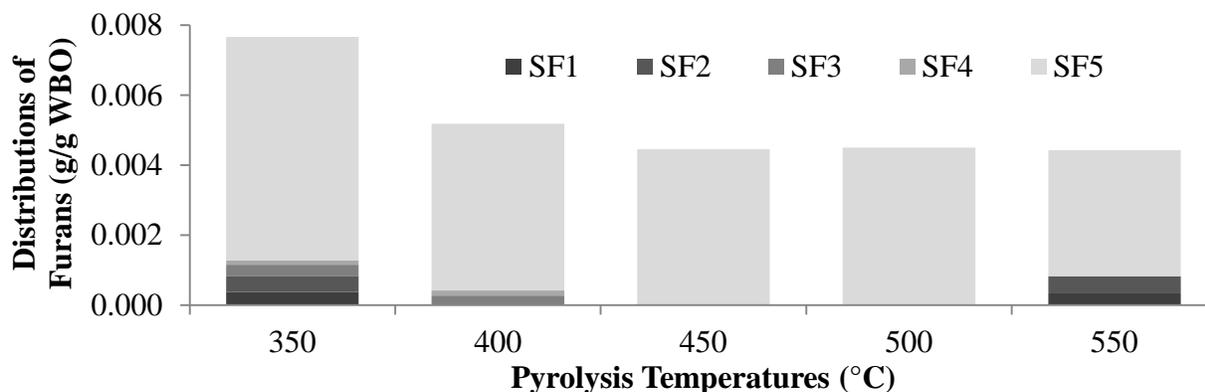


Figure 6. Distribution of furans (furfural, furfuryl alcohol and 5-methylfurfural) in stage fractions (SF) on the basis of whole bio-oil (WBO) as a function of pyrolysis temperatures (wb).

As shown in Figure 7, the fractionating bio-oil recovery system was particularly effective in concentrating water in the last bio-oil fraction (SF 5). Typically, none of the first four fractions contained more than 4% of the total water found in the bio-oil while SF 5 contained more than 90% of the water. The amount of water produced increased with pyrolysis temperature, increasing approximately 10% between 350°C and 550°C. Other researchers saw a more prominent increase with temperature. Heo et al. [116] observed an increase of 33% in the temperature range of 400-550°C when pyrolyzing waste furniture sawdust.

Garcia-Perez et al. [9] reported an increase of 43% in the temperature range of 450-580°C for mallee wood. This is likely due to differences in pyrolysis conditions rather than attributable to the fractionating bio-oil recovery system.

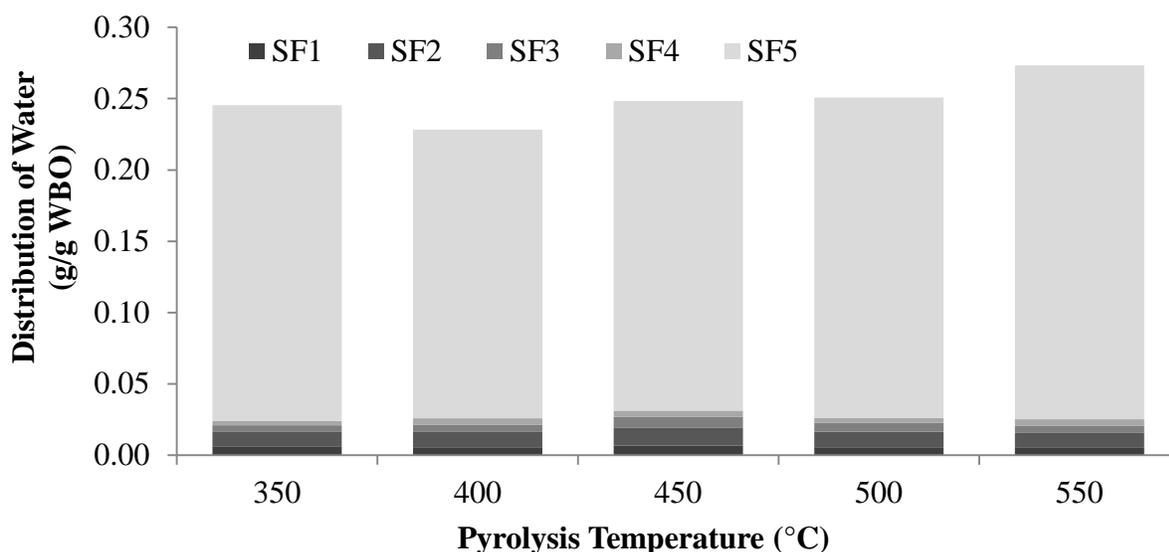


Figure 7. Distribution of water in stage fractions (SF) on the basis of whole bio-oil (WBO) as a function of pyrolysis temperature.

Carboxylic acids (acetic, formic, glycolic, and propionic) increased with increasing pyrolysis temperatures in the range of 350°C to 550°C (Figure 8). The majority of acids were captured in SF 5 (approximately 73% wb at 550°C). Pollard et al. [1], observed relatively high

concentrations of acetic acid in SF 4 as well as SF 5. The current study showed only a small percentage of the total acetic acid appeared in SF 4 at any temperature (about 3.4% wb). This difference was likely due to improvements in collection efficiency of the ESP.

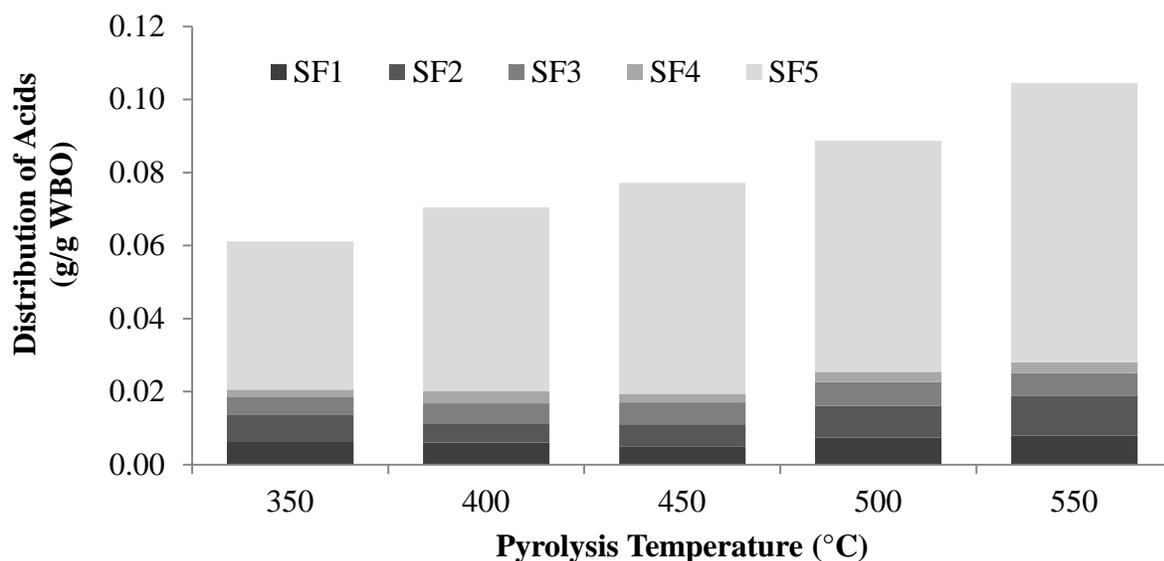


Figure 8. Distribution of acids (acetic, formic, glycolic, and propionic) in stage fractions (SF) on the basis of whole bio-oil (WBO) as a function of pyrolysis temperatures (wb).

Figure 9 shows the elemental analysis of whole bio-oil. For a given bio-oil fraction, there was no significant difference in the elemental composition as temperature increased. This suggested that little in the way of deoxygenation or dehydrogenation occurred during pyrolysis.

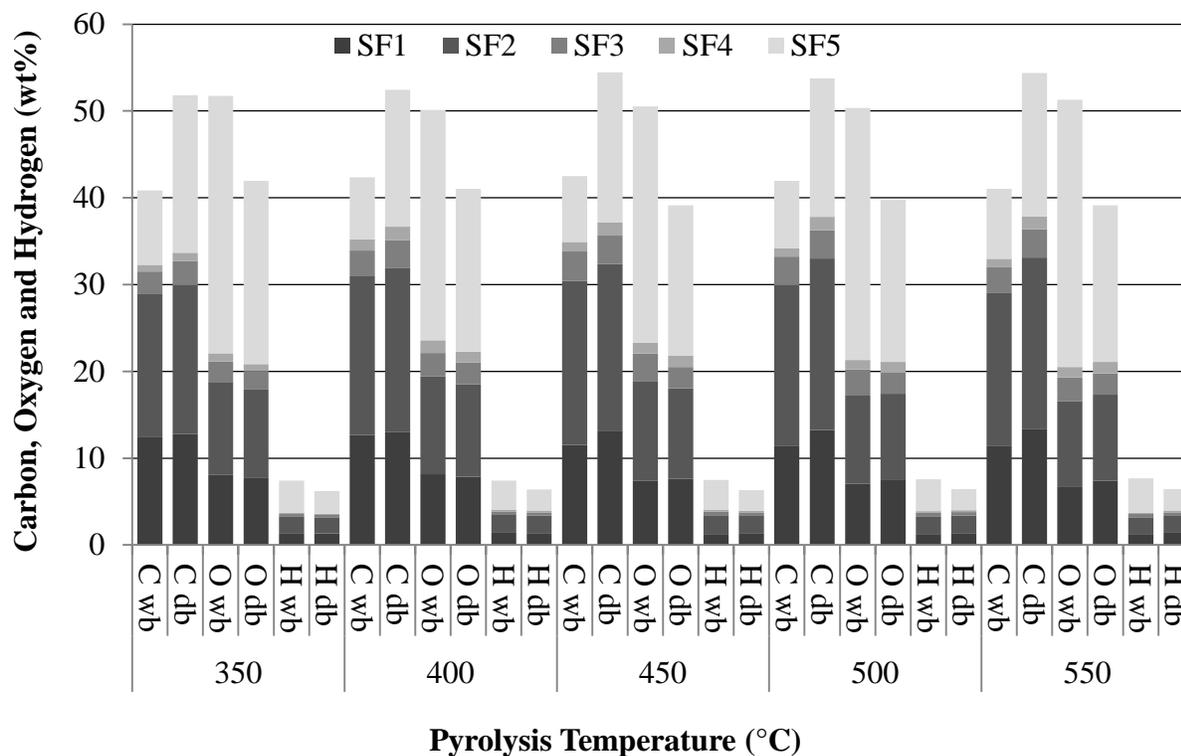


Figure 9. Yields (wt%) of carbon (C), oxygen (O), and hydrogen (H) on wet basis (wb) and dry basis (db) at different pyrolysis temperatures.

GPC was only performed on SFs 1 and 2 because these were the only two fractions to have significant water-insoluble content, the source of most of the high molecular weight material in bio-oil (see Figure 10). The molecular weight distributions of SF 1 range from 30 – 8,000 Da with several peaks consistent with SF 1 containing largely monomers, dimers, trimers, and tetramers derived from lignin [94]. SF 2 had a narrower molecular weight distribution, ranging from 30 - 5,000 Da but also included tetramers. As pyrolysis temperature increased, the prominent peaks in the distributions for both SF 1 and 2 shifted to higher molecular weights. This observation does not support the expectation that higher pyrolysis temperatures promote more complete depolymerization of lignin; however, it is consistent with repolymerization of monomers and small oligomers that originally formed from depolymerizing lignin.

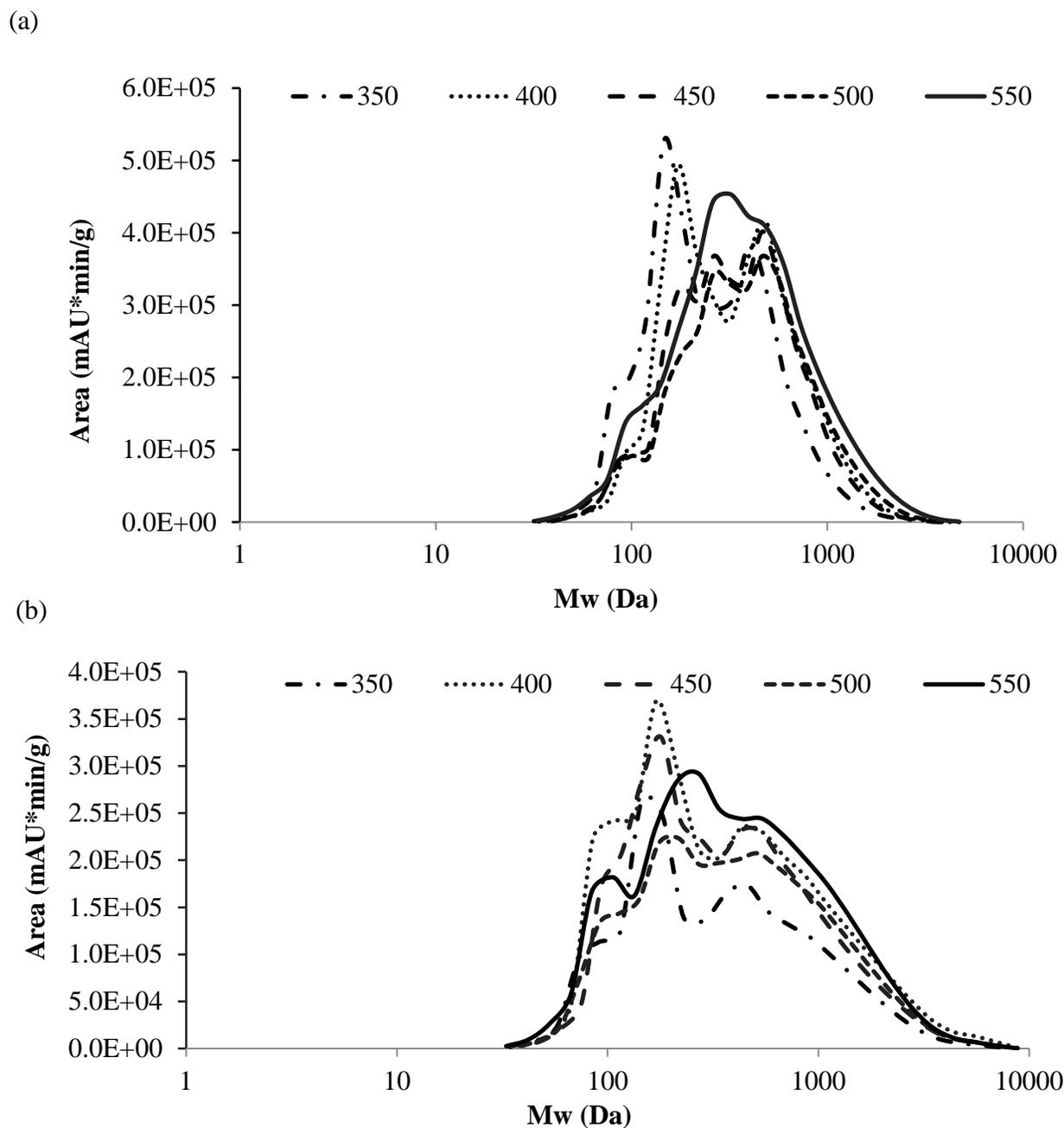


Figure 10. Molecular weight distributions of (a) SF 1 and (b) SF 2 using GPC (relative to polystyrene standards utilizing a diode array detector (DAD) at 254 nm).

Conclusions

As reported previously, we found that the yields of liquid, solid, and gaseous products from the pyrolysis of lignocellulosic biomass were strongly influenced by pyrolysis temperature.

However, in the range of 300-550°C, pyrolysis temperature had relatively small effect on the

yield of specific chemical constituents in the bio-oil or their distribution across the SFs of the fractionating bio-oil recovery system, with a few exceptions. Carboxylic acids increased by 41% in this temperature range, especially appearing in SF 5. Other constituents showed various temperature dependencies but insignificant shifts in distributions among the SFs. The water-insoluble content of bio-oil also increased with temperature before peaking at 500°C. Water increased with temperature, but only by 10% in the temperature range tests. Furan yield decreased at increasing temperature from 300°C to 350°C but yield was independent of temperature beyond that. Yields of levoglucosan and total water soluble sugar showed a discernible peak at 450°C, but the total effect was small. The results indicate that the bio-oil separation system, in terms of its ability to recover distinctive stage fractions, is robust to changes in pyrolysis temperature.

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CHAPTER 8. CONCLUSIONS AND FUTURE WORK

My overall research goal was to develop analysis methods for quantifying sugars and phenolic compounds in bio-oil, recover these two valuable products, and explore pyrolysis temperature effects for possible product optimizations. Specific goals included (1) adapting analytical methods developed for sugar analysis in the food industry to measure total water-soluble sugars in the aqueous phase of bio-oil; (2) adapting analytical methods developed for quantification of total phenols in wine to measure the total phenolic content of bio-oil; (3) separating the heavy fraction of bio-oil into a concentrated sugar-rich solution and a phenolic oligomer-rich raffinate; and (4) determining the effect of pyrolysis temperature on the yield of sugars and phenolic compounds in bio-oil. Bio-oils are recognized as potential resources for drop-in fuels and chemicals. Bio-oil chemistries are very complex but offer a vast resource with potential applications in many areas of industry. This provides opportunities to exploit this valuable resource.

8.1 Total Water-Soluble Sugars Quantification in Bio-Oil Using the Phenol-Sulfuric Acid Assay

I demonstrated AOAC Method 988.12 (44.1.30) Phenol-Sulfuric Acid Assay for Total Carbohydrate Determination, originally designed to evaluate sugars in food products, can be used to measure water-soluble sugars in bio-oil with $\pm 1.7\%$ uncertainty of measurement. Both whole bio-oil and SFs 1-5 bio-oil were examined and all show similar results in comparison to a hydrolysis method utilizing HPLC for quantification. The present research showed that the typical liquid-liquid extraction bio-oil method greatly overestimated the total water-soluble sugars and should not be used for these analyses. Interferents included furans, therefore a correction factor of 0.76 wt% was established for use with bio-oil. Present research demonstrated

that AOAC Method 988.12 (44.1.30) gave highly reproducible results while providing a reliable standardized test method for quantifying total water-soluble sugars in bio-oil.

8.2 Quantification of Total Phenols in Bio-Oil Using the Folin-Ciocalteu Method

I quantified total phenolic content in bio-oil by using an established food methodology, the Folin-Ciocalteu (FC) colorimetry method, with a ± 1.1 wt% uncertainty of measurement. Three whole bio-oils and SF 2 WIF were examined and all show virtually the same result by the FC method in comparison to liquid-liquid extractions. This research has shown the FC method gave highly reproducible results and provided a reliable standardized test method for quantifying total phenolic compounds in bio-oil.

8.3 Sugar and Phenolic Oligomer Recovery from the Heavy-ends of Fractionated Bio-Oil

I demonstrated the ability to separate sugars and lignin-derived phenolic oligomers from the heavy fractions of bio-oil produced by pyrolysis of lignocellulosic biomass. This was made possible by the large difference in water solubility of the sugars and phenolic oligomers. The sugars were extracted effectively at over 93 wt% with two water washes. Approximately 3-7 wt% of other water-soluble or partially soluble constituents were removed with the water-soluble sugars. This research has shown that sugars and phenolic oligomers can be separated, providing two separate streams for fermentation, catalytic upgrading, or other kinds of conversions to value-added products.

8.4 Effects of Pyrolysis Temperature on Recovery of Bio-Oil as Distinctive Stage Fractions

As reported by previous researchers, we found that the yields of liquid, solid, and gaseous products from the pyrolysis of lignocellulosic biomass were strongly influenced by pyrolysis temperature. However, in the range of 300-550°C, pyrolysis temperature had relatively small effect on the yield of specific chemical constituents in the bio-oil or their distribution across the

stage fractions of the fractionating bio-oil recovery system, with a few exceptions. Carboxylic acids increased by 41% in this temperature range, especially appearing in SF 5. Other constituents showed various temperature dependencies but insignificant shifts in distributions among the stage fractions. The water insoluble content of bio-oil also increased with temperature before peaking at 500°C. Water increased with temperature, but only by 10% in the temperature range tests. Furan yield decreased as temperatures increased from 300 to 350°C but yield was independent of temperature beyond that. Yields of levoglucosan and total water soluble sugar showed a discernible peak at 450°C, but the total effect was small. Overall, the results indicate that the bio-oil separation system, in terms of its ability to recover distinctive stage fractions, is robust to changes in pyrolysis temperature.

8.5 Future Work

Many challenges (related to my research work) still remain with bio-oil before it finds commercial applications and production .

1. Lack of standardized test methodology causes many issues. Identical test methods must be used for bio-oil analyses throughout the world. For example, bio-oil lacks stability which can create quality issues for producers, retailers, and consumers. Without standard test methods, results will vary which will lead to inconsistencies and poor quality bio-oil. Unfortunately, this ultimately limits wider use acceptance. Additional existing standardized test methods should be explored to determine if they can be used effectively for bio-oil characterization.
2. Lack of easy and fast testing methods causes difficulties. Bio-oil testing protocol needs to be faster and easier. Analytical instrumentation and personnel to operate the instrumentation comes at a high cost. Testing that can be accomplished using less expensive instrumentation and operated by personnel that require minimum training is essential. These types of methods

can be used during production for quality control. This is extremely important because bio-oil properties can vary significantly. Further development/identification of fast easy test methodology is vital for bio-oil commercialization.

3. Removal of specific families/chemicals from bio-oil is critical: allowing for higher value end-products thus making economic feasibility more likely. The fractionated condenser system has progressed us toward this end goal. There are a variety of features requiring investigation, such as cleaning the sugar rich solution during production, isolation of phenol, furfural, levoglucosan, etc. It is important to look at other cost effective separation techniques.
4. It is essential to change the traditional objective of maximized bio-oil yields. We need to emphasize quality versus quantity. Process requirements for end-use products resulting in the desired bio-oil properties is extremely important for fast pyrolysis commercialization.

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