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Summative and Ultimate Analysis of Live Leaves from Southern U.S. Forest Plants for Use in Fire Modeling

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ABSTRACT: Laboratory-derived composition and pyrolysis data are essential inputs for the modeling of fire behavior. Recently, fire research has focused on live fuels including living wood and leaves, which exhibit sharp differences in moisture levels and chemical composition as compared to dead fuels. These leaf components have fuel properties that function in the spread of wildfire and must be considered in order to produce nuanced predictive models that reflect real life conditions. The goal of this study was to assemble a suite of methods that would achieve summative mass closure for analysis of live leaves from 12 tree and plant species from the southeastern United States (consisting of broadleaves, conifers, grasses, and palmettos). Most of the procedures used were adapted from standard methods commonly used for biomass analysis at the Forest Products Laboratory (FPL), National Renewable Energy Lab (NREL), and others. A mass closure (aka mass balance) of 95 to 100% was achieved for 10 of the 12 species the other 2 were both 91%. This required measuring of 12 parameters which are lipids, nonstructural sugars, protein, pectin, hemicellulose, cellulose, starch, phenol, structural lignin, silicates, and minerals. When burned, these components span a wide range of pyrolysis temperatures from 70 to 600 °C, posing a challenge for pyrolysis measurements. Observable differences in leaf composition were noted within groups of plant types as well as between them—with grasses being most similar and palmettos being most dissimilar. A rigorous statistical analysis was out of the scope of this study (involving analysis of 12 matrices of 12 plants); instead, validated standard analytical protocols (with known % error) were used in most cases. These error percentages (and sources) are reported with data presented in this study. The overriding goal of reaching summative mass closure (for all plant species) was however achieved, serving as a validation of the reported methodology for use in pyrolysis modeling. To predict the ultimate analysis data for leaf elemental composition and the heat of combustion, the empirical formula for each components was identified to provide for summation over all components. A formula for heat of combustion based on the oxygen consumption principle was found to be adequate to within 4% error.

INTRODUCTION

Prescribed burning is a wildland vegetation management technique used to accomplish many objectives, including the reduction of the potential of uncontrolled wildland fire in areas of dense vegetation as well as site preparation for reforestation, habitat restoration and management for threatened and endangered species dependent upon fire, and mimicking the natural role of fire in North American ecosystems.[1] To predict fire behavior, some models rely on pyrolysis data based on measured thermal degradation properties under laboratory conditions. Pyrolysis is the first step in the initiation of combustion and has been widely studied on dried biomass and wood but not on live leaves, which have been shown to burn differently.[2] The early pyrolysis work typically determined characteristics for live fuel samples which were dried and then ground to be analyzed thus eliminating the effects of water content and shape. Live leaves contain more water, lipids, nonstructural carbohydrates, protein and higher levels of mineral components than dried wood.[3] Furthermore, models such as Gpyro, Gpyro-Fire Dynamics Simulator (FDS), and the FDS (stand alone) consider components of cellulose, hemicellulose, and lignin (on dead wood) as the major predictive variables from which fire behavior is based.[4−6]Less is known about pyrolysis properties of intact live leaves, which are the primary fuel in the spread of fire in the canopy of forest and shrub lands.[7] As part of a large study examining and modeling pyrolysis of intact fuels in order to develop improved models of prescribed fire behavior,[8] we performed a series of measurements to derive many fundamental properties of vegetation burned in prescribed fires in the southern U.S. In the U.S. approximately 3.6 million hectares are burned for forestry purposes annually; 2.5 million hectares are burned in the southern U.S. alone.[9] This manuscript describes the methods and results of these analytical measurements.

Background. Pyrolysis lab instrumentation such as a thermogravimetric analyzer (TGA) measure thermal degradation of a fuel over time at a programmed rate of heat increase in inert or air flows. The differential scanning calorimeter (DSC) measures heat capacity and pyrolysis of a material. These instruments are designed to measure physical properties

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related to heat transfer, combustion, and conversion of mass. For these data to be meaningful, a suite of lab analytical methods is needed that can characterize close to 100% of the dry fuel mass. In laboratory terminology this is referred to as "summative analysis" or "summative mass closure". One such suite of methods has been published by the National Renewable Energy Laboratory (NREL) in lab analysis procedures (LAP) for use in biomass analysis.[10−12] NREL methods are similar to National Institute of Standards and Testing (NIST) methods with adaptation to biomass feedstocks and serve as a measure of feedstock quality. NIST published the results of an inter laboratory study of 11 laboratories including the Forest Products Laboratory (FPL) which demonstrated successful summative mass closure on standard biomass feedstocks (using NREL LAP methods).[10] This study was used to recharacterize four NIST reference feedstocks (sugar cane bagasse (SB) RM8491, eastern cottonwood (EC), RM8492, Monterey pine RM8493 (MP), and wheat straw RM8494 (WS) (for use as a reference standard for analytical laboratories). Ten major components were studied: ethanol extractives, water extractives, whole ash, structural sugars (glucan, xylan, mannan, and arabinan), lignin (klason and acid soluble lignin), and glucuronic acid. Total mass closures and standard deviations of recharacterized values were as follows: SB, 102% ± 2; EC, 99% ± 2; MP, 100% ± 1; and WS, 100.9% ± 5. It was noted that previously characterized values of the same standard feedstocks were as follows: SB, 94% ± 6; EC, 91% ± 7; MP, 94% ± 5; and WS, 96% ± 5. NREL further studied method uncertainties on two feedstocks (bagasse NIST standard control and a corn stover unknown sample) and reported both data with (uncensored) and without outliers (censored) for the corn stover unknown. From this NREL was able to measure the effect of uncertainties due to instrumental and equipment error by using statistical analysis from 154 replicate samples in 13 batches, by seven analysts in two laboratories. They reported standard deviations (SD) for the main components (lignin, extractives, xylan, and glucan) at 1−3% and minor components (i.e., protein, whole ash, and acetyls) at 4−10% for the censured and noncensured data. (Recoveries ranging from 95% to 105% were considered to be the target for mass closure.) They concluded that uncertainties were due to the analytical method being used rather than chemical differences in the feedstocks. Another conclusion reached was that summative mass closure of close to 100% (95−105%) was an indication that most of the components were accounted for, and little (if any) redundant analysis was occurring (which will be referred to as "interferences").[13]

The NREL LAPs provide a comprehensive resource of available standardized methods for biomass analysis. These methods provided the framework for much of our analysis. This study however targeted live leaves for pyrolysis testing. It was expected that components such as extractives, nonstructural carbohydrates (NSC), phenols, protein, and lignin components in live leaves would differ considerably from those in dried biomass materials. Furthermore, it was unknown to what extent interferences would occur using standard methods validated for biomass and wood samples. One example of interferences occur in the measuring of acid insoluble (Klason) lignin (AIL) in leaves. Hatfield and Fukushima[14] have suggested the difficulties of accurately measuring lignin in forage plants due to high levels of precipitated protein. Klason lignin is also known to contain phenolics in leaves (phenylpropanoids).[15] Similarly, NSC(s), pectin, and starch levels are indistinguishable from total sugars measured to calculate cellulose/hemicellulose in biomass feedstocks. These distinctions are important when determining mass balances for pyrolysis studies which focus on predicting ignition, fire growth, and fire emissions on fuels.

Halls, Knox, and Lazar[16] reported compositional values for forage plants. This was an early report of mass closure for the purposes in evaluating seasonal contribution to cattle diet. It measured crude protein, lignin, ash, cellulose, ether extractives, and so-called "other carbohydrates"—which were determined by difference of percentages of measured components. They used some of the same test plants selected for this study (i.e., inkberry, saw palmetto, and wild oak). Other papers reviewed also reported compositional data where the mass closure of unknown components was reported by difference[3,16−18]

Recent work has shed light on plant composition vs pyrolysis characteristics. In a review paper, Debiagi and others[17] have proposed components of lignin, lipids, and tannins (polyphenols) as model compounds considered to calculate biomass pyrolysis kinetics during heating. Jolly and others[19] considered the dry leaf mass components (in measuring seasonal moisture of neutral detergent fiber carbohydrates (NDF), nonfiber carbohydrates (NFC), crude fat (lipids) (CF), crude protein (CP) and ash content (AC), and total nonstructural carbohydrates (NSC) in seasonal moisture fluctuations in leaves). The formula: NFC = 100 − NDF + CF + CP + AC (AOAC 1984) is used to calculate an unknown component of "non-fiber carbohydrates". Jolly and others[19] also measured crude fat (CF) (method AOCS Aa) 4−38) to determine extractive fraction. The notion of leaf extractives as being largely lipid/fat based has been suggested in the literature elsewhere.[15,16] Lipids are considered a "primary plant metabolite" and would be expected to be abundant in leaf cells.[20] Further literature reviews emphasized the importance of cell disruption in the extraction of lipids from algae.[21,22] This led us away from use of the Soxhlet extraction method (AOCS NREL) to borrowing the biochemical extraction method of rupturing (lysing) cells with a bead mill (often used to extract cellular DNA and protein).[23]

Because unknowns cannot be pyrolysis tested, evaluated, or modeled, the goal of this study was to develop a protocol for the compositional analysis of live leaves capable of attaining close to 100% mass closure (95% to 105%) mass balance. A chemical composition has not been done previous to this paper on live leaves to obtain such a summative mass balance for a pyrolysis study. Furthermore, chemical composition has been restricted to cellulose, hemicellulose and lignin when used to model wood fuels and feedstocks. Since live leaves and litter are the fuels that accelerate forest fire canopy spread it is important to study the pyrolysis behavior of all components that can be measured.

Plant Primary and Secondary Metabolites. Plant components can be categorized as primary or secondary metabolites.[20] Primary metabolites are synthesized by plants and consist of sugars, amino acids, and nucleotides which are essential to formation of the polymeric (structural) plant components. They generally have a simpler metabolic pathway than secondary metabolites. Examples of primary metabolites include the following: carbohydrates, lipids, lignin, and amino acids. Secondary metabolites are synthesized in specialized plant cells during specific stages of plant formation and do not aid in growth or development of plant. They are harder to

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extract and purify than primary metabolites and are present in much lower concentrations in plants.[24] There were reported seasonal fluctuations in total phenolics in apple leaves which added up to less than 1% of DM at their peak.[25] Secondary metabolites can be classified in three major groups:[20] (1) Terpenoids (consisting mostly of carbon and hydrogen), (2) phenolics (made from simple sugars, consist of benzene rings, hydrogen and oxygen, and (3) nitrogen containing compounds. Secondary metabolites are found in natural products such as essential oils, sweeteners, and flavoring. They also function as protective agents against pests in the plant. Minerals, though not a plant metabolite serve vital function in plant metabolism and are important pyrolysis components that need to be considered from a mass balance standpoint.[20]

Leaf Composition and Model Compounds for Pyrolysis Modeling. On the basis of standard methods available and literature reviewed, the following measurable components (of mostly primary metabolites) were targeted to obtain the full summative mass closure in live leaves: lipophillic extractives (lipids), nonstructural carbohydrates (glucose, fructose, and starch), pectin, structural carbohydrates (cellulose, hemicellulose), protein, lignin, phenols, silicates, and minerals. Essential to pyrolysis kinetics modeling (i.e., in ref 17 for biomass) is the identification of model compounds and empirical formulas that correlate to the ultimate analysis data (CHON composition) of the fresh leaves. Once this is determined heats of combustion can be calculated. Analysis of live leaves presents an additional challenge in that their lipids, protein, phenols, and nonstructural carbohydrates can change via enzymatic reactions into simpler compounds shortly after sampling.[26−30] The empirical formulas of the above listed carbohydrates and lignin are well-known, whereas the lipids, protein, and phenols have a range of subcompounds, such that representative compounds of a specific empirical formula would be needed for them to predict the ultimate analysis data of the biomass (organic) material. From this, the individual heats of combustion can be calculated for the components that make up the majority of plant mass. This data can be used to provide inputs for a predictive fire model. It is our hope that mass closure data from live plant leaves will add important "real world" elements to models used in controlled burns.

MATERIALS AND METHODS

Plant Species and Growing Conditions. Twelve live plant species native to the southeastern US were received from a nursery via express shipment to the USDA Forest Products Lab (FPL) in Madison, WI. The plants were transplanted and placed under grow lights at room temperature then set on a 12-h light cycle and watered and fertilized as needed and grown to maturity for leaf sampling. (Figure 1) The list of plants is summarized in Table 1 according to common and scientific name as well as plant type. The description of the live plant seedlings shipped to our laboratory from the nurseries are identical to that in the companion papers.[31,32] Matured leaves were snipped with a scissors to fit in small sealed containers for the various tests. To ensure leaf freshness, and to avoid initiating leaf rancidity, the various tests were begun immediately.

Standard Methods Used. Most of the analytical methods described here were adapted from standard methods and/or protocols published by NREL and NIST as well as the FPL in house methods. In the case of pectin analysis, an HPLC-AEC method was developed based on an FPL house method for uronic acids (this method is described in the Materials and Methods).

Dry Weight and Absorbed Water Weight Determination. Accurate dry weight and water weight measurements were the starting point for mass balance determination. When drying samples for analysis, several requirements were considered: (1) evaporate only water and minimize loss of volatile organic compounds (VOCs), (2) curtail enzymatic activity so as to preserve the samples for storage, and (3) release absorbed water in the leaves for an accurate measurement of dry mass.

Figure 1. Transplanted fetterbush under grow lights at FPL.

Table 1. Common and Scientific Names of Selected Plants Native to the Southeastern U.S. Used in the Pyrolysis Study

no.	common name	scientific name	type
1	saw palmetto	*Serenoa repens*	palmetto
2	dwarf palmetto	*Sabal minor*	palmetto
3	swamp bay	*Persea palustris*	broadleaf
4	yaupon	*Ilex vomitoria*	broadleaf
5	inkberry	*Ilex glabra*	broadleaf
6	wax myrtle	*Morella cerifera*	broadleaf
7	live oak	*Quercus virginiana*	broadleaf
8	fetterbush	*Lyonia lucida*	broadleaf
9	water oak	*Quercus nigra*	broadleaf
10	longleaf pine	*Pinus palustris*	needlelike
11	wiregrass	*Aristida stricta*	grass
12	little bluestem	*Schizachyrium scoparium*	grass

To fulfill the drying requirements a number of standard methods were considered. The NREL Lab analytical procedure NREL/TP-510-42620 suggests three techniques for drying biomass feedstocks. (1) air drying at room temperature (RT) suitable for large samples; (2) convection oven drying at temperatures <45 °C, as suitable for drying small samples where microbial degradation is an issue; (3) lyophilization (freeze-drying), for very wet samples where microbial degradation is an issue.[30] NREL/TP-510-42618 also suggests drying biomass at 40 °C under vacuum for carbohydrate and lignin analysis.[9]

The results of air, microwave, and vacuum drying for preservation and storage of collard greens have been compared by Alibas (2009),[26] and these were used to establish our drying method. The vacuum drying technique was preferred over the others for rapid transfer of moisture due to the vacuum pressure gradient. Vacuum drying at 50 °C performed the best in preserving taste and color and reducing the loss of aromatics for collard green storage.[26] Vacuum drying at low temperature has also proven to be effective for preserving essential oils of oregano.[27] These results agree with those of Rahimmalek et.al.,[28] who found oven drying (at 50 °C) effective in preservation of essential oils when comparing six treatments (second to freeze-drying). Lindroth and Koss[33] investigated preservation of quaking aspen (*Populus tremuloides*) leaves for phytochemical analysis by comparing vacuum drying (at RT) with freeze-drying. They found that freeze-drying caused a small decline in nitrogen and soluble

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protein whereas vacuum drying at room temperatures following flash freezing allowed a 38% decline in starch concentration.

Plant enzymes promote rapid breakdown of components such as starch, sugars, lipids, proteins, and chlorophyll. The breakdown of chlorophyllases takes place at temperatures up to 55 °C.[24] Lipids (lipases) have been reported to be active up to 45 °C in the degradation of the lipid tributrin.[30] Tobacco sugar conversion by flue curing is maximized at 40 °C as a result of promoting enzymatic hydrolysis while the leaf is moist but avoiding the negative oxidation processes, and yet the amylase will deactivate at the higher temperatures.[29] Finally, protein (proteases) enzymes are deactivated at 60 °C.[14] Among the drying methods reviewed, it was clear that the air-dry method was least optimal for maintaining compositional integrity since enzymatic hydrolysis and microbial degradation processes progress rapidly upon harvesting of the live leaves thereby releasing moisture.

Figure 2 illustrates the effects of various drying conditions on fresh yaupon leaves. The conventional drying conditions of air drying for

Figure 2. Illustration of ground vacuum-dried yaupon that was kept in dry storage for 2 months as well as the same samples after room air drying over several days, oven drying overnight at 103 °C, and vacuum drying overnight at 60 °C.

several days and oven drying overnight at 103 °C both resulted in the browning of the leaves. Vacuum drying of yaupon leaves at 60 °C resulted in the leaves remaining green, but very brittle. Meanwhile the live sample that was vacuum-dried at 45 °C and ground also remained green and did so for months in a sample desiccator. This was taken to be an indication that chlorophyll (and interrelated compounds) remained intact during the vacuum drying process at a low enough temperature.

On the basis of literature reports and the experiment reported above, it seemed that the "best" preservation choices were freeze-drying or vacuum oven drying. Given the emphasis on determining an accurate dry weight for summative mass balance, vacuum drying overnight at 45 °C was chosen as the optimum (and simplest) method to preserve samples for long-term storage. Although technically not warm enough to deactivate all enzymes (chlorophyllase, amylase, or protease deactivate at 60 °C), the drying temperature was adequate to remove absorbed water under vacuum and minimize loss of volatile components of interest. It was thought that the rapid vacuum removal of water at 45 °C would also adequately curtail enzyme activity thereby minimizing degradation of starch (a side effect proposed by Lindroth and Koss for room temperature).[33] Since freeze-drying has been shown to cause a decline in nitrogen and protein and dry only to around 7% moisture content,[30] it was thought to have a greater effect on summative mass balance.

Plant Sampling Procedure. The samples to be extracted were either cut with a pair of scissors to fit in the vials (longleaf pine and grasses) or punched with a 1 mm paper punch (most of the broadleaf plants and palmettos). At each sampling two subsamples were taken and preweighed; i.e., both samples were immediately weighed on an analytical balance to obtain their wet weights. The first subsample was placed directly into tared Beadbug (bead mill) vials—600−800 mg on a wet weight (ww) basis for extraction. The extraction scheme described below was performed immediately. The extracted samples were allowed to air-dry, then dried in a vacuum oven at 45 °C, and then weighed for determining the weight of extractives. The second preweighed subsamples (3 g ww) were placed directly into 50 mL tared test tubes then dried in a 45 °C vacuum oven overnight to determine water weight (by weight loss). The dried samples were stored in a desiccator for other analyses. Both the extraction procedure and water weight determination are shown as the top hierarchy branch in Figure 3.

Lipid Selective Leaf Extraction Method. Leaf extractives are rich in lipids that are likely to play a major role in leaf combustion due to their low pyrolysis temperature and significant concentrations in leaves.[35−37] Lipids are fats and fat-like substances that are generally insoluble in water and contain a large number of carbon−hydrogen bonds; they play roles as structural materials and energy reserves.[37] Fuller and Stumph distinguished plant lipids as compounds that are "highly soluble in non-polar solvents and insoluble in water which can be classified as derivatives of fatty acids or other compounds not containing a fatty acid moiety".[36] Phospholipids and triglycerides which are the major plant lipids found in cell walls and chloroplasts are derived from fatty acids.[34,35] Other plant lipids such as sterols and terpenes are derived via the isopentenoid pathway and are derived from mevalonic acid. These lipids belong to the plant secondary metabolite group and are not derived from fatty acids.[20]

A number of parameters were considered in choosing a solvent system/method for leaf extraction for purposes of summative mass balance (SMB): (1) cell disruption—enabling complete solvent contact for extraction of lipid components known to reside in cell walls of leaves.; (2) polarity of solvents—capable of dissolving extractive compounds with a broad spectrum of polarities ranging from water-soluble (phenolics) to volatile oils and volatile organic compounds (VOCs); (3) compatibility with LC/MS lipid analysis protocol used by the contract laboratory (Creative Proteomics-Shirley, NY) and (4) room temperature extraction—which would minimize mass loss of volatile oils and VOCs; (5) use of nonchlorinated solvents which are less toxic.

The standard method for biomass extractives involves a 16 to 24 h Soxhlet extraction with water, ethanol, or acetone.[10,38,39] This method has been used to extract biomass feedstocks and other industrial uses, particularly in the natural products field. Byreddy and others[22] studied cell disruption techniques for maximizing lipid yields from algae for the production of biofuel. They concluded that to extract lipids a combination of polar and nonpolar solvents was required and that 2:1 chloroform: methanol on a volumetric basis (v:v) worked the best which is the basis of the Bligh and Dyer method commonly used in lipid partitions. More recently rapid extraction techniques have been used in areas of systems biology and cell metabolomics where many samples are extracted for liquid chromatography/mass spectroscopy (LC/MS).[40] For these applications halogenated solvents cause interferences with LC/MS. Furthermore, less toxic solvents have been substituted for chlorinated solvents. Mixtures of hexane, alcohols, acetone, and water have been selected as alternatives.[41−43]

For pyrolysis testing and quantitative chemical analysis, it was important to select a method that removed all relevant extractives while not causing interference with subsequent analytical procedures. Additionally, it was suspected that high levels of lipids would be present since many of the plants selected have noticeable cuticles.

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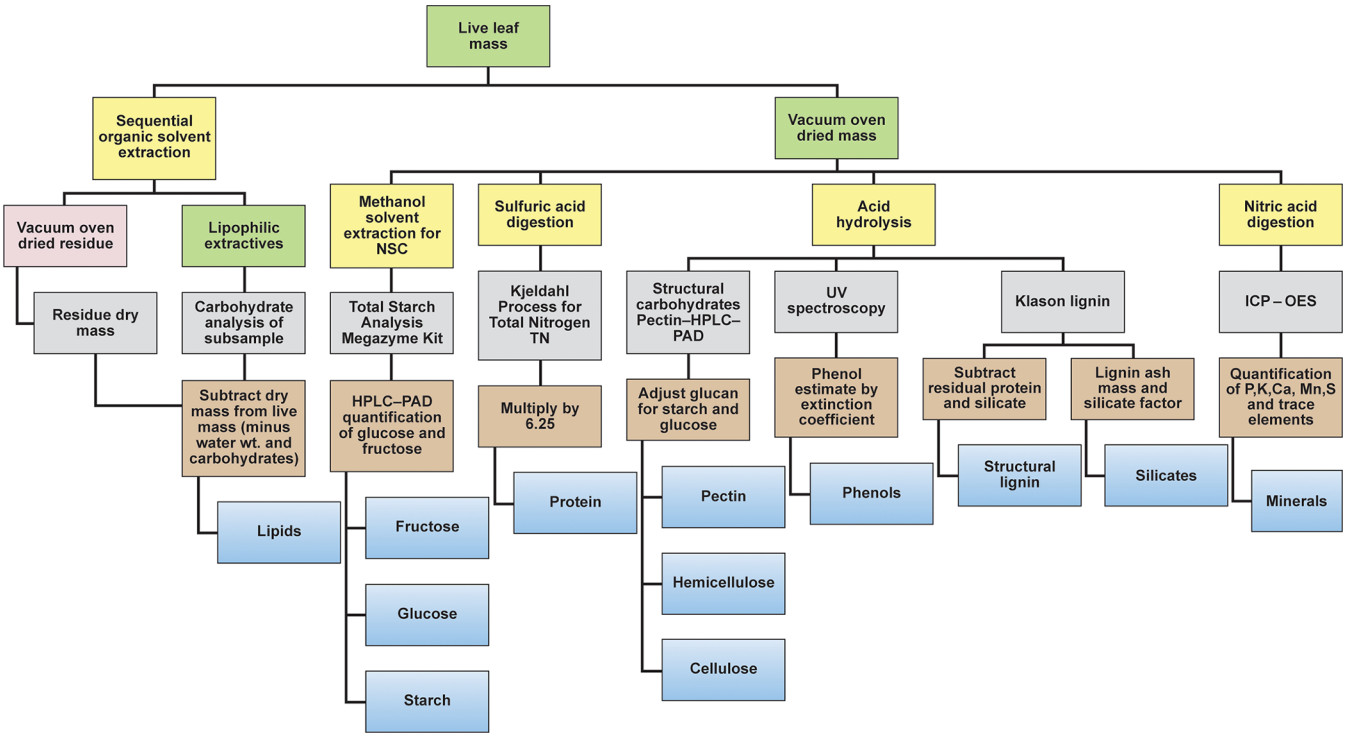

Figure 3. Flow diagram of methods used to isolate live leaf components for summative mass balance. Isolation methods are shaded in gray. Measurement methods are shaded in yellow. Isolated products are shaded in graduated blue. Quantitative adjustments for analytical duplications (interferences) are shaded in brown. Components containing chlorophyll pigment are shaded in green.

Precautions were taken to not expose extractives to air to avoid lipid peroxidation. Additionally a 10 mL subsample was taken and preserved with sodium benzoate for LC/MS analysis. Repetitive bead mill extractions (Bead Bug) with 1:1 hexane/2-propanol followed by 9:1 acetone/water was selected. The procedure is outlined below:

Leaf Extraction Procedure.

1 Weigh approximately 0.6−0.8 g wet leaves into tared Bead Bug (BB) bead mill vials containing steel beads (three vials were required to obtain enough mass for analysis).
2 Set BB to 2 min at 400 rpm, fill up vials with 1:1 hexane/2-propanol, and then cap. Then start cycle.
3 When cycle is done, place vials in desk top centrifuge set at 12 000 rpm for 1−2 min.
4 Decant supernatant into a 10 mL glass syringe with a tared nylon 0.45 μM Luer-lock filter and then filter directly into a 50 mL volumetric flask.
5 Repeat steps 2 to 4 until leaves turn a brownish tan color and all of the green pigment is extracted (usually about sic replicates).
6 Repeat steps 2−4 with 9:1 acetone/water using a hand vortex for 50 s. At this stage, the leaf tissue is adequately pulverized. Usually do four repeats.
7 Collect 9:1 extract in another 50 mL volumetric flask.

Link to Beadbug Manufacturer Web Site Illustration. http://www.benchmarkscientific.com/BeadBug.html

Gravimetric Measurement of Extractives.

1 Take both extracts up to 50 mL mark and mix well.
2 Accurately remove a 25 mL subsample from each volumetric flask and pool extracts into a tared aluminum weighing dish. Then allow to air-dry. A 10 mL subsample was taken for LC/MS—TOF analysis and preserved with sodium benzoate (0.1%) for lipids analysis.
3 After pooled samples are air-dried, place dish in 45 °C vacuum oven along with extracted plant material and 0.45 μM nylon filter (containing extracted plant residues) overnight. Then immediately place all samples in desiccators the next day to cool.
4 Weigh all items to obtain a mass balance (on a dry weight basis) consisting of the following: extracted leaf weight and pooled extractive weights. The extractives were determined by weight loss (from the leaf wet weight) minus the water weight determined by vacuum drying of samples run in parallel. Extractives were measured in this manner due to potential loss of volatiles during dry-down. This was similar to the AOCS crude fat protocol where food is extracted with hexane and lipids are measured by difference.[42]
5 Obtain subsamples of extracts for the carbohydrate analysis (see below for nonstructural (NSC) and structural (SC) carbohydrate analysis) so that subtraction of carbohydrate mass can be used to arrive closer to the lipid mass.

Lipid Profile of Leaf Extractives. Subsamples of wax myrtle, saw palmetto, yaupon, fetterbush, longleaf pine, and inkberry extracts were preserved with sodium benzoate and sent to Creative Proteomics Laboratories in Shirley, NY for high performance liquid chromatography−mass spectrometry analysis (LC/MS). Samples were reconstituted in 2-propanol/acetonitrile/water (2:1:1) then injected onto a Waters ACQUITY UPLC (HPLC) interfaced with a SYNAPT G2 HDMS (time-of-flight mass spectrometer TOF-MS) using a method similar to that outlined in the Waters application note: "Lipid Separation using UPLC with Charged Surface Hybrid Technology".[40] MS data were processed using a program called mz Mine. Peak assignments were made with the aid of the Lipid Blast Lipidomics library.[44]

Nonstructural Carbohydrates. Nonstructural carbohydrates are accessible carbohydrates within the plant[12] used for metabolic purposes. For pyrolysis modeling, it is important to distinguish between NSC and SC since their pyrolysis temperatures differ. A different analytical approach is also required to distinguish NSC (starches and free sugars) and pectin from SC (cellulose, hemicellulose) sugars. Plant starches are composed of linked glucose carbohydrates.[45] Starch determination methods rely either on acid hydrolysis or enzymatic procedures.[45,46] In the case of leaf/plant

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starches the acid hydrolysis procedures (commonly used to determine cellulose) are not useful because they quanitfy total sugars (both NSC and SC). Most enzymatic methods use α-amylase (a thermally stable enzyme) for starch analysis. To quantitate starch sugar and other leaf sugars, a Megazyme total starch assay kit was used.[46] This kit uses α-amylase and amyloglucosidase (thermostable enzymes) and is based on AOAC and AACC methods 996.11 and 76.13.01 respectively.

Soluble sugars (nonstructural sugars) were extracted from dried leaf samples by heating in 80 °C ethanol (80%) for 5 min and then repeating with fresh solvent (to remove soluble sugars). This ethanol and sugar mix was collected in a 25 mL volumetric flask for acid hydrolysis with 2% $H_2SO_4$for 1 h. Fructose and glucose (the components of sucrose) were quantitated using the IC-PAD method described below.

Crude Protein. Crude protein determination was performed at the University of Wisconsin (UW) Soil and Forage Lab on vacuum-dried leaves using the Kjeldahl process for total N. The procedure consists of three basic steps: (1) sulfuric acid digestion with a catalyst which converts N to ammonia; (2) distillation of ammonia into a trapping solution containing an indicator; (3) quantitation by titration. Crude protein is then determined by multiplying total N by 6.25.[47,48]

Structural Carbohydrate Analysis. Most carbohydrate methods for biomass analysis target the primary components of cellulose and hemicellulose. The ASTM method E1758-01 "Standard Method for the Determination of Carbohydrates by HPLC" is often cited as is NREL/TP-510-42618 which references the ASTM method. Both methods use a 2-step heated sulfuric acid hydrolysis, which breaks all sugars down to monomer units of: arabinose, galactose, glucose, xylose, and mannose. Concentrations of cellulose in wood or biomass can be calculated reliably by multiplying by an "anhydro correction factor" to obtain a cellulose (or hemicellulose) mass fraction. Additionally, leaves contain carbohydrates in various forms other than cellulose and hemicellulose. The major types of leaf carbohydrates are SC (celluloses, hemicelluloses, and leaf pectin) and NSC (starches and soluble sugars).[20]

Structural Carbohydrate Method. Hydrolysis in H_2SO_4 was carried out similarly to the standard method described by Effland[49] using the FPL in-house protocol for SC.[50] Samples were milled to pass a 1.00 mm (20 mesh) screen and vacuum-dried at 45 °C overnight in a vacuum oven. Primary hydrolyses of 80−100 mg subsamples were performed with 1.00 mL of 72% (w/w) H_2SO_4 for 1 h at 30 °C. Hydrolysates were diluted to 4% (w/w) H_2SO_4 with distilled water, and a secondary hydrolysis was performed for 1 h at 120 °C. After cooling, aliquots of the supernatant (hydrolysate) were taken for chromatographic analysis and acid soluble lignin analysis (ASL).

Ion Chromatography HPLC System. Hydrolysates were analyzed on a Dionex ICS-3000 DP high performance anion exchange liquid chromatography system using pulsed amperometric detection (HPAEC-PAD) similar to the method described by Davis.[37] The system was equipped with a second pump to deliver a post column stream of 200 mM sodium hydroxide at a flow rate of 0.2 mL/min. (to raise the eluent pH to 12.4). The system was controlled by Chromeleon v. 6.8 software and programmed to condition a Dionex PA-1 Carbo Pac column (with guard) using aqueous 220 mM sodium acetate and 380 mM sodium hydroxide for a period of 2 min followed by an 8 min equilibration period with 100% water prior to injection (10 μL). The sample was then eluted with an isocratic system of 100% water. The column compartment was maintained at 22 °C throughout the run sequence.

Pectin. Pectin is a carbohydrate component that is found in the cell walls and intercellular regions of most leaves. Pectin is made up of 80−90% polygalacturonic acid (the polymerized form of galacturonic acid) with small amounts of neutral sugars.[51,52] Various hemicelluloses, particularly xylan, can be attached to the long pectin chain, and they often substitute for lignin as a binding material for the celluloses in the leaf.[53]

Pectin has been analyzed in the past by wet chemistry methods involving hot water/mild acid extractions and quantified using gravimetric methods or colorimetric methods.[54] Recently, pectin in fruit was estimated by measuring galacturonic acid content by high performance liquid chromatography with ultraviolet detection (UV-HPLC) in raspberries.[52] This was the basis for adapting an FPL in-house method (developed for measuring uronic acid oligomers) for analysis of leaf pectin by HPAEC-PAD. This separation and detection technique is specific to acidic carbohydrates (i.e., galacturonic acid) and less prone to interferences than UV-HPLC. This provided an opportunity to make an approximation of pectin in leaves by analyzing the leaf carbohydrate hydrolysate for galacturonic acid (GA).

The same hydrolysates that were injected to determine structural carbohydrates as cellulose and hemicellulose were again injected into the Dionex ICS-3000 only eluted with the following mobile phase program, shown in Table 2. Galacturonic acid concentrations were measured against a standard curve. Figure 4 presents the separation of galacturonic acid in a sulfuric acid hydrolysate of inkberry leaf.

Table 2. Mobile Phase Program in Dionex ICS-3000 for GA Determination

time (min)	flow (mL/min)	water (vol. %)	NaOAC[a] (vol. %)	NaOH[b] (vol. %)	gradient curve[c]
0.0	0.5	90	0.0	10	L
10.0	0.5	80	10	10	L
25	0.5	60	30	10	C
30	0.5	10	80	10	C
30.1	0.5	90	0	10	L
37	0.5	90	0	10	L

[a]1 N tribasic sodium acetate. [b]1 N sodium hydroxide. [c]L = linear; C = concave.

Link to Dionex ICS-3000 HPLC-PAD Manufacturer Web Site Illustration. https://www.thermofisher.com/us/en/home/industrial/chromatography/ion-chromatography-ic/ion-chromatography-systems/modular-ic-systems.htm.

Lignin and Phenolic Components. Lignin in woody and herbaceous plants is a macromolecule formed of phenylpropanoid units. These units are derived from the cinnamyl alcohols (monolygnols) *p*-coumeryl, coniferyl, and synapyl alcohols.[55] Plant lignin aids in maintaining cell wall strength and in water transport and impedes the degradation of cell wall polysaccharides acting as a defense against plant pathogens.[17] The most common method for determining lignin is the Klason 2-step acid hydrolysis procedure described in published methods such as NREL/TP-510−42618 and was described above in the carbohydrate analysis sections. This method was developed by Klason in the early 1900s[56] and required digestion in 72% sulfuric acid to dissolve all the carbohydrates followed by autoclaving at 120 °C for an hour (after diluting the acid to 4%) to hydrolyze the sugars producing an insoluble residue filtrate (so-called "acid insoluble lignin"). The resulting supernatant was subsampled for analysis of sugars and acid soluble lignin (ASL) or the portion of lignin that is solubilized during the second hydrolysis step in dilute acid.[57]

Acid Soluble Lignin (ASL)/Polyphenols. Acid soluble lignin was analyzed similarly to the NREL method: (LAP) NREL/TP-510-42618 (April 2008). An aliquot of the hydrolysate was diluted 1:3 and read in a spectrophotometer at (240 nm) after subtracting a similarly diluted aliquot of hydrolysis solution as a blank. ASL was then calculated as a percentage by using the molar absorptivity coefficient of 25 L/(g cm) that is used for a variety of feedstocks.[57]

Quantitative data for lignin tends to show considerable variation relying heavily on parameters of the analytical method used. This is due to inherent difficulties in isolation, purification and characterization.[14,58] Similar difficulties apply to measuring ASL particularly in leaves which are biochemically diverse. Constantines and Fownes studied N mineralization rates vs lignin and polyphenols levels in Hawaiian palm leaf litter in Hawaii.[59] They used hot methanol/water to extract polyphenols which ranged from 2 to 9% of the fresh leaf weight, but there was no value of ASL reported. For this study, it was

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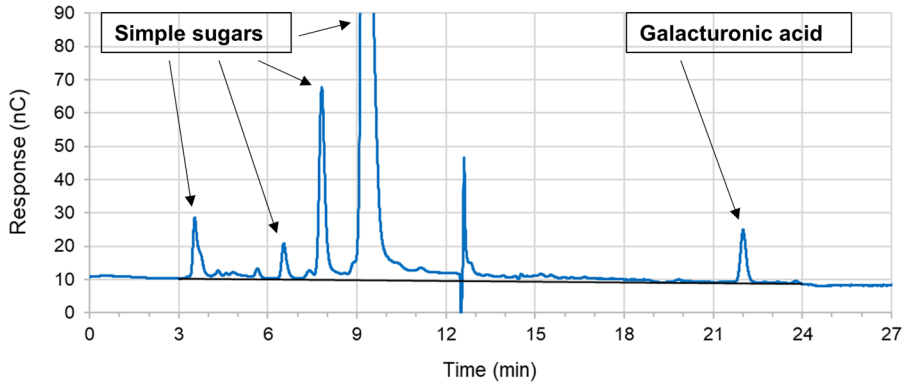

Figure 4. Separation of galacturonic acid in a sulfuric acid hydrolysate of inkberry leaf.

Table 3. Percent Moisture in Live Leaves

plant species	vacuum dry at 45 °C, MC%[a] (% water wt.)	oven dry at 102 °C, MC%[a] (% water + volitile extractives wt.)	% moisture difference[a]
saw palmetto	61.6	62.2	0.6
dwarf palmetto	65.3	68.2	2.9
swamp bay	54.2	56.4	2.2
yaupon	61.8	63.1	1.3
inkberry	57.5	57.6	0.2
wax myrtle	59.0	60.5	1.5
live oak	57.7	59.4	1.8
fetterbush	59.2	58.5	−0.7
water oak	56.0	58.2	2.2
longleaf pine	64.3	70.6	6.4
wiregrass	61.2	NA[b]	NA[b]
little bluestem	67.6	NA[b]	NA[b]

[a]Gravimetric measurement accurate to ±0.5% (NREL/TP-510-42621) [b]NA, no sample available.

assumed that the ASL fraction (commonly measured in biomass analysis) is actually a polyphenol-rich fraction which contained the majority of the phenolic material. ASL has been reported to contain dissolved lignin, polyphenols, and tannins and is best characterized by a phenolic model compound.[59−61]

Protein Content of Klason Lignin. Herbaceous plants such as forage plants can contain 15−25% protein on a dry weight basis (DW).[14] This was taken into consideration for summative compositional analysis since the pyrolysis temperature as well as chemical makeup differs considerably between the protein and lignin. To accomplish this, the Klason lignin fraction of all plants was subsampled for crude protein and analyzed using the Kjeldahl method (KM)—performed at the UW Soil and Plant Analysis Lab. This protein value was subtracted from the Klason lignin value since it was measured in the protein determination for the whole plant leaf.

Lignin Ash/Silicate Correction. Another correction was made for ash content of lignin. This was obtained by placing the Klason lignin residue in a 560 °C muffle furnace (for 3 h) to obtain an ash weight. The dry weight percentage of ash was calculated, then converted to silicate dry weight percentage, and subtracted from the Klason lignin weight along with the protein weight subtraction. Silicate values were calculated by multiplying the ash weight by 1.6, which is the ratio of molecular weight of silicates in Klason lignin to silicon dioxide in Klason lignin ash.

Leaf Silicate Content. Both sulfuric acid (used in Klason lignin) and nitric acid (used in the mineral analysis) do not dissolve silicon and aluminum. The ash weight of the Klason lignin was considered to be mostly silicon dioxide. The aluminum concentration in the leaves was considered to be negligible.

Mineral Analysis. Elemental analysis was performed by the UW Soil and Plant Analysis Lab and included N, P, K, Ca, Mg, S, Zn, Mn, B, Fe, and Cu. Total nitrogen (TN) was performed by the Kjeldahl method as described above. Samples were submitted on a vacuum-dry weight basis. A dried and ground sample was weighed into a Folin tube and digested until ∼1 mL of solution remained and then diluted to a known volume and mixed. The sample was analyzed by ICP-OES (inductively coupled plasma emission spectroscopy), which measures the elemental content of the sample.[62]

Figure 3 shows a hierarchy flowchart of the isolation and analytical procedures followed for quantitation of the 12 components used to determine summative mass balance.

Ultimate and Proximate Analysis. Proximate (ASTM D7582) and ultimate analysis (ASTM D5291, ASTM D4239, and ASTM E711) were performed commercially at Keystone Laboratory in Newton, IA. Desiccants and oxygen absorbers were inserted in the shipped aluminized bags containing the vacuum oven-dried samples stored in labeled plastic bags. Exposure to normal humidified air still occurred both in the greenhouse preparation and in the contract laboratory, meaning that the lipids, protein, pectin, and starch present in the leaves could undergo some amount of oxidation and/or enzymatic hydrolysis, thus changing the live composition somewhat. Duplicate samples were prepared, as set A and set B. Ultimate analysis using ASTM standard methods for total CHON (D5291) and S (D4239) was applied to the samples on a "as is" basis. Low and high heat values (corresponding to heats of combustion) were measured by ASTM Method E711. The proximate analysis for moisture, ash, volatile matter, and fixed carbon contents on "as is" basis followed ASTM D7582. The Results and Discussion provides a discussion of how ultimate analysis supports the composition data and provides additional data needed for pyrolysis and combustion modeling.

RESULTS AND DISCUSSION

Moisture Measurements and Volatiles in Leaves. Table 3 shows water weight measurements obtained by vacuum drying (at 45 °C) and oven drying (at 103 °C). The percent difference in the two measurements appears in the last column. In most cases, the oven-dried measurement is

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Table 4. Lipophilic Extractives of Live Leaves

plant species	lipophilic extractives (% dry wt. basis)	adjusted lipid extraction[a] (% dry wt. basis)	coextraction sugar (% dry wt. basis)
saw palmetto	13.1	11.9	1.2
dwarf palmetto	8.0	6.8	1.2
swamp bay	13.3	11.3	2.0
yaupon	31.3	31.3	0.0
inkberry	31.9	28.1	3.8
wax myrtle	17.5	15.6	1.9
live oak	11.6	9.9	1.7
fetterbush	33.3	30.0	3.3
water oak	17.7	15.1	2.5
longleaf pine	24.3	22.3	2.0
wiregrass	3.8	3.4	0.4
little bluestem	4.8	4.8	0.0
variance (±)[b,c]	0.5[b]	CV[d]	0.2[c]

[a]Dry weight % minus coextracted carbohydrate interferences. [b]Gravimetric measurement accurate to ±0.5% (NREL/TP-510−42621). [c]Standard deviation for measuring monomer sugars by HPLC-PAD-from FPL database. [d]CV = calculated value.

slightly higher than that for the vacuum-dried samples. The exceptions were fetterbush, which was approximately the same, and longleaf pine, which was considerably higher at 6.5%. The longleaf pine result was most likely due to volatile oil loss during oven drying at 102 °C which did not occur during vacuum drying at 45 °C. Dwarf palmetto was the next highest at 2.9%. Based on these results, it was concluded that the vacuum drying method (45 °C) used in the NREL method was best suited for sample preservation by minimizing potential loss of volatiles while still curtailing enzyme activity.

Lipophilic Extractives. Table 4 presents lipophilic extractives expressed on a dry weight basis and corrected extractive weight adjusted for coextracted carbohydrates. The broadleaf plants contained the highest quantities of extractives, while the grasses (little bluestem and wiregrass) were the lowest. Longleaf pine needles also contained high levels of extractives similar to many broadleaves tested.

The bead mill 2-step extraction method proved to be effective for extracting live leaves. It worked rapidly to minimize biological degradation of sample components and provided adequate cell disruption to release lipids from the plant cells. Figure 5 shows combined filtered extracts of yaupon, water oak, fetterbush, and little bluestem using the 2-step extraction process outlined above. The extracts shown had been stored in the dark for 5 months and maintained their original color and clarity. The lack of turbidity suggested that the 0.45 μM filter was effective in removing particulates.

Identification of Extractive Components. For this study, attempts to characterize the extractives were restricted to lipid content. Lipids are highly soluble in the solvent system used and would likely comprise the major dry mass fraction since they are a primary plant metabolite which includes a wide range of compounds.[40] Table 5 presents the major classes of lipids found by LC/MS from Creative Proteomics Laboratories in order of % signal abundance.

Extracted Lipids and Model Compounds. Although the lipid profiles differed among the plants selected for lipid analysis, the galactosyl lipids (MGDG and DGDG) were consistently most abundant whereas ratios of phosphotidyl lipids varied considerably among plant species. Inkberry, longleaf pine, and yaupon are the most abundant in PC, PE, and PI. These results are in agreement those found by Browse and Sommerville[3] who reported glycerolipid content in *Arabidopsis* leaf chloroplasts as MGDG, DGDG, PC, and PG—with MGDG and DGDG levels being the highest in 7-week-old leaves. Halls, Knox, and Lazar[16] reported MGDG, DGDG, PG, PC, PI, and PA in thin-layer-chromatography

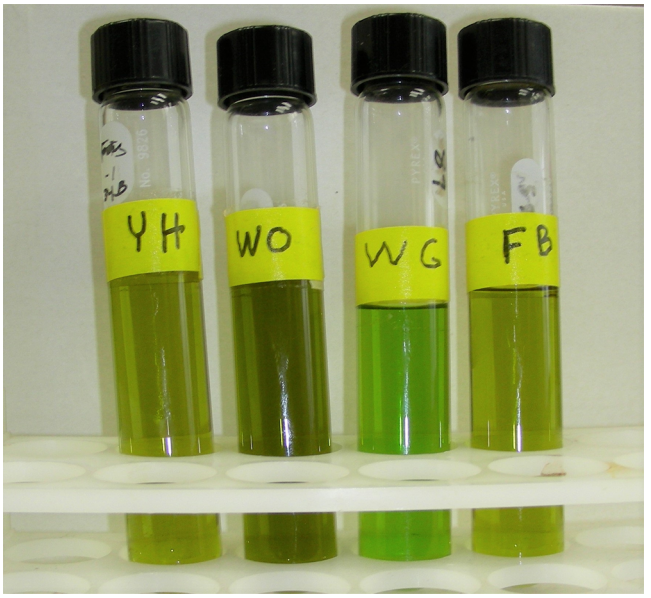

Figure 5. Combined 1:1 hexane/2-propanol and 9:1 acetone/water bead mill extracts of yaupon (YH), wild oak (WO), fetterbush (FB), and bluestem grass (BG).

Table 5. Lipid % of Total LC/MS Signal[a]

lipid species	saw palmetto	yaupon	wax myrtle	fetterbush	longleaf pine	inkberry
DGDG[b]	3.6	7.6	1.8	11.1	19.0	6.8
MGDG[b]	89.7	68.6	96.4	69.4	45.4	73.0
PC[c]	1.4	17.5	0.4	4.7	20.1	8.8
PE[c]	0.4	1.8	0.1	0.4	3.7	2.1
PI[c]	1.1	0.6	0.3	3.4	3.4	1.0
PA[c]	1.8	0.7	0.0	1.7	1.5	3.8
tot.	98	97	99	91	93	95

[a]Single analysis of samples using the following Waters method: lipid separation using UPLC with charged surface hybrid technology. [b]DGDG and MGDG = mono- and digalactosyl diglycerides. [c]PC, PE, PI, and PA: phosphotidyl = choline, ethanolamine, inositol, and phosphatidic acid.

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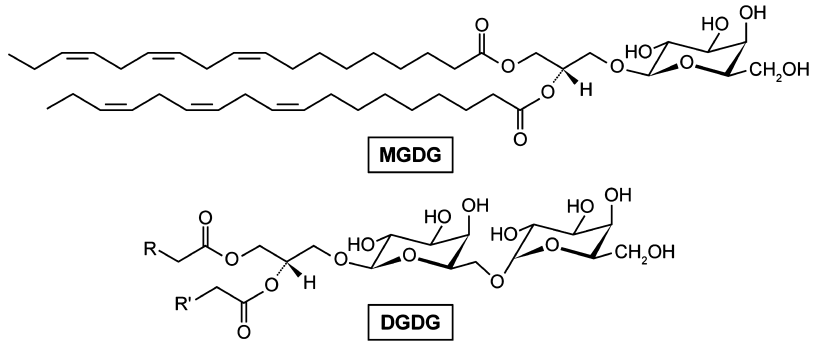

Figure 6. Potential model lipid compounds: MGDG, 1,2-dioctadecatrienoyl-3-O-β-galactosyl-sn-glycerol; DGDG, digalactosyldiacylglycerol.[64]

(TLC) analysis of browse plants: inkberry, saw palmetto, and running oak. Debiagi and others[17] chose two model compounds for hydrophobic and hydrophyllic extractives of biomass (wood) feedstocks. Triglycerides (hydrophobic) were chosen based on available wood extractives data and high C/H content which best fit available ultimate analysis data. MGDG and DGDG were also reported by Aziz and Larher[63] as the main lipids in rapeseed leaves as part of a study measuring lipid changes as a response of moisture deficit. The authors used the AOCS Am-04 protocol (a crude fat method for food analysis) to extract leaf lipids.[57] This method uses a soxhlate extraction to remove fat from the sample and measured crude fat gravimetrically by measuring weight loss of the dried extracted sample. The empirical formulas of MGDG and DGDG best fit the ultimate analysis data (presented in Table 10) and they also agreed with the results obtained by LC/MS (Table 4) hence, they were picked as potential model compounds. Their chemical structures are presented in Figure 6.

Co-extracts of Lipids. Given the wide polarity range of solvents used for extraction it was likely that extractives contain small amounts of nonstructural carbohydrates (NSC) and polyphenols. The extractive samples were analyzed for carbohydrates and adjusted. Table 4 presents the extractive data minus coextracted NSC. No further attempt was made to characterize phenols in the extractives, although in a related study using tandem gas chromatography with time of flight mass spectrometry detection (GC x GC TOF-MS) several identified phenols in negligible amounts in comparison to other chemicals in the extractives were found (private communication with R. Moore, Research Chemist at FPL, see Acknowledgments).

Carbohydrate Analysis. NSC are subdivided into sugars and starches, and each had their own method of analysis recapped below. The NSC components are summarized in Table 6. Collectively they ranged from 1 to 6%. Monomers of glucose and fructose were chosen as model compounds representing the NSS fraction.

Dissolved sugars are found in plants in the form of sucrose (fructose + glucose) and monomeric glucose. They are reported as fructose and glucose in Table 5 and after extraction with 80 °C ethanol. A subsample was taken and combined 2:1 with 2% H_2SO_4 for hydrolysis so as to break down sucrose into glucose and fructose for the forms which can be analyzed by ICP-PAD. Dissolved sugars ranged from 1.1% in little bluestem and wiregrass to 4.2% in inkberry.

Starches required enzymatic hydrolysis with amyloglucosidase and amylase to break down starch into glucose units (AOAC method 996.11) Starch levels ranged from nondetectable (ND) in wiregrass to 2.2% in inkberry using a Megazyme total starch assay kit. The starches were within the 1% level.

Table 6. Summary of Non-structural Carbohydrates in the Live Leaves

plant species	fructose (% dry wt)	starch (% dry wt)	glucose (% dry wt)
saw palmetto	0.6	1.0	0.7
dwarf palmetto	1.0	0.6	1.3
swamp bay	1.9	0.8	2.2
yaupon	0.3	ND	3.4
inkberry	0.8	2.2	3.4
wax myrtle	0.8	0.3	0.9
live oak	1.1	0.7	1.5
fetterbush	0.5	ND	3.5
water oak	1.3	0.9	1.6
longleaf pine	0.8	0.7	1.4
wiregrass	0.1	ND	1.0
little bluestem	0.4	0.1	0.7
variance (±)[a,b]	0.2[a]	0.04[b]	0.2[a]

[a]Standard deviation for measuring monomer sugars by HPLC-PAD-from FPL database. [b]Standard deviation for measurement of total starch in green pea using AOAC method 996.11.

Structural Carbohydrates (SC). Structural carbohydrates are fibrous components of the plant that consist of cellulose, hemicellulose, and pectin. Pectin ranged from ND in wiregrass to higher values in wax myrtle but never exceeded 3% as shown in Table 7. The values in broadleaf plants tended to be higher than the grasses with the palmetto plants falling in between. Cellulose consists mostly of glucose whereas hemicellulose consists of primarily xylose and arabinose with small amounts of galactose, glucose and mannose. Normally the cellulose content is set equal to the measured glucan content for biomass, but the presence of glucan units also in starch, glucose, and mannan[59] in the live leaves, result in the derived cellulose content generally less than the glucan content value. The hemicellulose can be described as combination of a hexosan (combination of galactan and mannan with adjustment for glucan within mannan) and a pentosan (combination of arabanan and xylan) using the factors from Jones and others.[65]Table 7 shows the resulting percentages of cellulose and hemicellulose on a DW basis. For pyrolysis study this is an important ratio to know since it aids in the interpretation of ultimate analysis data and in the choice of a model compound. Additionally levels of cellulose vs hemicellulose affect the C:H:O ratio which in turn affects combustion properties of leaves. Cellulose and hemicellulose also differ in levels of crystallinity in wood and biomass. Typically, hemicellulose is a

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Table 7. Percent Hydrolyzed Carbohydrates (Anhydrous Form) as Related to Cellulose, Hemicellulose, and Pectin Contents (Dry Basis)

plant species	pectin (%)	arabanan (%)	galactan (%)	xylan (%)	mannan (%)	glucan (%)	cellulose (%)	hexosan (%)	pentosan (%)	hemicellulose (%)
saw palmetto	0.7	4.3	0.9	12.5	0.7	12.9	11.0	1.9	16.8	18.7
dwarf palmetto	2.0	3.0	1.5	13.6	0.0	29.7	28.0	1.5	16.5	18.0
swamp bay	1.8	1.5	1.5	7.5	0.0	21.0	18.3	1.5	9.0	10.5
yaupon	1.9	1.5	2.5	2.6	0.5	12.0	11.8	3.2	4.1	7.3
inkberry	2.6	2.3	1.9	1.2	0.0	16.4	11.1	1.9	3.5	5.4
wax myrtle	2.7	1.1	1.8	7.6	0.0	18.7	17.6	1.8	8.7	10.5
live oak	1.7	1.9	1.9	7.0	0.0	21.4	19.2	1.9	8.9	10.9
fetterbush	1.8	1.9	1.5	3.5	0.0	19.8	19.8	1.5	5.4	6.9
water oak	2.9	2.7	2.3	0.0	0.6	18.2	15.7	3.0	2.7	5.8
longleaf pine	2.0	2.0	2.5	3.0	5.6	22.8	19.0	10.0	5.1	15.0
wiregrass	0.0	3.2	0.9	22	0.0	34.8	34.8	0.0	25.2	22.8
little bluestem	0.5	3.2	1.3	17.0	0.0	32.0	31.3	1.3	20.2	21.5
variance	ND[a]	0.1[b]	0.2[b]	0.3[b]	0.5[b]	0.8[b]	1.2[b]	CV[c]	CV[c]	CV[c]

[a]Not currently determined. [b]Values from FPL Standard Feedstock database for Loblolly pine. [c]CV = calculated value.

Table 8. Summary of Measurements for Lignin, Phenolic, and Silicate (Dry Basis)

plant species	Klason lignin (KL) (%)	KL ash (%)	KL silicates (%)	KL protein (%)	phenols (%)	structural lignin (%)
saw palmetto	35.79	3.02	4.83	1.19	4.82	29.77
dwarf palmetto	22.27	0.70	1.13	4.06	7.94	17.09
swamp bay	34.94	0.00	0.00	2.05	5.28	32.89
yaupon	35.19	0.24	0.38	9.63	6.60	25.18
inkberry	25.94	0.00	0.00	0.97	9.00	24.96
wax myrtle	34.11	0.16	0.25	5.78	2.39	28.09
live oak	27.51	0.00	0.00	3.76	5.82	23.74
fetterbush	33.29	0.21	0.33	2.69	3.45	30.27
water oak	33.27	2.70	4.31	3.64	13.49	25.32
longleaf pine	27.50	0.39	0.62	3.34	3.09	23.54
wiregrass	18.70	1.50	0.00	0.00	2.8	18.70
little bluestem	21.2	1.70	0.03	0.00	3.10	21.2
variance (±)[a]	0.4	0.1	CV[b]	CV[b]	0.1	CV[b]

[a]Values from FPL Standard Feedstock database for loblolly pine. [b]CV = calculated values.

shorter chained polymer containing more branches and is more amorphous than cellulose. The anhydrous monomer of glucose (glucan) was chosen as a model compound to represent cellulose. Similarly the anhydrous monomer of xylose (xylan) was selected as a model compound for hemicellulose for application to modeling of live leaves.

Lignin, Phenols, and Silicates. Table 8 presents mass percent(s) of Klason lignin and including associated compounds of ash, silicate, protein, and phenols (ASL) and finally the structural lignin. The silicate fraction will transform to silicon dioxide by loss of water. Silicates (H_4O_4Si) in the fresh leaf has a molecular weight that is 1.6 times that of the ashed form of Klason lignin, as SO_2, is presented in Table 8. The highest level of silicates were found in saw palmetto and water oak at mass fractions of 4.8% and 4.3% respectively. (Table 8). The protein content of KL is significant as it varied in the plants tested from 1% in inkberry to 9.6% in yaupon with none detected in the two grasses. KL protein and silicate percentages were subtracted from that of Klason lignin to obtain "structural lignin" (SL). SL levels were generally highest in broadleaf and longleaf pine and lowest in the two grasses. Palmettos as a group varied the most. A lignin reference compound high in oxygen levels (LIGO: $C_{20}H_{22}O_{10}$) of the three lignin references proposed by Debiagi et al.,[17] for biomass was determined as best suited for predicting the fresh leaf ultimate analysis data and was chosen as a model compound.

Polyphenols are a broad range of compounds including flavonoids, phenolic acids, stilbenes, tannins and lignans in plants. They are secondary plant metabolites and are present in quantities of around 1% of leaf dry mass.[66] For the purpose of this study given its' emphasis on measuring summative mass fractions for pyrolysis study and selecting model compounds representing those fractions the phenolic rich ASL fraction was grouped as phenols. Phenols were assigned the empirical formula of $C_{15}H_{12}O_7$ using a reference material of condensed tannin monomer.[13]

Protein and Minerals of Live Leaves. Minerals and silicates were measured in varying amounts and are components of interest in pyrolysis. Mineral fractions (excluding silicon and aluminum) were measured at UW using vacuum-dried leaf samples. The protein content was estimated by multiplying TN by 6.25 and is shown in Table 10. The leaf crude protein varied from 5.3% in inkberry to 14.1% in wax myrtle. Mineral fractions ranged from 2% DW in saw palmetto to 4% in water oak (individual list in Table 9 and overall in Table 10). The mineral content is largely noncombustible at temperatures below 950 °C (the temperature at which the carbonate will burn).

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Table 9. Mineral Contents Measured for Live Leaves (Dry Basis) That Are Significant

plant species	TN (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
saw palmetto	0.86	0.19	0.60	0.59	0.22	0.16
dwarf palmetto	2.06	0.25	2.10	0.41	0.14	0.33
swamp bay	1.34	0.24	0.64	0.61	0.22	0.14
yaupon	1.39	0.78	2.35	0.71	0.43	0.21
inkberry	0.86	0.07	0.58	0.95	0.20	0.11
wax myrtle	2.25	0.17	1.26	0.62	0.25	0.17
live oak	2.24	0.17	0.96	1.21	0.30	0.16
fetterbush	0.78	0.05	0.52	1.21	0.35	0.10
water oak	1.41	0.15	1.11	1.28	0.34	0.15
longleaf pine	1.19	0.11	0.92	0.64	0.16	0.12
wiregrass	1.51	0.16	1.30	0.36	0.14	0.18
little bluestem	2.00	0.18	1.56	0.30	0.13	0.13

Analytical Interferences and Summative Mass Balance (SMB). Methods of chemical isolation were chosen to minimize analytical interference/duplication among the components. However, in some cases, isolation methods were adjusted to maintain mass balance. For example, the crude protein content is calculated by multiplying the total nitrogen of the dried sample by 6.25. Since the acid insoluble lignin (AIL) fraction also contains protein it was dried and analyzed for crude protein then subtracted from the AIL fraction to obtain "structural lignin". The final step at deriving the structural lignin content was the subtraction of the silicate content in the AIL. Other interferences were observed in the following measurements: 1) Structural and nonstructural carbohydrates— SC standard methods commonly used in analysis of wood and biomass measure total carbohydrate and needed to be adjusted for starch, pectin and NSCs. 2) NSC in extractives—NSCs were coextracted during the exhaustive live leaf extraction. Summative Mass Balance of the measured live leaf components are displayed in Table 10. The data are presented graphically in Figure 7, for comparison of composition. Empirical formulas for individual components are also presented in Figure 7. Observable differences in leaf composition were noted within groups of plant types as well as between them—with grasses being most similar and palmettos being most dissimilar.

Summative Mass Balance Expected Error. Despite correcting for the few analytical interferences, most of the mass recoveries were between the 95 to 100% range with the exception of live oak and saw palmetto which were 91%. The recoveries indicate that mass closure was reached using the 12 measured parameters which are arranged in order of increasing pyrolysis temperature (L to R). It is noteworthy that SMB was reached with analysis of primary plant metabolites and/or their derivatives and that secondary plant metabolites were not present in levels of interest for pyrolysis [<1−5% of dry matter (DM)]. Some of the components were measured at lower values (i.e., the NSC, pectin, and lipids) than had been reported in the literature for similar foliage, providing potential challenges to the current methods. However, since 95 to 100% mass balances were reached by measuring with 12 components in most cases, the methods used were considered adequate.

The SMB in this study was similar in most cases to those reported in the NIST biomass characterization round robin study[67] which were 102.4 ± 1.8, 99.4 ± 1.5, 100.2 ± 0.2, and 100.9 ± 5.4% (% DM) for bagasse, cottonwood, pine, and wheat straw, respectively. The average of all live leaf closures was 96.5 ± 3%, which is within the range of those found in the NIST recertification study. (The NIST study was intended to provide "true values" of the feedstocks to assist in QA/QC programs of analytical laboratories.) The two lowest recoveries of 91.4 (live oak) and 91.2 (saw palmetto) did not qualify as outliers. (Dixon outlier test). The Dixon test (or Q-test) is often used in analytical measurements and defines the range of values that occur within a normal statistical distribution of measurements corresponding to a given sample size and confidence interval (95% in this case). Results that fall outside of the normal distribution can be considered true outliers.[68] Given the scarcity of mature leaf specimens at sampling times (along with inherent difficulties of raising plants in an improvised greenhouse), samples could not be run in triplicate nor was there any statistical justification to exclude lab results obtained. However, the summative analysis values were consistent over all plant species, which replicated results in the NIST study of analytical variance (in biomass). That previous study found that sources of variance arise from analytical methods being used rather than differences in feedstocks.[67] This, for now, serves as a reasonable validation of the method. A more rigorous statistical validation of the method is out of the scope of this study. Indeed, this is an advancement over many previously reported SMB analyses that often do not achieve full measurable mass balance thus necessitating the introduction of unmeasured components to make up the mass balance by difference.

Table 10. Summative Analysis of Leaf Components

dry basis	lipids (%)	glucose (%)	fructose (%)	protein (%)	pectin (%)	hemi cell (%)	cellulose (%)	starch (%)	phenols (%)	lignin structural (%)	minerals (%)	silicates (%)	total (%)
saw palmetto	11.9	0.7	0.6	5.4	0.7	18.6	11.0	1.0	4.8	29.8	1.8	4.8	91.2
dwarf palmetto	6.8	1.3	1.0	12.9	2.0	18.0	28.0	0.6	7.9	17.1	3.3	1.1	100.0
swamp bay	11.3	2.2	1.9	8.3	1.8	10.5	18.3	0.8	5.3	32.9	1.9	0.0	95.2
yaupon	31.3	3.4	0.3	8.7	1.9	7.3	8.8	0.0	6.6	25.2	4.5	0.4	98.4
inkberry	28.1	3.4	0.8	5.3	2.6	5.4	11.1	2.2	9.0	25.0	1.9	0.0	94.9
wax myrtle	15.6	0.9	0.8	14.1	2.7	10.5	17.6	0.3	2.4	28.1	2.5	0.2	95.7
fetterbush	30.0	3.5	0.5	4.9	1.8	6.9	16.6	0.0	3.4	30.3	2.2	0.3	100.6
live oak	9.9	1.5	1.1	14.0	1.7	10.9	19.2	0.7	5.8	23.7	2.9	0.0	91.4
water oak	15.1	1.6	1.3	8.8	2.9	5.8	15.7	0.9	13.5	25.3	3.1	4.3	98.2
longleaf pine	22.3	1.4	0.8	7.4	2.0	15.0	19.0	0.7	3.1	23.5	2.0	0.6	97.9
wiregrass	3.8	1.0	0.1	9.5	0.0	22.8	34.8	0.0	2.8	18.7	2.2	0.0	95.7
little bluestem	4.8	0.7	0.4	12.5	0.5	21.5	31.3	0.1	3.1	21.2	2.3	0.0	98.4

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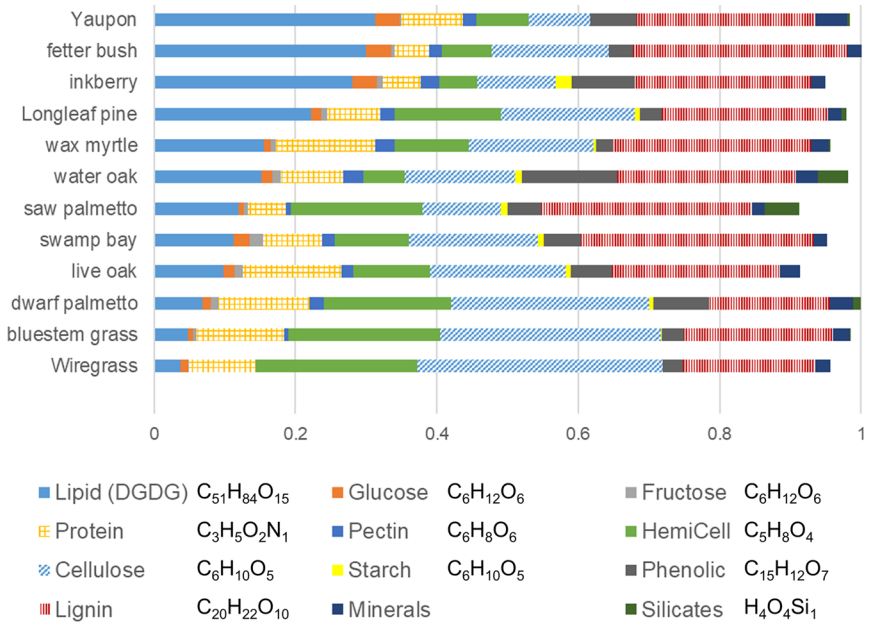

Figure 7. Leaf components with species arranged in the order of increasing lipid content with empirical formulas of modeling compounds.

Ultimate Analysis and Heats of Combustion of Vacuum-Dried Leaves. Leaf components spanned a wide range of pyrolysis temperatures from 70 °C (lipids) to 600 °C (structural lignin). Minerals and silicates dehydrate and/or oxidize to form ashes as their pyrolysis products. All other components form volatile matter at their respective pyrolysis temperatures, while some components, primarily lignin and celluloses, will form char (fixed carbon), as listed for the 12 species in Table 11 (also Safdari et al.[27]). As flammability was

Table 11. Proximate (ASTM D7582) Data on Set B on as Received Basis (Keystone Laboratory, Newton, IA)

plant species: as received basis	moisture (%)	ash (%)	volatile matter (%)	fixed carbon (%)
saw palmetto	7.21	4.94	67.78	20.07
dwarf palmetto	6.59	7.67	76.71	9.03
swamp bay	6.52	4.11	72.16	17.21
yaupon	6.47	5.62	73.96	13.95
inkberry	4.5	2.55	74.66	18.30
wax myrtle	7.31	4.54	65.33	22.82
fetterbush	5.16	2.94	70.68	21.21
live oak	7.4	4.98	69.55	18.06
water oak	6.54	3.94	70.99	18.53
longleaf pine	5.92	2.15	73.52	18.41
wiregrass	5.55	3.96	73.74	16.74
little bluestem	6.34	3.99	76.53	13.14

predicted by the piloted ignition criteria of the heat release rate of 24 kW m^{-2},[69] it is necessary to know the net heat of combustion H_c (kJ g^{-1}) of each significant volatile component. When the volatile mass rate (g s^{-1} m^{-2}) of each component is known, the potential heat release rate can then be predicted and compared to the piloted ignition criteria for establishing a stable flame. One complication of this analysis is that volatile components of organic materials can undergo further secondary pyrolysis at the high temperatures (near a diffusion flame sheet) thus forming simpler molecules that are flammable as well and possess their own heats of combustion. Using gas phase kinetics to predict the secondary pyrolysis can be feasible,[70] but it is not practical for most situations, as also in this case. However, it can be shown that the overall heat of combustion changes little during the secondary pyrolysis process, even at the limit of producing syngas and soot at very high temperatures (between 8% and 13% increases in H_c for the idealized conversion) as discussed later (in heats of combustion calculation section). It is therefore sufficient, for flammability considerations, to only derive the heat of combustion of each leaf component without consideration for the secondary pyrolysis.

Since the heat of combustion (whether gross or net) has been correlated well with the elemental formula of the fuel,[71] there is the need to define the model compound for the 11 leaf components (it is not needed for the minerals which are nonflammable), as provided in Figure 7. Correlation of ultimate analysis results with the leaf components was best obtained when the ultimate analysis raw data shown in Table 12 are rescaled on the dry and ash free (daf) basis. However, elemental formulas were not clear-cut for complex model compounds of lipids, proteins, lignin, and phenolic material given the uncertainties provided in the literature[13] and the inability to obtain pure component extracts in general. This was resolved empirically in this work by comparing reported literature values (coupled with LC/MS analysis in the case of lipids and Kjehldal analysis in the case of proteins) to the ultimate analysis test results for the leaves on a dried and ash free basis as follows.

Conversion to Dry and Ash-Free Basis. The process for converting the ultimate analysis data to dry and ash free basis is as follows. We note that the value for O is the least accurate as it was determined by difference, and it was not directly measured (according to ASTM D5291). Since the samples were analyzed on an "as-is" basis, it was necessary to convert the results to a dry ash-free (daf) basis to be equivalent to measurements taken on all other sample components. The first conversion step was to a dry basis that involved adjusting H and O to a slightly lower value comparatively to correspond to the dried fuel and then renormalizing all elements, CHONS, and ash to the dried basis. The total N (TN) on a dried basis

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Table 12. Ultimate Analysis (ASTM D5291, ASTM D4239, and ASTM E711) Data on Set B on as Received Basis (Keystone Laboratory, Newton, IA)

plant species: as received basis	sulfur (%)	carbon (%)	hydrogen (%)	nitrogen (%)	oxygen (%)	LHV (BTU/lb)	HHV (BTU/lb)
saw palmetto	0.15	47.21	5.91	0.67	41.12	7155	7703
dwarf palmetto	0.5	45.09	6.35	1.46	38.94	6981	7569
swamp bay	0.22	49.39	6.36	0.9	39.03	7816	8405
yaupon	0.16	47.84	6.56	1.2	38.63	7643	8251
inkberry	0.06	52.49	6.67	0.39	37.83	8301	8919
wax myrtle	0.18	48.14	5.65	1.07	40.43	7162	7685
fetterbush	0.13	50.14	6.15	0.36	40.28	7770	8340
live oak	0.18	45.86	5.96	1.42	41.59	6938	7491
water oak	0.17	48.9	6.13	0.74	40.12	6929	7497
wiregrass	0.25	43.3	5.84	2.71	43.94	7023	7564
little bluestem	0.18	46.75	6.35	2.72	40.01	7023	7612

Table 13. Ultimate Analysis of Vacuum-Dried Plants on an Ash Free Basis (Derived from Table 12)

plant species	carbon (% (daf))	hydrogen (% (daf))	oxygen (% (daf))	nitrogen (% (daf))	sulfur (% (daf))	LHV (kJ/g (daf))	HHV (kJ/g (daf))
saw palmetto	50.88	5.51	42.57	0.89	0.16	18.94	20.40
dwarf palmetto	48.27	6.01	43.06	2.13	0.54	18.94	20.53
swamp bay	52.83	6.03	39.55	1.36	0.24	20.34	21.88
yaupon	51.14	6.24	40.98	1.46	0.17	20.22	21.83
inkberry	54.97	6.46	37.64	0.87	0.06	20.77	22.32
wax myrtle	51.93	5.22	40.35	2.31	0.19	18.90	20.28
fetterbush	52.87	5.88	40.31	0.80	0.14	19.67	21.11
live oak	49.53	5.55	42.42	2.30	0.19	18.42	19.89
water oak	52.32	5.78	40.24	1.47	0.18	18.00	19.48
longleaf pine	51.06	5.93	41.68	1.22	0.11	18.64	20.10
wiregrass	45.84	5.53	45.49	2.87	0.26	18.05	19.44
little bluestem	49.91	6.03	40.96	2.90	0.19	18.22	19.75

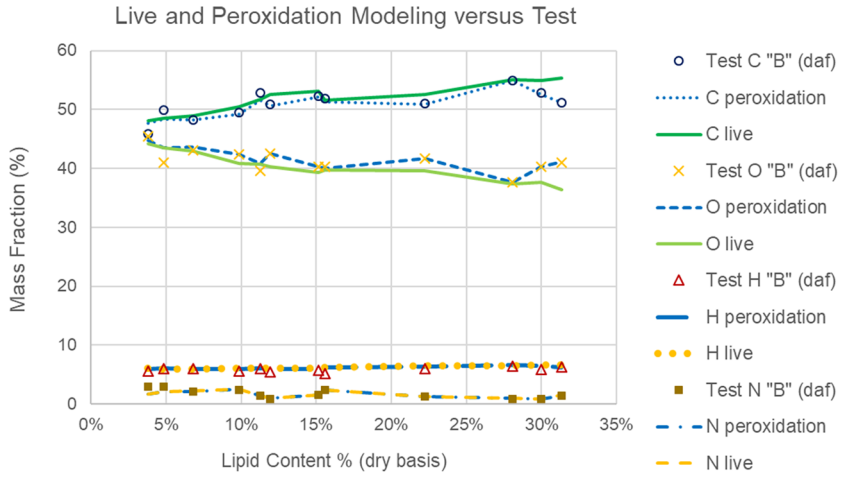

Figure 8. Leaf elemental composition (% mass) for C, H, O, and N, including lipid peroxidation model plotted versus lipid content on dry ash free (daf) basis.

was available from the more accurate mineral analysis discussed above. Since TN was typically greater than the N from the ASTM method D5291, the second conversion step was to reduce O value lower by TN − N difference value and the value for N was then replaced by the TN value, to maintain the mass balance. The third conversion step was renormalizing the adjusted CHONS further to the ash-free basis (daf). Table 13 provides the result of these conversions for the 12 species, and the data are also plotted in Figure 8, as elemental compositions of C, H, O, and N versus the lipid content (S is negligible and because it is associated with crude protein in any case, it is lumped with N in the figure), and in Figure 10, as HHV plotted versus the lipid content.

Prediction of Elemental Mass of Leaf Components. During the proximate and ultimate analysis of the leaves as sent to a contract laboratory for standard testing (Tables 11 and 12), it was likely that hydrolysis and/or peroxidation had occurred to some degree in the samples, despite the insertion of portable desiccants and oxygen absorbers in the shipped aluminized bags of the vacuum oven-dried samples. Samples were exposed to normal humidified air during greenhouse preparation and at the contract laboratory. This left lipid, protein, pectin, and starch components in the leaves

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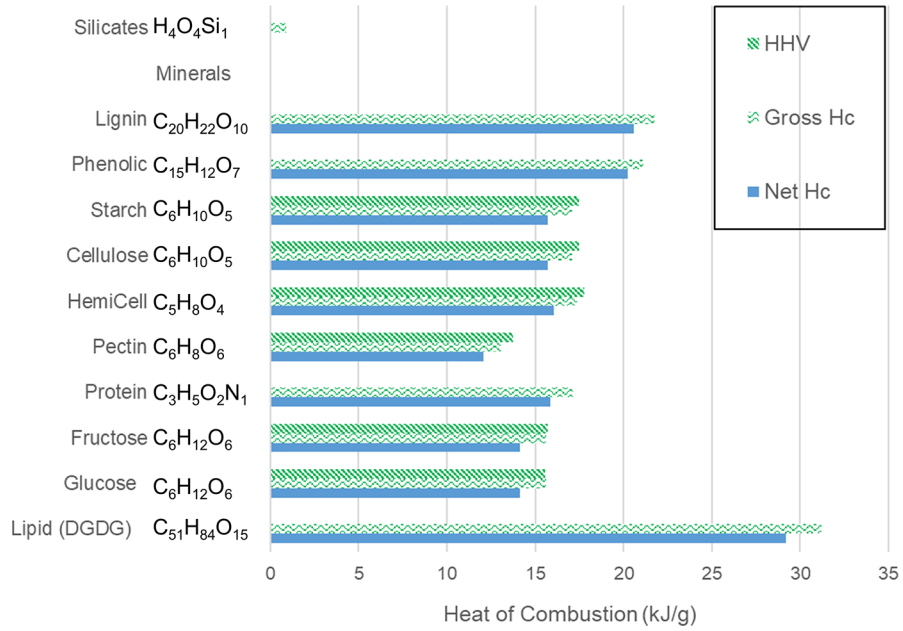

Figure 9. Predicting the heats of combustion for plant leaf components by comparing gross heat of combustion to the higher heating value (HHV). Net heat of combustion is provided for computer modeling of fire.

susceptible to oxidation (i.e., lipid peroxidation—a.k.a. rancidity) and other forms of biochemical degradation (i.e., enzyme hydrolysis), thereby partly changing the live composition by the time of the ultimate analysis measurements. To accommodate the possible peroxidation/rancidity factor, we merely added oxygen, O_2 (up to a theoretical limit), to the elemental formula of the lipid model compound due to the initiation of lipid peroxidation.[72] Additions of H_2O to other components (protein, pectin, and starch) were found to be unnecessary for modeling their potential change since degradation was minimized due to the very low moisture content of the samples.

Figure 8 shows prediction of the elemental mass formula on the dried and ash free basis (eq 1) using the data in Table 13 (as normalized to 100%) for comparison to the ultimate analysis data for the tested dried leaves, showing the live and peroxided condition. Note that each mass fraction, f, for C, H, O, and N from the elemental formula along with corresponding molar values, n, and molecular weights, M, for each component in eq 1 is in turn weighted by the composition mass fractions of 10 components (lipid, glucose, fructose, protein, pectin, hemicellulose, cellulose, starch, phenolic, and lignin) and summed to obtain the overall mass fractions (daf) of each atomic element in the leaves.

$$f_C = n_C M_C/(n_C M_C + n_H M_H + n_O M_O + n_N M_N)$$
$$f_H = n_H M_H/(n_C M_C + n_H M_H + n_O M_O + n_N M_N)$$
$$f_O = n_O M_O/(n_C M_C + n_H M_H + n_O M_O + n_N M_N)$$
$$f_N = n_N M_N/(n_C M_C + n_H M_H + n_O M_O + n_N M_N) \quad (1)$$

There were six species that did not need the lipid peroxidation, and the best agreement of those to the data was obtained by the elemental model compound formulas for lipid, protein, phenolic, and lignin provided in Figure 7. Model compounds for phenolic chosen as condensed tannins and for lignin chosen as with high oxygen content (i.e., known as LIGO) was obtained from ref 17. In the case of protein model compound, the serine polypeptide, $(C_3H_5O_2N)_x$, was chosen to provide a molecular weight close to 6.25 times the total N content, which is routinely used in test methods as an approximation of "crude protein". The lipid formula was particularly challenging to assign to a model compound. This was due to uncertainty in knowing the amount of peroxidation that occurs on the six double carbon bonds on the digalactosyldiacylglycerol (DGDG) lipid model compound chosen. Six O_2 per DGDG model compound is the theoretical limit for its full peroxidation and is shown by the straight black line in Figure 10. The Excel Spreadsheet Solver was used to optimize the lipid oxidation mass fractions of each sample (given by the yellow dots in Figure 10) for the best fit to the CHON data in Figure 8 to within 0.6% standard deviation of all data in Set B. In this way the additional six samples that required lipid peroxidation could get close agreement with the converted ultimate analysis data (daf) without compromising those other six samples that did not have apparent lipid peroxidation. Of particular note in Figure 8 is the trend of increasing carbon mass fraction and decreasing oxygen mass fraction with the lipid content. This is consistent with the lipid having the lowest relative oxygen content of all the leaf components. This also has an impact on the heat of combustion calculation, which is presented next.

Calculation of Heats of Combustion of Leaf Components. The lower and higher heats of combustion (LHV and HHV) from ultimate analysis are presented in Table 13 in SI units and the HHV versus lipid content is plotted in Figure 10. To predict the heats of combustion, we first determined the net and gross heat of combustion using the oxygen consumption correlation,[71,73] for each composition in the order of lipid, glucose, fructose, protein, pectin, hemicellulose, cellulose, starch, phenolic, lignin, minerals, and silicates, as

$$H_{c,net} = 13.27 \times \left(\frac{2M_o}{M_c} f_c + \frac{M_o}{2M_h} f_h - f_o\right) \quad (2)$$

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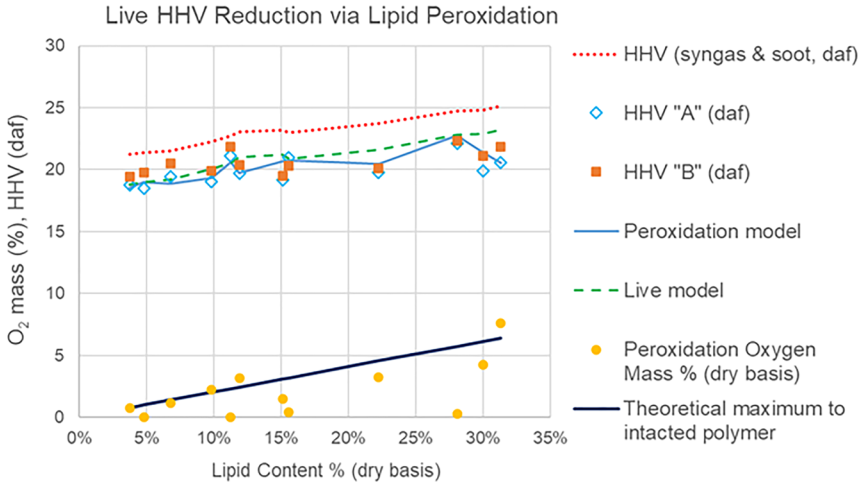

Figure 10. Live leaves HHV plotted versus lipid content for sets A and B and comparison to predicted gross heat of combustion for live, peroxidation, and idealized syngas conditions. Presented on a dry ash free basis (daf).

$$H_{c,gross} = H_{c,net} + 21.96 f_h \quad (3)$$

These are plotted in Figure 9 for each plant component. Also plotted in Figure 9 are the well-known HHV of a few components (glucose, fructose, pectin, starch, cellulose, and hemicellulose (i.e., xylan)).[73,74] It is seen that only small percentage errors are involved with the oxygen consumption correlation with these known components HHV. Since the other components are generally not able to be extracted purely, the HHV values for them are inherently not available, nor reliable. The crude lipid obviously has the highest heat of combustion, at around 30 kJ g^{-1}, with lignin being the next highest, at around 20 kJ g^{-1}. Since the crude lipid is likely to pyrolyze before the other combustible volatiles, it may very well be the parameter, with its high heat of combustion and its high content in some leaves, controlling the flammability (i.e., piloted ignition). However, for the live leaves this notion is made complicated by the water evaporation of the live leaf that lowers the overall volatile heat of combustion, possibly obscuring the flammability of the various species.[75]

Summing these heats of combustion as weighted by their composition mass fractions (provided in Table 10 and normalized) obtains the overall heat of combustion. The comparison of the predicted gross heat of combustion to the higher heating value (HHV) (Figure 10) includes the live peroxidation condition, confirming the use of eqs 2 and 3 for estimating heats of combustion of the leaf elements. It is seen that the gross heat of combustion for the live dried leaves, increases from 19 to 23 kJ g^{-1} as the lipid content increases from 4% to 31%. The variation of gross heat of combustion with other components, such as cellulose, lignin, and protein, were not as prominent as the lipid. The stoichiometric heat of combustion per oxygen mass consumed was optimized to $H_{c,ox}$ = 13.27 ± 0.56 kJ g^{-1}, for the best fit to both set A and set B samples in Figure 10. This can be compared to the cone calorimeter standard (ASTM E1354) of $H_{c,ox}$ = 13.1 ± 0.7 kJ g^{-1} and that of forest materials, $H_{c,ox}$ = 13.23 ± 0.66 kJ g^{-1} (Dietenberger[69]). Finally, shown in Figure 10 is the calculated gross heat of combustion in the case of the dried fresh leaf material converted via secondary pyrolysis at very high temperatures entirely to solid carbon, CO, and H_2 resulting in an averaged positive shift of 2 kJ g^{-1} for this idealized conversion into syngas, which is within 10% of the averaged heat of combustion of about 20 kJ g^{-1} for the dried leaves. Indeed, a reasonable secondary pyrolysis into high production of tar at around 500 °C as indicated by Table 11, may only result in negligible shift in the heat of combustion for flaming. Thus, any issues relating to the leaf flammability can adequately be addressed by primary pyrolysis compounds' mass flow rate and their corresponding heat of combustion, helping to simplify CFD modeling of controlled burns.

SUMMARY AND CONCLUSIONS

Chemical composition analysis was performed on live leaves from 12 plants native to the southeastern US, in order to obtain a summative mass balance. These data are intended to support pyrolysis tests such as ultimate analysis, TGA, DSC, and cone calorimetry. Composition analysis is used to determine empirical formulas, pyrolysis temperatures, heats of combustion, and other properties for use in combustion modeling within computational fluid dynamics models (e.g., FDS) for prescribed burns. Analytical methods were adapted from standard protocols commonly used in the evaluation of wood and feedstocks (particularly NREL ASTM and TAPPI). Ten of the mass balances ranged from 95 to 100%, while two others were 91%. Summative mass closure was reached by measuring 12 components: lipophilic extractives, glucose, fructose, protein, pectin, hemicellulose, cellulose, starch, phenols, lignin, silicates, and minerals. All components fell into the following primary plant metabolite groups: lipids (extractives), structural carbohydrates (cellulose, hemicellulose), additional carbohydrates (pectin, glucose, fructose, and starches), protein, structural lignin, and phenols. Although most standard biomass methods were adaptable to live leaf analysis, some modifications/adjustments were made: (1) Acid insoluble lignin (Klason) was analyzed/adjusted for protein and silicate content. (2) Extractives were analyzed/adjusted for carbohydrates. (3) Structural carbohydrates were adjusted for additional carbohydrates (glucose, fructose, pectin and starch). (4) Acid soluble lignin was assumed to be largely phenolic in nature and represented by a polyphenolic unit for a reference compound. (5) Filtration of extracts took place through a 0.45 μM filter to remove structural particulates. These relatively exhaustive adjustments were necessary to obtain mass balances mostly >95% and <100% by avoiding redundant analysis. Additionally the method for extraction (commonly done with

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soxhlate extractions) was modified (for live leaves) by using a bead mill assisted extraction with 1:1 hexane:2-propanol followed by a 9:1 mixture of acetone−water. This was the most complete method for cell disruption capable of releasing lipids and absorbed moisture and separating structural components of the leaves.

Of particular note was the extent to which composition of live leaves differs from typical biomass feed stocks by reaching extractives levels of 30% of DM (inkberry and fetterbush). Differences were also noted in protein, sugar, and phenolic composition of live leaves. These data when presented with pyrolysis tests (outside the scope of this paper) suggest that predictive fire modeling for prescribed burn applications would benefit by including these additional parameters (that will likely affect ignition and flammability behavior, particularly with the variation to the heat of combustion). Further compositional differences between leaves and biomass noted within the structural carbohydrates were (1) hemicellulose of the leaf is composed primarily of xylose/arabinose whereas woody biomass has higher levels of mannan, (2) pectin as a polysaccharide which functions in maintaining leaf cell structure was present in levels up to 3% of DM and is reported to be in highest concentrations in leaves,[76] and (3) cellulose of the leaf has a significant amorphous structure (private communication with U. Agarwall, Raman Spectroscopist at FPL, see Acknowledgments). These compositional differences in dried leaves and woody biomass are likely to affect pyrolysis behavior in the various tests (TGA and cone calorimeter tests performed as part of larger pyrolysis study) and modeling parameters derived from data obtained.

By employing a summative analysis approach to leaf characterization, it was concluded that additional analysis parameters to commonly measured components of; lignin, cellulose, and hemicellulose more completely describe the biochemical components of live leaves. Such an approach reveals observable differences within and between plant species which could be extended to ecosystems where fire management and modeling practices are in use. Once mass fractions of live leaf components are obtained, these data can be combined with data from carefully selected model compounds to arrive at a chemical empirical formula from which leaf empirical formula and heats of combustion can be calculated. These data along with TGA measurements of pyrolysis profiles are the basis for predicting ignition. We believe this suite of analyses could be adapted to field use since relatively simple wet chemical procedures were often used to obtain mass balance. Given the current technologies available for portable field instrumentation/analysis, a rich data sample representing the forest ecosystem under study could be obtained in situ. This would in turn provide more real-time and seasonal fire modeling data when considering forest management techniques such as controlled burning.

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Notes

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REFERENCES

(1) Waldrop, T. A.; Goodrick, S. L. *Introduction to Prescribed Fires in Southern Ecosystems, Science Update SRS-054*, USDA Forest Service, Southern Research Station: Asheville, NC, 2012; p80.

(2) Countryman, C. M.; Charles, W.; Philpot, C. W. *Physical Characteristics of Chamise as a Wildland Fuel*, Research Paper PSW-66, USDA Forest Service, Pacific Southwest Forest and Range Experiment Station: Berkeley, CA, 1970, 1−16.

(3) Li-Beisson, Y. Ch. 4 Summary of Arabidopsis Lipid Composition. Acyl-Lipid Metabolism. *The Arabidopsis Book* **2013**, *11*, e0161.

(4) Shotorban, B.; Yashwanth, B. L.; Mahalingam, S.; Haring, D. J. An Investigation of Pyrolysis and Ignition of Moist Leaf-Like Fuel Subject to Convective Heating. *Combust. Flame* **2018**, *190*, 25−35.

(5) Rahimi Borujerdi, P.; Shotorban, B.; Mahalingam, S.; Weise, D. R. Modeling of Water Evaporation from a Shrinking Moist Biomass Slab Subject to Heating: Arrhenius Approach vs. Equilibrium Approach. *Int. J. Heat Mass Transfer* **2019**, *145*, 118672.

(6) Mulky, T. C.; Niemeyer, K. E. Computational Study of the Effects of Density, Fuel Content and Moisture Content on Smoldering Propagation of Cellulose and Hemicellulose Mixtures. *Proc. Combust. Inst.* **2019**, *37*, 4091−4098.

(7) Chandler, C.; Cheney, P.; Thomas, P.; Trabaud, L.; Williams, D. *Fire in Forestry*; Wiley: New York, 1983; Vol. *1.2*.

(8) Weise, D. R.; Fletcher, T. H.; Johnson, T.J.; Hao, W. M.; Dietenberger, M.; Princevac, M.; Butler, B. A Project to Measure and Model Pyrolysis to Improve Prediction of Prescribed Fire Behavior. *Advances in Forest Fire Research* **2018**, 308.

(9) Melvin, M. A.; *2015 National Prescribed Fire Use Survey Report*, Technical Report 02-15. Coalition of Prescribed Fire Councils, Inc.: 2015.

(10) NREL Laboratory Analytical Procedures; Online http://www.nrel.gov/biomass/analytical_procedures.html, Dec 2009.

(11) ASTM Standards; http://www.astm.org/Standard/index.shtml; Dec. 2009.

(12) *Determination of Structural Carbohydrates and Lignin in Biomass NREL/TP-510−42618*; June 2010.

(13) Templeton, D. W.; Scarlata, C. J.; Sluiter, J. B.; Wolfrum, E. J. Compositional analysis of Lignocellulosic Feedstocks. 2) Method Uncertainties. *J. Agric. Food Chem.* **2010**, *58*, 9054−9062.

(14) Hatfield, R.; Fukushima, R. S. Can Lignin Be Accurately Measured? *Crop Sci.* **2005**, *45*, 832−839.
(15) Sariyildiz, T.; Anderson, J. M. Variation in the Chemical Composition of Green Leaves and Leaf Litters from Three Deciduous Tree Species Growing on Different Soil Types. *For. Ecol. Manage.* **2005**, *210*, 303−319.
(16) Halls, L. K.; Knox, F. E.; Lazar, V. A. *Common Browse Plants of the Georgia Coastal Plain—Their Chemical Composition and Contribution to the Cattle Diet*. Station Paper No 75, USDA: 1957.
(17) Debiagi, P. E. Q.; Pecchi, C.; Gentile, G.; Frassoldati, B. A.; Cuoci, A.; et al. Extractives Extend the Applicability of Multistep Kinetic Scheme of Biomass Pyrolysis. *Energy Fuels* **2015**, *29*, 6544−6555.
(18) Miranda, I.; Sousa, V.; Ferreira, J.; Pereira, H. Chemical Characterization and Extractives Composition of Heartwood and Sapwood from *Quercus Faginea*. *PLoS One* **2017**, *12* (6), No. e0179268.
(19) Jolly, W. M.; Hadlow, A. M.; Huguet, K. De-coupling Seasonal Changes in Water Content and Dry Matter to Predict Live Conifer Foliar Moisture Content. *Int. J. Wildland Fire* **2014**, *23*, 480−489.
(20) Chinou, J. Primary and Secondary Metabolites and Their Biological Activity. *Thin Layer Chromatography in Phytochemistry* **2008**, *99*, 59−75.
(21) Guiheneuf, F.; Schmid, M.; Stengel, D. B. Lipids and Fatty Acids in Algae: Extraction, Fractionation into Lipid Classes, and Analysis by Gas Chromatography Coupled with Flame Ionization Detector (GC-FID). *Methods Mol. Biol. (N. Y., NY, U. S.)* **2015**, *1308*, 173−190.
(22) Byreddy, A. R.; Gupta, A.; Barrow, C. J.; Puri, M. Comparison of Cell Disruption Methods for Improving Lipid Extraction from Thraustochytrid Strains. *Mar. Drugs* **2015**, *13*, 5111−5127.
(23) Benchmark Scientific Product Note: Benchmark Bead Beating Guide.
(24) Fishwick, M. J.; Wright, A. J. Comparison of Methods for the Extraction of Plant Lipids. *Phytochemistry* **1977**, *16*, 1507−1510.
(25) Mikulic Petkovsek, M.; Stampar, F.; Veberic, R. Seasonal changes in phenolic compounds in the leaves of scab-resistant and susceptible apple cultivars. *Can. J. Plant Sci.* **2009**, *89*, 745−753.
(26) Alibas, I. Microwave, Vacuum, and Air Drying Characteristics of Collard Leaves. *Drying Technol.* **2009**, *27* (11), 1266−1273.
(27) Figiel, A.; Szumny, A.; Gutierrez-Ortíz, A.; Carbonell-Barrachina, A. Composition of oregano essential oil (Origanum vulgare) as affected by drying method. *J. Food Eng.* **2010**, *98*, 240−247.
(28) Rahimmalek, M.; Goli, S. A. H. Evaluation of Six Drying Treatments with Respect to Essential Oil Yield, Composition and Color Characteristics of Thymys Daenensis Subsp. *Ind. Crops Prod.* **2013**, *42*, 613−619.
(29) Davis, D. L., Nielson, M. T., Eds., *Tobacco: Production, Chemistry, and Technology*; Blackwell Science (Pub.): 1999.
(30) Abascal, K.; Ganora, L.; Yarnell, E. The Effect of Freeze-Drying and Its Implications for Botanical Medicine: A Review. *Phytother. Res.* **2005**, *19* (8), 655−660.
(31) Safdari, M. S.; Rahmati, M.; Amini, E.; Howarth, J.; Berryhill, J. P.; Dietenberger, M.; Weise, D. R.; Fletcher, T. H. Characterization of Pyrolysis Products from Fast Pyrolysis of Live and Dead Vegetation Native to the Southern United States. *Fuel* **2018**, *229*, 151−166.
(32) Amini, E.; Safdari, M. S.; DeYoung, J. T.; Weise, D. W.; Fletcher, T. H. Characterization of Pyrolysis Products from Slow Pyrolysis of Live and Dead Vegetation Native to the Southern United States. *Fuel* **2019**, *235*, 1475−1491.
(33) Lindroth, R. L.; Koss, P. A. Preservation of Salicaceae Leaves for Phytochemical Analyses: Further Assessment. *J. Chem. Ecol.* **1996**, *22* (4), 765−71.
(34) Liu, Y.; Wang, X. Extraction and Profiling of Plant Polar Glycerol Lipids. *Bio-protocol* **2016**, *6* (12), 1849 DOI: 10.21769/BioProtoc.1849.
(35) Roughan, P. G.; Batt, R. D. The Glycerolipid Composition of Leaves. *Phytochemistry* **1969**, *8*, 363−369.
(36) Fuller, G.; Stumpf, K. D. Fatty Acids in Plants: A Model System. *Plant Lipids and Their Interactions* **1987**, *325*, 44−52.
(37) Raven, P. H.; Evert, R. F.; Curtis, H. *Biology of Plants*, 3rd ed.; Worth Publishers: New York, 1981.
(38) Miranda, I.; Sousa, V.; Ferreira, J.; Pereira, H. Chemical Characterization and Extractives Composition of Heartwood and Sapwood from Quercus Faginea. *PLoS One* **2017**, *12* (6), No. e0179268.
(39) *Solvent Extractives of Wood and Pulp: Test Method T 204 cm-17*. TAPPI Journal: 2007; T 204 cm-97.
(40) Giorgis, I.; McDonald, S.; Astarita, G. *Lipid Separation using UPLC with Charged Surface Hybrid Technology*; Waters Corporation: Milford, MA, Application note.
(41) Guckert, J. B.; White, D. C. Evaluation of a Hexane/Isopropanol lipid solvent system for analysis of bacterial phospholipids andapplication to chloroform-soluble Nuclepore (polycarbonate) membranes with retained bacteria. *J. Microbiol. Methods* **1988**, *8* (3), 131−131.
(42) AOCS. Rapid Determination of Oil/Fat Utilizing High Temperature Solvent Extraction. *Ancom user manual*; 2017; AOCS AM 5-04.
(43) Axelsson, M.; Gentili, F. A Single-Step Method for Rapid Extraction of Total Lipids from Green Microalgae. *PLoS One* **2014**, *9* (2), e89643.
(44) Kind, T.; Liu, K.-H.; Lee, D. Y.; DeFelice, B.; Meissen, J. K.; Fiehn, O. LipidBlast in Silico Tandem Mass Spectrometry Database for Lipid Identification. *Nat. Methods* **2013**, *10* (8), 755−758.
(45) Bellasio, C.; Fini, A.; Ferrini, F. Evaluation of a High Throughput Starch Analysis Optimised for Wood. *PLoS One* **2014**, *9* (2), e86645.
(46) *Megazyme Total Starch Assay Procedure Amyloglucosidase α-Amylase Method*. K-TSTA-50A/K-TSTA-100A Assay Booklet: 2019.
(47) AOACProtein (Crude) Determination in Animal Feed: $CuSO_4/TiO_2$ Mixed Catalyst Kjeldahl Method. (988.05) *Official Methods of Analysis*; Association of Official Analytical Chemists: Washington, DC, 1984.
(48) Bremmer, J. M. Total nitrogen. *Agronomy* **2016**, *9*, 1149−1178.
(49) Effland, M. J. procedure to determine acid-insoluble lignin in wood and pulp. *Tappi J.* **1977**, *60* (10), 143−144.
(50) Davis, M.W. A. Rapid Modified Method for Compositional Carbohydrate Analysis of Lignocellulosics by High PH Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC/PAD). *J. Wood Chem. Technol.* **1998**, *18* (2), 235−252.
(51) Monsoor, M. A.; Kalapathy, U.; Proctor, A. Determination of Polygalacturonic Acid in Pectin Extracts by Diffuse Reflectance Fourier Transform Infared Spectroscopy. *Food Chem.* **2001**, *74*, 233−238.
(52) Vazquez-Blanco, M. E.; Vazquez-Oderiz, M. L.; Lopez-Hernandez, J.; Simal-Lozano, J.; Romero-Rodriguez, M. A. HPLC Determination of Pectins in Raspberries as Galacturonic Acid and Optimization Using Forward Optical Scanning. *J. Chromatogr. Sci.* **1993**, *31*, 477−479.
(53) Voragen, A.; Coenen, G.-J.; Verhoef, R.; Schols, H. A. A Versatile Polysaccharide Present in Plant Cell Walls. *Struct. Chem.* **2009**, *20*, 263−275.
(54) Muller-Maatsch, J.; Bencivenni, M.; Caligiani, A.; et al. Pectin content and composition from different food waste streams. *Food Chem.* **2016**, *201*, 37−45.
(55) Fagerstedt, K. V.; Saranpaa, P.; Tapanila, T.; Immanen, J.; Serra, J.; Nieminen, K. Determining the Composition of Lignins in Different Tissues of Silver Birch. *Plants* **2015**, *4*, 183−195.
(56) Klason, P. *Svensk Papperstidn* **1923**, *26*, 319.
(57) Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. *Determination of Structural Carbohydrates and Lignin in Biomass, online LAP method*, June 2010; www.nrel.gov/biomass/analytical_procedures.html.
(58) Nicholson, D. J.; Leavitt, A. T.; Francis, R. C. A Three-Stage Klason Method for More Accurate Determinations of Hardwood Lignin Content. *Cellulose Chem. Technol.* **2014**, *48* (1−2), 53−59.

(59) Constantinides, M.; Fownes, J. H. Nitrogen Mineralization from Leaves and Litter of Tropical Plants: Relationship to Nitrogen, Lignin and Soluble Polyphenol Concentrations. *Soil Biol. Biochem.* **1994**, *26*, 49−55.
(60) Hattenschwiler, S.; Hagerman, A. E.; Vitousek, P. M. Polyphenols in Litter from Tropical Montane Forests across a Wide Range in Soil Fertility. *Biogeochemistry* **2003**, *64* (1), 129−148.
(61) *Quantification of Tannins in Tree Foliage*; FAO/IAEA Working Document; 2000; pp 4−6.
(62) Mills, H. A.; Jones, J. B. *Plant analysis handbook II. A practical sampling, preparation, analysis, and interpretation guide*. Micro-Macro Publishing, Inc., Athens, GA, 1996.
(63) Aziz, A.; Larher, R. F. Osmotic Stress Induced Changes in Lipid Composition and Peroxidation in Leaf Discs of Brassica napus L. *J. Plant Physiol.* **1998**, *153*, 754−762.
(64) *Lipid Web*: online www.lipidhome.co.uk/lipids/complex.
(65) Jones, D.; Schimleck, L. R.; Peter, G. F.; Daniels, R. F.; Clark, A. Nondestructive estimation of wood chemical composition of sections of radial wood strips by diffuse reflectance near infrared spectroscopy. *Wood Sci. Technol.* **2006**, *40*, 709−720.
(66) Jakobek, L. Interactions of polyphenols with carbohydrates, lipids and proteins. *Food Chem.* **2015**, *175*, 556.
(67) Templeton, D. W.; Wolfrum, E. J.; Yen, J. H.; Sharpless, K. Compositional Analysis of Biomass. *BioEnergy Res.* **2016**, *9* (1), 303−314.
(68) Dixon, W. J. Processing Data for Outliers. *Biometrics* **1953**, *9* (1), 74.
(69) Lyon, R.; Quintiere, J. Criteria for piloted ignition of combustible solids. *Combust. Flame* **2007**, *151*, 551−559.
(70) Ranzi, E.; Cuoci, A.; Faravelli, T.; Frassoldati, A.; Migliavacca, G.; Pierucci, S.; Sommariva, S. Chemical Kinetics of Biomass Pyrolysis. *Energy Fuels* **2008**, *22* (6), 4292−4300.
(71) Dietenberger, M. Update for combustion properties of wood components. *Fire Mater.* **2002**, *26*, 255−267.
(72) Ghnimi, S.; Budilarto, E.; Kamal-Eldin, A. The New Paradigm for Lipid Oxidation and Insights to Microencapsulation of Omega-3 Fatty Acids. *Compr. Rev. Food Sci. Food Saf.* **2017**, *16*, 1206−1218.
(73) Ioelovich, M. Comparison of methods for calculating the combustion heat of biopolymers and vegetable biomass. *ChemXpress Research* **2016**, *9* (6), 107−115.
(74) Babrauskas, V. Chapter 8: Related Quantities (a) Heat of Combustion and Potential Heat. In *Heat Release in Fires*; Babrashskas, V., Grayson, S. J., Eds.; Elsevier Applied Science: London and New York, 1992.
(75) Weise, D. R.; Fletcher, T.; Mahalingam, S.; McAllister, S.; Shotorban, B.; Jolly, M. *Determination of the Effects of Heating Mechanisms and Moisture Content on Ignition of Live Fuels*, Final Report, Project 11-1-4-19, Joint Fire Science Program.
(76) Brown, A. L.; Hames, B. R.; Daily, J. W.; Dayton, D. C. Chemical Analysis of Solids and Pyrolytic Vapors from Wildland Trees. *Energy Fuels* **2003**, *17*, 1022−1027.

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