ANALYSIS OF NON-CARBOHYDRATE BASED LOW-MOLECULAR WEIGHT ORGANIC COMPOUNDS DISSOLVED DURING HOT-WATER EXTRACTION OF SUGAR MAPLE

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The present paper is part of an ongoing study undertaken to evaluate the effect of hot-water extraction on sugar maple (*Acer saccharum*), for extending the scope of ESF biorefinery. The final objective is to assess the contribution of hot-water extracted non-carbohydrate-based organic compounds to generate a stream of platform chemicals. The hot-water extracts (HWEs) were subjected to ultrafiltration to remove the insoluble solids and the clear permeate was extracted with organic solvents, derivatized and analyzed by GC/MS. The organic extracts of the HWEs were composed mainly of phenolics. These compounds were compared to the extractives obtained from native sugar maple wood. Our results indicate that the aromatic compounds present in the organic extracts of HWE may be extractive-based or products of lignin acidolysis generated primarily by cleavage of the α-O-4 linkages.

Keywords: sugar maple, hot-water extraction, GC/MS, biorefinery, extractives, lignin degradation products

INTRODUCTION

With increasing fuel prices and depletion of fossil fuel, the demands for alternative fuel and energy sources are growing. The use of biomass to produce fuel and energy is therefore attracting attention. Lignocellulosic biomass is probably the most abundant naturally occurring biomass.[1] Tapping into such biomass to generate fuel and value-added products is the basis of a biorefinery, more specifically, of the so-called "lignocellulosic feedstock biorefinery".[2] The trees that can be used as a renewable and sustainable source to generate energy-producing chemicals, bioplastics and value-added chemicals are an integral part of such a biorefinery. The East coast of the United States, including the state of New York, where ESF is located, is abundant in sugar maple *Acer saccharum.*[3] Therefore, it is important to explore if this locally abundant species, known for its use in food products and as lumber for furniture, can be also used to generate useful value-added products. Based on these principles, a biorefinery scheme has been devised at ESF, with support from the US Department of Energy. The first step in the ESF biorefinery begins with hot-water extraction of hardwood chips, performed under mild acidic conditions, due to *in situ* deacetylation of xylans (pH at the end of extraction is ~3.5).[4] The hemicellulosic sugars removed in this step are fermented to produce biofuels (ethanol, butanol) or bioplastics (polylactic acid, PLA).[5,6] The chips can be subsequently used for pulping to make paper, for the production of reconstituted wood products or for the generation of combined heat and power.[6] Along with sugars, hot-water extraction results in the removal of other organic

compounds from wood, for example lignin/lignin degradation products and extractive-based organic compounds.[5,6] GC/MS analysis was performed on the extracts obtained from both native wood and HWE as they were (*i.e.* the derivatization and analysis were carried out on the extracts as a whole, without purification or separation of compounds from the mixture) to give an insight into the nature and abundance of the compounds present.

Extractives are low-molecular weight (LMW) organic compounds present as non-structural components of plants/trees, with different chemical composition and, possibly, of considerable taxonomic value. Different classes of organic compounds constitute the extractives, for example fatty acids, monolignols, polyphenols, flavonoids, sterols and terpenoids. Free phenolics, such as vanillin, syringaldehyde, coniferyl alcohol, coniferaldehyde and scopoletin; fatty acids, such as palmitic acid, linolenic acid, and sterols, such as stigmasterol and β-sitosterol, are some of the extractives known to be present in sugar maple syrup and sapwood.[7,8] The importance of vanillin as a component in the food and flavor industry, and of syringaldehyde as a component in dyes and as a pharmaceutical precursor has been well documented.[9] The nature of the LMW organic compounds detected in the present study after hot-water extraction and extraction of native wood will be used to determine feasible means to sequester the extracts and to provide streams of compounds that may be used as value-added chemicals. This is an effort to enhance and broaden the scope of the ESF biorefinery, and also to understand the extent of the structural changes taking place in the wood matrix during extraction.

EXPERIMENTAL

Materials and method

The reagent grade (A.C.S) ether was purchased from Mallinckrodt chemicals. Spectrophotometric grade chloroform (99.8%, A.C.S.) and *N,O*-bis(trimethylsilyl)trifluoroacetamide + trimethylchlorosilane (BSTFA + TMCS) were purchased from Sigma-Aldrich. Anhydrous sodium sulfate was purchased through EMD Chemicals and HPLC grade methylene chloride was purchased from Pharmco Chemicals. Sugar maple wood, obtained from forest properties of ESF, was debarked and chipped. The sugar maple woodchips were extracted with hot-water (4:1 water-to-solid ratio) at 160 °C for 120 min, in an M-K digester system. The HWE collected was allowed to sit for 48 h at <8 °C, before it was passed through an ultrafiltration membrane under N_2, in a solvent-resistant stirred cell (Millipore UF stirred cell, Cat. No. XFUF 076 01). The membrane was a Millipore, 1000 Da nominal molecular weight limit, regenerated cellulose acetate membrane. The precipitate (lignin/lignin degradation products of higher molecular weight, LHMW) was analyzed for the lignin content (Klason and acid-soluble lignin). The clear permeate was then extracted with chloroform and ether. The organic extracts were dried using anhydrous sodium sulfate, filtered, and the solvent was evaporated under vacuum. The residue was dissolved in a minimum volume of methylene chloride and silylated using BSTFA, in the presence of pyridine. Maximum care was taken to ensure that silylation was performed under "dry" conditions. The silylated extracts were analyzed by GC/MS, using a Thermo PolarisQ GC/MS Ion Trap Mass Spectrometer and a TR5 column. The oven-temperature was held at 60 °C for 2 min, raised to 300 °C at a rate of 8 °C/min and maintained at a final temperature (300 °C) for 30 min. Identification of the individual peaks was performed using ESF's mass spectral library and published data. Henceforth, the organic extracts of the HWE sugar maple will be referred to as OHWE. Extraction of native wood was performed with chloroform, acetone: water (9:1), and 95% ethanol, the common organic solvents for wood extraction. Sugar maple woodchips were milled (30 mesh) and extracted individually with the solvents (1:5 wood-to-solvent ratio), in an ultrasonication bath (Bransonic® - Model 3510) 3 times. All three extracts of each solvent extraction were mixed (to give a total of 3 extracted samples of chloroform, acetone:water, and ethanol), dried using anhydrous sodium sulfate, filtered, evaporated, then the residue was silylated and analyzed by the same method mentioned for OHWE.

RESULTS AND DISCUSSION

Ultrafiltration of HWE resulted in a solid retentate in an amount of ~1.8% of OD wood, composed mainly of lignin (Klason + acid-soluble lignin accounted for ~75% of the OD retentate).[10] The chloroform

extractions of the resulting permeate yielded OHWE in an amount of ~1.5% of OD wood, whereas extraction with ether was less effective, of ~0.7% of OD wood. Vanillin and syringaldehyde were the major compounds in both OHWEs (both refer to chloroform and ether OHWE), based on their relative abundance in the respective GCs. Shown in Table 1 are the aromatic compounds detected in the extracts of native wood and in the OHWEs, whereas Table 2 lists the non-aromatic compounds identified in the same material. Likewise, Figures 1 and 2 present the structures of the compounds listed in Tables 1 and 2, respectively. The major peaks in the chloroform extracts of native wood were fatty acids, with trace amounts of aromatics. A list of non-aromatic compounds extracted with chloroform from native sugar maple is presented in Table 2. Common sugar dehydration products, such as furfural and furfural derivatives (not reported here), were observed in OHWEs, along with several sugars.

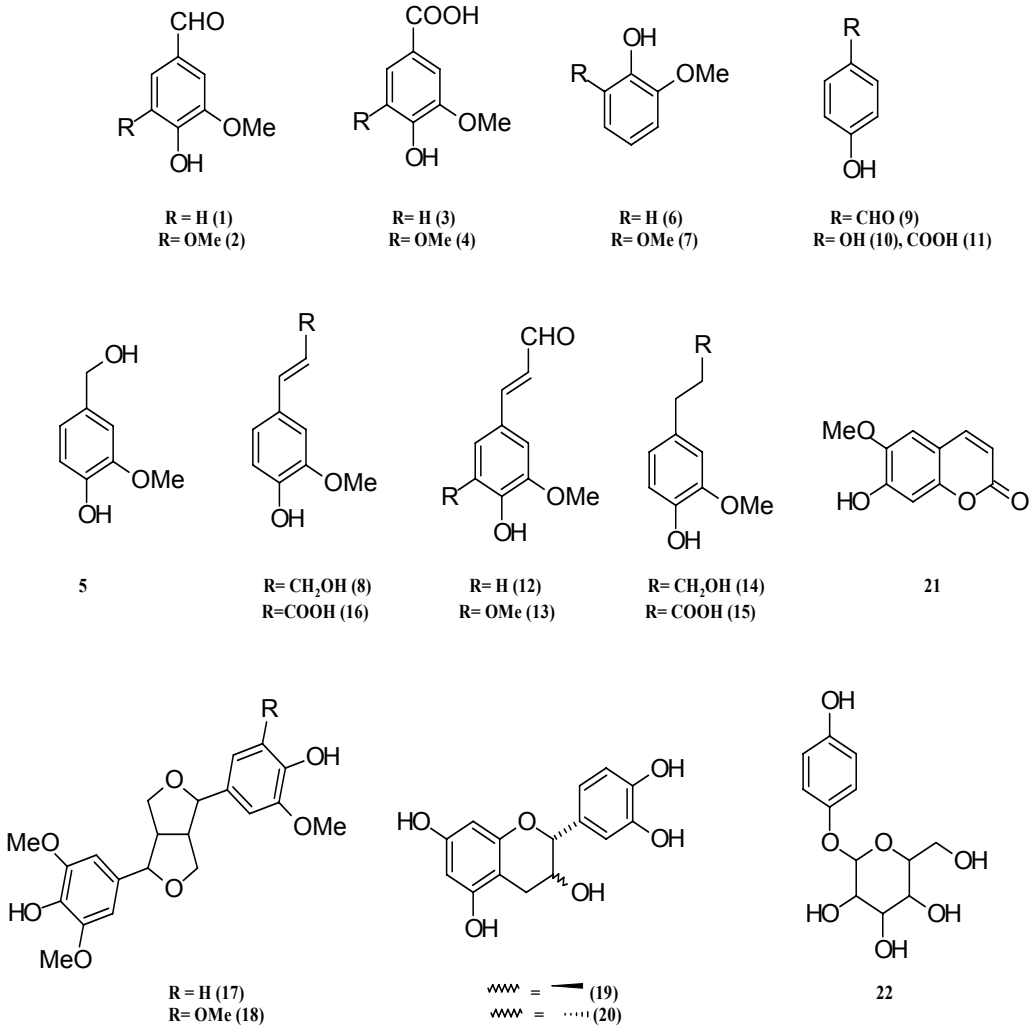

Figure 1: Structures of aromatic compounds reported in Table 1

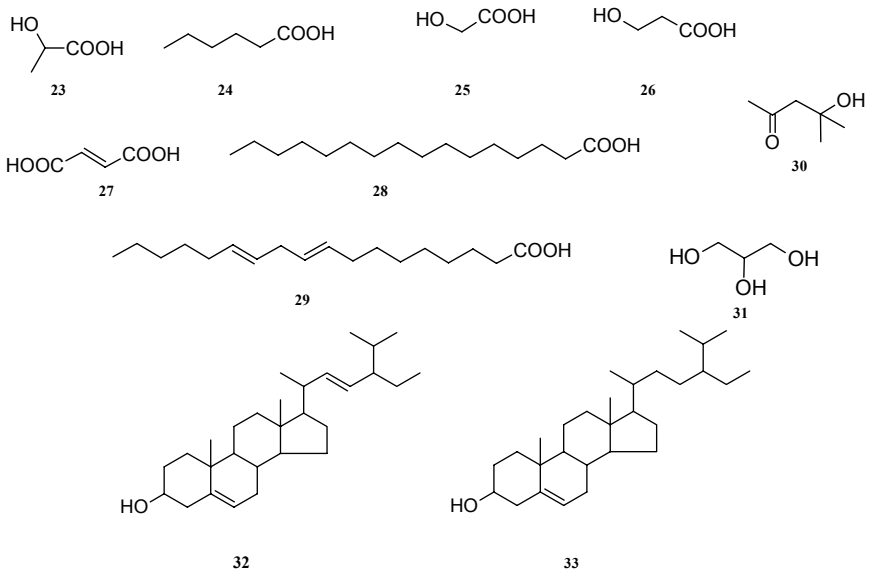

Figure 2: Structures of non-aromatic compounds reported in Table 2

Table 1
Organic compounds detected as extractives in hot-water extracts of sugar maple

Aromatic compounds	Extractives		HWE of sugar maple		MW*
	Ac_2O:H_2O	EtOH	$CHCl_3$	Ether	
Simple Phenolics					
Vanillin (1)	+	+	+++	+++	224
Syringaldehyde (2)	-	-	+++	+++	254
Vanillic acid (3)	+	-	+	++	312
Syringic acid (4)	-	-	++	++	342
Vanillic alcohol (5)	+	+	-	-	298
Guaiacol (6)	+	-	+	+	196
Syringol (7)	-	+	+	+	226
Coniferyl alcohol (8)	++	++	-	-	324
p-hydroxybenzaldehyde (9)	-	-	-	+	194
p-hydroquinone (10)	-	-	-	+	254
p-hydroxybenzoic acid (11)	-	-	-	++	282
Coniferaldehyde (12)	-	-	++	++	250
Sinapaldehyde (13)	-	-	++	+	280
Dihydroconiferyl alcohol (14)	-	-	-	+	326
Dihydroferulic acid (15)	-	-	+	-	340
Ferulic acid (16)	-	-	-	+	338
Lignans					
Medioresinol (17)	-	-	-	+	532
Syringaresinol (18)	-	-	++	++	562
Flavonoids					
Catechin (19)	+++	++	-	-	650
Epicatechin (20)	++	++	-	-	650
Coumarins					
Scopoletin (21)	++	++	-	-	264
Phenolic glycosides					
Arbutin (22)	+	+	-	-	633

+++ 50-100% relative abundance; ++ 5-50% relative abundance; + 0-5% relative abundance/trace
*TMS derivatives wherever applicable

Phenolics and phenolic glycosides

Free phenolics such as **1**, **2**, **3**, **4**, **6**, **7**, **12** and **13**, observed in the OHWEs, may be either extractives or products of lignin acidolysis.[11] Vanillin (**1**) and guaiacol (**6**) were the only phenolics detected in native wood and in both OHWEs. Coniferyl alcohol (**8**) in native wood was not surprising, as it is one of the three cinnamyl alcohols from which lignin biosynthesis proceeds, although it was absent in the OHWEs. Also, there was observed *p*-hydroquinone (**10**) in trace amounts in the ether OHWE, which may be an acid hydrolysis product of *p*-hydroquinone glycoside (arbutin), identified earlier in *Pyrus* species and birch leaves.[12,13] This agrees with the presence of arbutin (**22**) detected in this study in the native sugar maple wood extracts (characteristic *m/z* – 361, 254, 217, 147, 73).[14] Peaks displaying a fragmentation pattern similar to that of BSTFA derivatized sugars in close proximity of arbutin in the GC/MS of native wood suggest that other phenolic glycosides or other glycosides may be present in the extractives of sugar maple.[15] The dihydroconiferyl alcohol (**14**) detected in the ether OHWE is most likely a result of lignin acidolysis, as this compound was not identified in native sugar maple organic extracts. It has been previously discussed as an unusual structure incorporated in lignin.[16]

Table 2
Non-aromatic compounds detected as extractives and in HWE of sugar maple

Non-aromatic compounds	Extractives			HWE of sugar maple		MW*
	$CHCl_3$	Ac_2O:H_2O	EtOH	$CHCl_3$	Ether	
Carboxylic acids						
Lactic acid (23)	-	+	+	-	+	234
Hexanoic acid (24)	+	+	+	-	+	188
2-hydroxyacetic acid (25)	-	-	-	-	+	220
3-hydroxypropanoic acid (26)	-	-	-	-	+	234
Fumaric acid (27)	-	-	-	-	+	260
Palmitic acid (28)	+++	++	++	-	-	328
9,12-octadecadienoic acid (29)	+++	++	+++	-	-	325
Alcohols and ketones						
4-hydroxy-4-methyl-2-pentanone (30)	+	+++	-	-	-	188
Glycerol (31)	-	++	++	-	-	308
Sterols						
Stigmasterol (32)	-	+	++	-	-	484
β-sitosterol (33)	-	+	++	-	-	486

+++ 50-100% relative abundance; ++ 5-50% relative abundance; + 0-5% relative abundance/trace
*TMS derivatives wherever applicable

Lignans, flavonoids and coumarins

It is interesting to note that, along with lignan syringaresinol (**18**), which has been shown to be present in hardwoods,[17] medioresinol (**17**) was also identified among the LMW organic compounds in HWE. Medioresinol (characteristic *m/z* – 532, 253, 223, 209, 73) was observed in the ether extract of HWE, whereas syringaresinol (characteristic *m/z* – 562, 253, 239, 223, 209, 73) was observed in both OHWEs. The fragmentation pattern is comparable to that identified in pomegranate extracts.[18] To the best of our knowledge, no previous citation of medioresinol as an extractive in sugar maple had ever been mentioned. Flavonoids, such as catechin (**19**) and epicatechin (**20**), along with coumarin and scopoletin (**21**), were detected only in the organic extracts of native wood.

Sterols and other non-aromatics

The GC/MS also indicates the presence of stigmasterol (**32**) and of β-sitosterol (**33**) in

native wood, with fragmentation patterns similar to those shown in literature.[19] The presence of β-sitosterol in the sugar maple sticker stain was reported previously by Miller.[8] Stigmasterol and β-sitosterol were absent in both OHWEs. The non-aromatic carboxylic acids shown in Table 2 – **23**, **25** and **26** – could be either carbohydrate or lignin degradation products,[20] whereas the fatty acids **28** and **29** are not unusual in hardwoods. Lactic acid (**23**), reported to be naturally accumulating in plants under anaerobic conditions,[21] is considered a building block chemical for biorefineries.[22]

Most of the compounds present in both OHWEs of sugar maple and native wood were aromatic in nature. Common extractives, such as sinapaldehyde and syringaldehyde,[23] were observed in the OHWEs, but they were absent in the organic extracts of native sugar maple. Conversely, vanillin was observed in the organic extracts of native sugar maple and even in higher amounts in both OHWEs. These results indicate that some of the organic compounds in the OHWEs may be lignin degradation products rather than extractives. At elevated temperature and pressure in a mildly acidic aqueous medium, lignin may undergo acidolysis by hydrolysis of the ether bonds.[11] Aromatic compounds, **2**, **4**, **11**, **12**, **13**, **14**, **15**, **16**, **17** and **18** were detected in the OHWEs, but not in native wood. These compounds may be attributed to the cleavage of the α-O-4 ether linkages in lignin, in a mild acidic environment of hot-water extraction, since characteristic lignin products of acidolysis proceeding *via* the β-O-4 bond cleavage, such as Hibbert ketones, were absent in both OHWEs.[24] Medioresinol (**17**) and syringaresinol (**18**) are most likely lignin-derived, since they were absent in the native wood extracts. Evidence of syringaresinol formation from birch lignin during acidolysis has been documented by Lundquist;[25] however, the presence of medioresinol has not been reported until now. The cinnamyl aldehyde structures (**12**, **13**) identified in both OHWEs may be products of the β-O-4 bond cleavage in lignin, as a certain amount of hydroxycinnamyl aldehydes is incorporated in lignin *via* the β-O-4 linkage.[26] In addition, the units with reduced side chains, such as dihydroconiferyl alcohol (**14**) and dihydroferulic acid (**15**), could be also products of the β-O-4 bond cleavage.[23] Therefore, based on such results, cleavage of β-O-4 linkage during hot-water extraction cannot be ruled out.

So far, not all peaks observed by GC-MS could be identified. Since the extracts were silylated as they were, without any separation or purification step, there is a cluster of peaks in the resulting GC/MS of all samples. Such close clusters could be and have been observed to cause overlaps, which could render some inaccuracy. To ascertain such data, separation and purification of the compounds present in the extracts, followed by identification and confirmation with standards of all peaks, is currently underway. The profile of compounds generated will indicate the nature of the major compounds formed and/or released during extraction, which can be consequently used in devising a strategy to separate individual or bulk streams of value-added platform chemicals from sugar maple extracts, to enhance the efficiency of biorefinery. Consequently, it would also reveal information on whether organosolv extraction of wood chips could be a viable option, along with hot-water extraction prior to pulping. Critical to the success of these pre-treatments is the repeatability and consistency of the profile of compounds extracted from sugar maple. Therefore, a series of extractions are concomitantly performed, to determine the consistency of the profile of the extracted compounds.

CONCLUSIONS

During hot-water extraction of sugar maple, two fractions of non-carbohydrate based compounds were generated; an insoluble one, which is filtered off and contains lignin as a major component, another fraction, composed of dissolved LMW organic compounds, extracted from HWE, with organic solvents. Understanding the composition of these LMW compounds is expected to provide an insight into the effect of HWE on sugar maple wood. As potential value-added chemicals, these compounds are expected to enlarge the

palette of the ESF biorefinery products. The comparative study of LMW compounds present in the HWE of sugar maple and of native sugar maple indicates that the compounds dissolved during HWE are of both extractive and lignin origin. Vanillin and syringaldehyde were established as the major compounds present in native sugar maple, as well as in the HWE of sugar maple. Other aromatic compounds were also detected in native sugar maple and in OHWEs, albeit at a much lower scale. The phenolic glucoside arbutin, detected in native sugar maple extracts, and *p*-hydroquinone, detected in ether OHWE, indicate cleavage of the glycosidic bond during HWE. Based on the abundance of vanillin and syringaldehyde among the OHWEs, and also on the possibility of producing them by oxidation of the insoluble lignin-rich fraction of the HWE precipitate, we suggest that the most important non-carbohydrate-based value-added products of the ESF biorefinery could be vanillin and syringaldehyde.

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