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Quantification of total phenols in bio-oil using the Folin–Ciocalteu method

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ABSTRACT

Bio-oil from fast pyrolysis of biomass contains phenolics derived from the lignin portion of the biomass. Traditional testing for total phenolics 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 interferents 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 interferents 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 ≤5.8%. Total phenolic content in bio-oil detected by the FC method was comparable to the quantity of total phenolics 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 ±1.1% at the 95% confidence level.

1. 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 phenolics 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 [1–3]. Little deoxygenation occurs during fast pyrolysis, producing bio-oil with an elemental composition closely resembling the original biomass [3,4]. Bio-oil is considered a possible alternative to petroleum as a source of liquid fuels [4] and chemicals [5].

Bio-oil is a complex mixture of water (15–30%), ketones, acids, aldehydes, sugars, phenolics and other oligomeric lignin derivatives. Approximately 35–50% of bio-oil is comprised of constituents that are nonvolatile [1,6]. Softwoods have the highest lignin content (25–35%), mainly the guaiacyl type, while hardwoods contain from 16 to 25% lignin comprised of the guaiacyl-syringyl type [6,7]. Bio-oil characteristics, which include extreme complexity, instability, heterogeneity, and low pH [8], 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 [9]. The high content of oxygenated compounds in bio-oil makes it a potential source for these organic compounds [1], either from whole bio-oil or major fractions of bio-oil [6]. One

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important product from the lignin-derived fraction of bio-oil is phenolic replacement in phenol-formaldehyde resins [6], which is utilized as a raw material for laminate industries and specialty chemical manufacturing [10].

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 [10], 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% [11]. Many phenolics 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) [9], which is adequately high enough that they cannot be analyzed by GC.

The WIF of bio-oil is often referred to as "pyrolytic lignin" [12] 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 [13]. 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 22–28% of the crude bio-oil [14]. Literature states the method for the determination of pyrolytic lignin requires improvement for better reliability [11,15]. This statement indicates that estimation of total phenolics by weight of the WIF is not reliable.

The wine industry utilizes the Folin–Ciocalteu (FC) colorimetry method to determine total phenolics 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 phenolics [16]. Slinkard and Singleton [17] 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 [18] could not recommend a single optimal protocol for the quantification of total phenols and condensed tannins (i.e. polyphenolics) 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 [18]. Derkyi [19] reported that different types of polyphenols react similarly with the FC reagent, making them more easily quantifiable. Chapuis-Lardy et al. [20] utilized the FC method to determine the water-soluble phenolics 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 phenolics. 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 phenolics was only about 10% of the water-soluble phenolic fraction estimated with the FC reagent [20].

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 [16]. 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 $(PMoW_{11}O_{40})^{4-}$. In principle, the addition of an electron to a nonbonding orbital reduces nominal MoO^{4+} units to isostructural MoO^{3+} blue species [21]. 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 [17]. 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 [16] explains that sugars create a complex issue because different sugars yield different interferences when using the FC method for total phenolics determinations in wine. Levoglucosan is the main sugar reported in literature at 3–6 wt% [22] while other sugars reported at low concentrations include xylose, arabinose, fucose, galactose, mannose, fructose, and ribose [11,22]. The FC reagent is commercially available but can be prepared in the laboratory [16].

Liquid-liquid extraction is time consuming, tedious, and can involve the use of many different hazardous solvents. Basing total phenolics on the WIF content of bio-oil is merely a rough estimation. A standardized test method that can be used to quantify total phenolics 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.

2. Materials and 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 450–500 °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. [3]. Stage fraction 1 was designed to capture levoglucosan and phenolic oligomers with high dew 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 fraction 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 SF2, as well. Stage fraction 3 was designed to capture compounds with dew 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 fraction 4, an insulated ESP, utilizes an operating temperature of about 77 °C. Larger molecular weight oligomers that escape SF2 are also collected in SF4. Stage fraction 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 [3]. 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 SF2 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 1307 × g force for 30 min. The water-soluble portion was decanted leaving behind the WIF.