ABSTRACT

Quantitatively Determining the Degradation of Waterlogged Archaeological Wood

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July, 2013

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Archaeological artifacts are an essential part of preserving history and culture, and can be valuable tools for enhancing education and contributing economic benefits through tourism. Of the artifacts that one may encounter, waterlogged archaeological wood is one of the most difficult materials to conserve. Without knowing the level of degradation of the wood, conservators cannot properly stabilize and conserve the waterlogged archaeological wooden artifact. Currently, an established method commonly used to determine the level of degradation of waterlogged archaeological wood is the pin test. Alternative and more quantitative methods are destructive, expensive, time consuming, and not readily available to archeologists or conservators. An analytical method that can quantitatively determine the degradation level accurately in order to properly stabilize and conserve the waterlogged archaeological wood would be a valuable tool for a conservation specialist.

The main goal of this project was to develop such a method. Lignin was chosen as the primary component of analysis in this study, since it is the last structural component within wood to degrade. Determining the concentration of lignin will result in a more quantitative measure of

the degree of degradation. Preliminary infrared data revealed a trend in lignin concentrations confirming that infrared spectroscopy has the potential to quantitatively determine the degradation of waterlogged archaeological wood. Two gravimetric methods were utilized to obtain percentages of lignin concentration within tongue depressors and compared to create an improved gravimetric analysis. The lignin quantities found from the modified gravimetric analyses were used as reference data with infrared spectroscopy so that known quantities of lignin may be compared with and verify the spectroscopic results. If the concentrations of lignin obtained by infrared spectroscopy are confirmed by the improved gravimetric analysis results, then a new nondestructive and quantitative method to determine the level of degradation of wooden waterlogged artifacts will only take minutes rather than weeks.

A QUANTITATIVE AND NONDESTRUCTIVE METHOD FOR DETERMINING THE DEGRADATION OF WATERLOGGED ARCHAEOLOGICAL WOOD

A Thesis

Presented to

the Faculty of the Department of Chemistry

East Carolina University

Greenville, North Carolina

In Partial Fulfillment

of the Requirements for the Degree

Master's of Science in Chemistry

By:

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July, 2013

A Quantitative and Nondestructive Method for Determining the Degradation of Waterlogged Archaeological Wood

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Acknowledgments

I would like to thank my advisor, Dr. Anne Spuches for her dedication, encouragement, and willingness to guide me through the development and creation of this thesis. There were difficult times during this process, but you helped me see the light. This thesis would never have been completed without you.

I also wish to acknowledge my thesis committee: Dr. Andrew Morehead, Dr. Colin Burns, Dr. John M. Kenney, and Susanne Grieve. Thank you for your patience, guidance, and constant optimism and contribution.

I would like to specifically thank Dr. Burns for giving me the opportunity to become involved in research as an undergrad. This opportunity provided insight to the direction of my future career.

Finally, I would like to express my gratitude to my family. Without their love, support, and enthusiasm, this process did not seem possible. Thank you, Mom and Dad.

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Abbreviations

IR	Infrared Spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy
ATR	Attenuated Total Reflectance
CE	Extraction #1 using cyclohexane and ethanol (2:1 v/v)
EE	Extraction #2 using ethanol ≥95%
W	Extraction #3 using boiling water
KLE	Extraction #4 using sulfuric acid to obtain Klason lignin
A	After extraction
B	Before extraction
tt	Test tube
TSL	Total sample loss during transfer
TWWt	Accurate total sample weight accounting for sample loss
WWt	Sample weight
E	Dry weight of extractives
%E	Percent extractive weight
F	Filter weight
tw	Thimble weight
P	500 mL pyrex bottle weight
KL	Klason lignin weight
%KL	Percent Klason lignin

Chapter 1: Introduction

1.1 General Background

Archaeological artifacts are an essential part of preserving history and culture, and can be valuable tools for enhancing education. Artifacts may also provide economic benefits such as stimulating tourism. [1] Ships, for examples of larger scale artifacts, have had a major impact on the development of civilization and communication throughout the world. [2] A shipwreck is a unique piece of history that should be considered as a time capsule. [2] Once a ship has been excavated, a wealth of artifacts may be obtained that can provide information regarding the path the ship traveled, which would support the purpose and period of time the vessel was in use. Metal artifacts such as weapons, instruments, jewelry, and fasteners will corrode from seawater and possibly become unrecognizable due to concretions. Organic materials, such as any wooden objects and the ship itself, will chemically degrade and potentially be consumed by marine life depending upon the environment in which it rests.

One of the most difficult materials to conserve is waterlogged archaeological wood. It is an extremely difficult material to handle due to its vulnerability to decay from the moment of excavation. Wood is anisotropic, meaning that its physical characteristics in different directions are not the same making its structure inconsistent. [3] The density, porosity, hardness, strength, and flexibility of fresh wood differ from species to species. There can even be enormous variability within a single species depending on the age of the wood and part of the tree the wood is taken from. [3]

When wood is submerged in water over a period of time the water will slowly diffuse into the pores of the wood until it is completely saturated. The water within the pores causes degradation and the breakdown and loss of structural components. This deterioration and loss of

structure is supplemented by water becoming a structural support. Once water is removed, water can no longer act as a structural support, therefore leaving the remainder of the degraded structural components to hold the function and purpose of the object.

Consequently, the fundamental challenge of conserving waterlogged wood is the drying process. Drying waterlogged wood must be done in a controlled manner to prevent collapse, shrinkage, distortion, splitting, and possibly complete disintegration. [2] The level of breakdown or loss depends upon the level of degradation. In order to prevent this, it is critical for conservation specialist to know the chemical nature and structure of these items to determine the proper method of preserving these finds.

1.2 Waterlogged Archaeological Wood

Waterlogged archeological wood refers to wood that has been subjected to a burial environment – regardless of size, species, age, or state of preservation – and is saturated with water. [4] Age alone is meaningless in determining the condition of the waterlogged archaeological wood as deterioration depends on a wide variety of variables. Some of these include the type of wood, the environment the wood has been exposed to, and the time spent in this environment.

Regardless of the environment, if the wood is allowed to air dry it will shrink, warp, and crumble. This is because the wood has undergone severe structural degradation and the cells within the wood lack structural support. As a result of degradation, distance between the cells and molecules increase, and the waterlogged wood becomes more porous and permeable to water. [5] These deteriorated areas of the wood, including cell cavities and intermolecular spaces, are filled with water, which is referred to as bound water. [6] It is the water that is within the wood that now acts as the structural support, which has been preventing drastic dimensional changes.

Once the water is released, the cells begin to collapse and/or shrink. Figure 1 demonstrates how uncontrolled drying of waterlogged wood can completely destroy a structure. The severity of this destruction is dependent upon how degraded the artifact has become.[4,7] In order to properly stabilize and conserve waterlogged archaeological wood, the level of degradation of the sample should be determined.

Figure 1. A screen shot from a video depicting the structural damage caused by uncontrolled drying of waterlogged wood. Image courtesy of the Mariners' Museum.

1.3 Factors that Influence the Degradation of Waterlogged Archaeological Wood

Not only does water itself facilitate degradation, but there are abundant environmental factors that influence the degradation of waterlogged wood as well. There are a plethora of chemical, biological, and physical degradation factors that include, but are not limited to, pH, oxidizing-reducing characteristics of sediment, temperature, pressure, depth of burial, and salinity. The microenvironment also has a tremendous effect on whether fungal or bacterial activity will affect the degradation of the wood as well. [8] Each microenvironment in a marine burial site (anaerobic sediments, aerobic sediments, sediment-seawater interface, and others) will degrade the archaeological wood differently. [7] Teredo worms (shown in Figure 2 below) cause irreversible damage to wooden materials in a maritime environment.

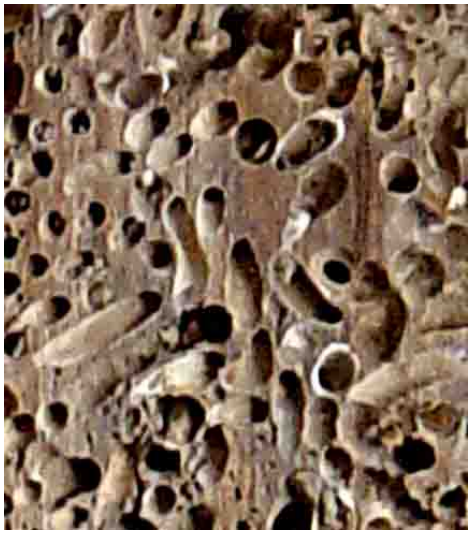


Figure 2. Teredo worms are bivalves that can live on wood and are a primary threat to wood stability in a marine environment.

Aerobic and anaerobic bacterial activity will occur in the sediment, depending on the oxidizing or reducing characteristics of the sediment. [7] Even smaller environments can exist

within one of these microenvironments, such as pieces of wood with metal attached. These regions can create an electric field that would cause hydrolytic or alkaline degradation of cellulose or lignin. [7] Not only are there environmental parameters that must be taken into consideration, but the wood species itself and its origin from the tree can also impact the rate of degradation. Although biological and physical degradation are also major contributors to severe degradation, this thesis specifically focuses on the chemical degradation of waterlogged archaeological wood. Hydrolysis is a major part of the break down and deterioration of wood, but the ions in the water and the bio deterioration is more damaging. For the purposes of this research, chemical degradation is the primary focus due to that it is the most critical interaction with wood and easiest to control, of the deteriorating factors, the rate and level of degradation.

1.4 Structural Components of Wood

The wood cell walls are composed of several layers, in descending order: middle lamella, primary wall, secondary wall 1, secondary wall 2, secondary wall 3. The void in the center of the wood cell is the lumen. This area is where "free" water collects and provides the structural support of the cell when it is waterlogged. In conservation, the goal is to replace the free water with products or remove it in a controlled manner. In fresh wood, bound water occurs naturally and it is not always beneficial to remove all of the bound water when performing treatments on wood. To conserve these wooden artifacts, it is critical to determine the level of degradation to remove the "free" water in a controlled manner.

To determine the level of degradation of waterlogged archaeological wood, three main structural wood components must be examined: cellulose, hemicellulose, and lignin. The chemical composition varies by the species of wood (hardwood or softwood), origin of the wood

from the tree (root, stem, or branch), type of wood (normal, tension, or compression) geographic location, climate, and soil conditions. [9,10] The structural components will vary quantitatively among tree species as shown in Table 1 found below. [9,11-15]

Table 1. A compilation of chemical composition of U.S. woods. [9,11-15]

Type	Extractives	Carbohydrates (Hemicellulose & Cellulose)	Lignin
Softwood	4-25%	55-71%	9-34%
Hardwood	4-16%	65-85%	16-30%
Birch Species (Yellow, River, & Paper)	5-7%	73-78%	17-21%

Northern white birch, a hardwood, is analyzed in this study. While birch is not a common shipbuilding material, it is easily obtained and artificially deteriorated for the purposes of experimentation. Hardwoods can contain anywhere from 16-30% Klason lignin, 40-50% cellulose, 25-35% hemicellulose, and 4-16% extractives. [9] It is thought that hemicellulose is the first to degrade, then cellulose, and lastly lignin.

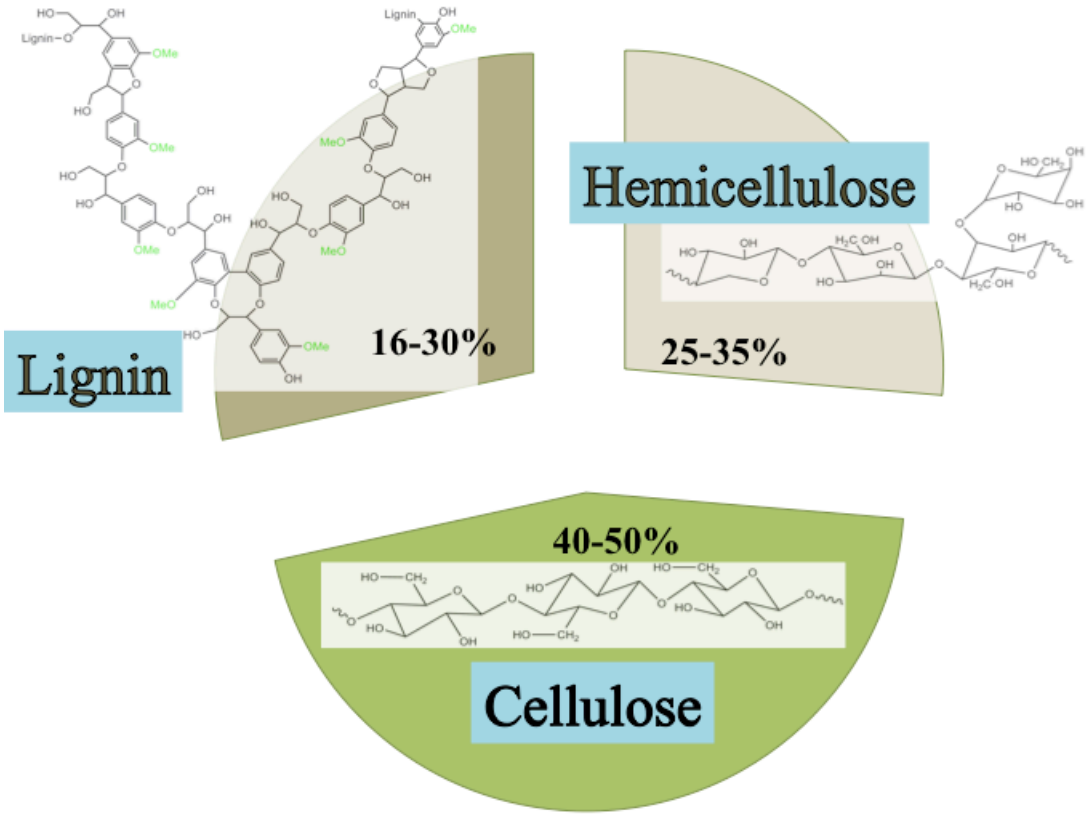


Figure 3. The structural composition of fresh hardwood.

Hemicellulose may account for 25-35% of dry hardwood weight and it is a relatively less organized structure than cellulose.[9,16] It is also more soluble, and more readily hydrolyzed than cellulose making it the first structural component to degrade.[16] Most importantly, the actual amount of hemicellulose varies widely between tree species and wood structures.[16]

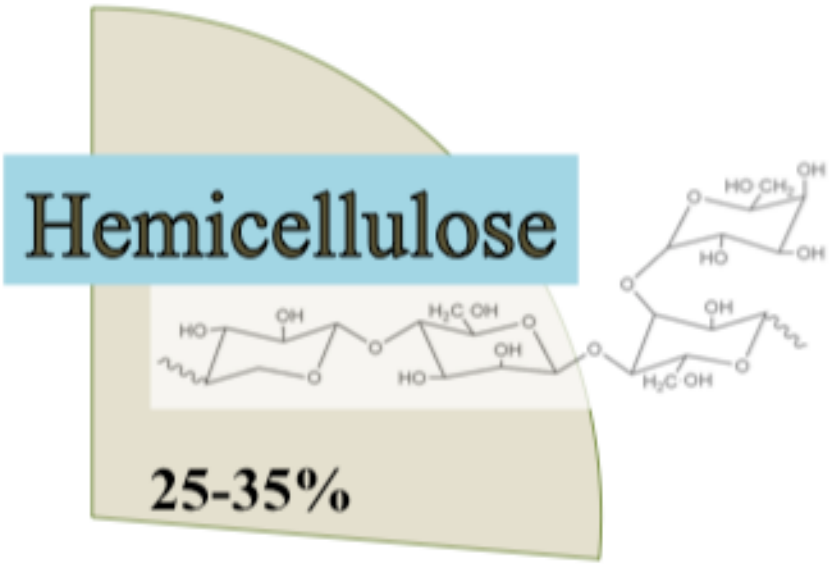


Figure 4. Hemicellulose is less organized, more soluble, and more readily hydrolyzed than cellulose.

Hemicellulose (Figure 5) consists of shorter carbohydrate chains of various sugar molecules 100-200 monomeric sugars per molecule, which are embedded in the primary cell wall and the secondary cell walls. Monomeric sugars that are found in hemicellulose are glucose, galactose, mannose, xylose, glucuronic acid, and arabinose (Figure 6).

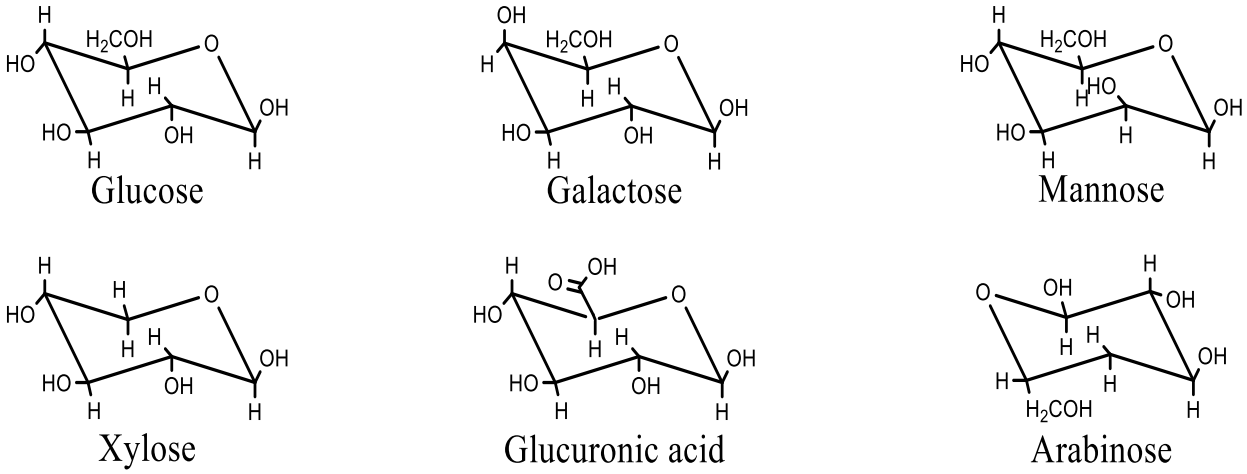


Figure 5. Common monomeric sugars found in hemicellulose. The concentrations and variety of sugars found in hemicellulose will differ between wood species.

Cellulose (Figure 6) is the most abundant and organized structural polysaccharide found in plant cells and it accounts for 40-50% of dry hardwood weight. [9]

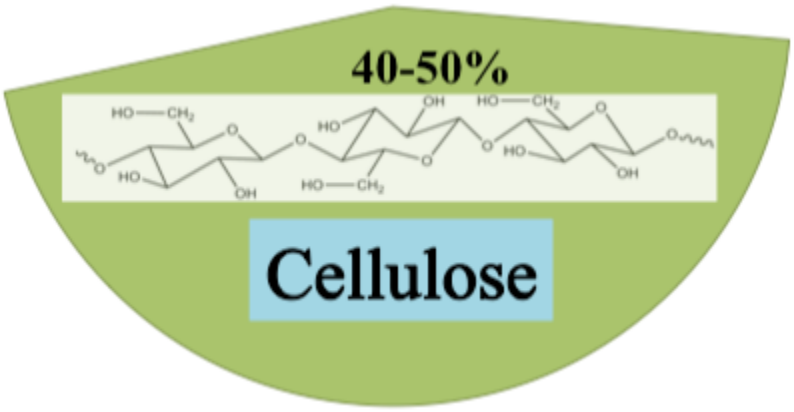


Figure 6. Cellulose is a linear molecule due to hydrogen bonding between hydroxyl groups within the sugar sequences.

Cellulose is the "reinforcing framework" of the primary cell wall that consists of straight chains of up to 15,000 D-glucose units linked together by glycosidic bonds (see Figure 7 below). These molecules are linear as a result of hydrogen bonding between hydroxyl groups within the sugar sequence and are more resistant to hydrolysis compared to hemicellulose. [16] The hydrogen bonding leads to the bundling of tightly packed cellulose molecules into microfibrils, which then create fibril bundles, and then cellulose fibers. [17]

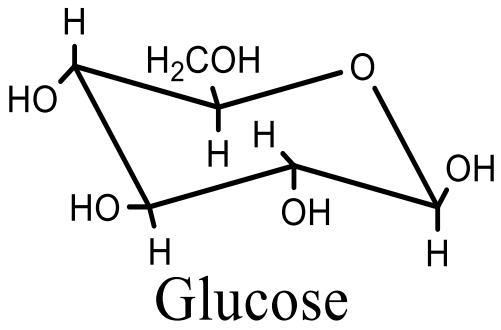


Figure 7. D-Glucose, shown above, links together by glycosidic bonds creating cellulose.

Lignin (Figure 8) is a highly branched heterogeneous high molecular weight polymer, which incorporates aromatic compounds such as guaiacyl, syringyl, and p-hydroxyphenyl

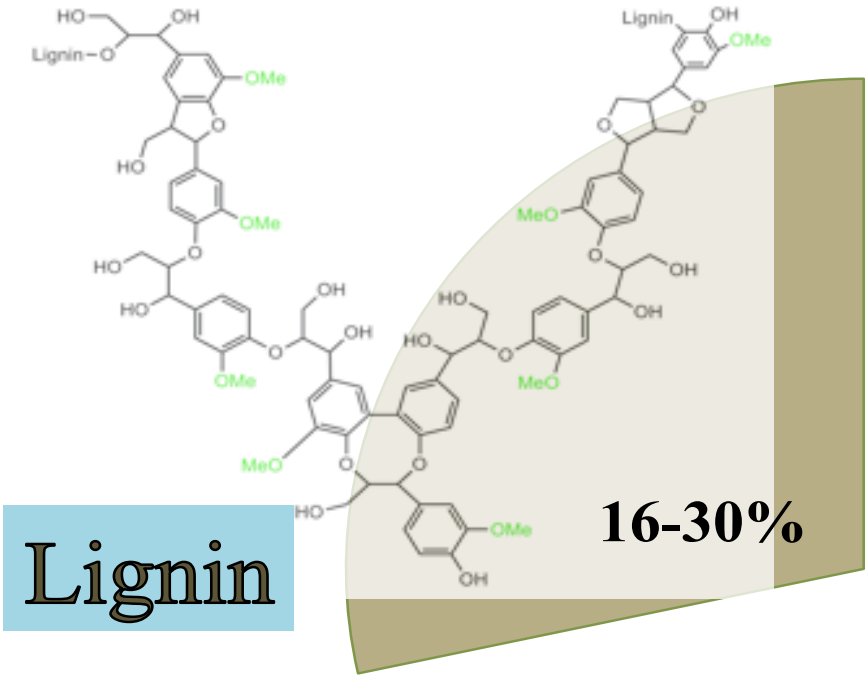


Figure 8. Lignin is a highly branched heterogeneous high molecular weight polymer that has no defined structure.

(Figure 9), which differ by the number of methoxy groups on the aromatic ring. It is thought that the relative proportions of syringyl structural units usually increase as the wood matures. [16] The polymer is held together by a wide variety of ether and carbon-carbon bonds that are intrinsically strong and resistant to hydrolysis. [16]

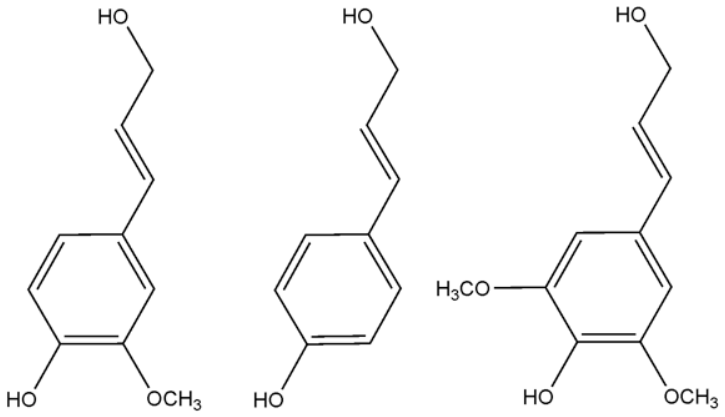


Figure 9. From left to right, coniferyl alcohol/guaiacyl, p-coumaryl alcohol/p-hydroxyphenyl, and sinapyl alcohol/syringyl, are the three building blocks of lignin.

The percent of lignin in fresh dry hardwood can range from 16-35%.[9] Usually, in heavily degraded wood, lignin is the most abundant of the three main components. It is thought that in the cell wall, lignin and hemicellulose fill the interstices between the cellulose microfibrils.[7] This is shown in Figure 10. The main function of hemicellulose and lignin is to strengthen the fibrils.[18] Degradation of any of these wood constituents results in a decrease in the strength of the material.

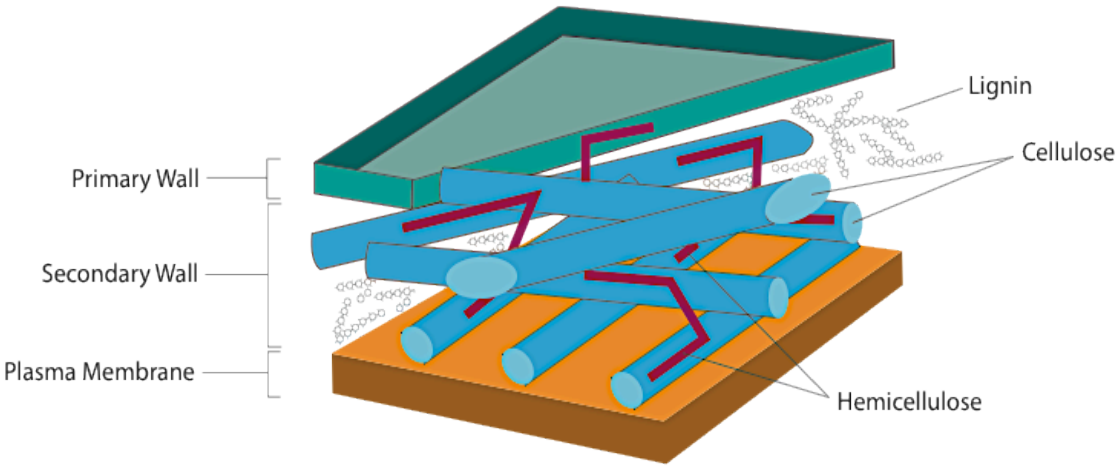


Figure 10. Hemicellulose, cellulose, and lignin within the cell wall.

1.5 Project Importance

The purpose of this research is to develop the methodology to quantitatively determine the degradation of waterlogged archaeological wood. Waterlogged archaeological wood by definition has been subjected to a submerged environment and is completely saturated with water. Once the archaeological wood is submerged in water, the water slowly diffuses into the pores of the wood until it has become completely saturated. Eventually, water facilitates the breakdown of the structural components of the wooden object. This continues until water itself has become the primary support of the object's shape. At this point, if the water were to be removed from the wood under uncontrolled condition, the object could potentially lose its shape completely causing its interpretative value to diminish. In order to prevent this from happening, it is critical for conservation specialist to know the chemical nature and structure of these degraded waterlogged wooden artifacts to establish the appropriate method of stabilizing and conserving these artifacts.

Currently, one method used to determine the level of degradation of waterlogged archaeological wood is the pin test (Figure 11). The pin test consists of using a pin to penetrate the wood to determine the level of degradation. This is determined by the ease and depth to which the pin penetrates the wood. Clearly, this method is subjective and difficult to maintain consistency between conservators. Therefore, an analytical method that can quantitatively determine the degradation and aid in properly stabilizing and conserving the waterlogged archaeological wood would be a valuable tool.

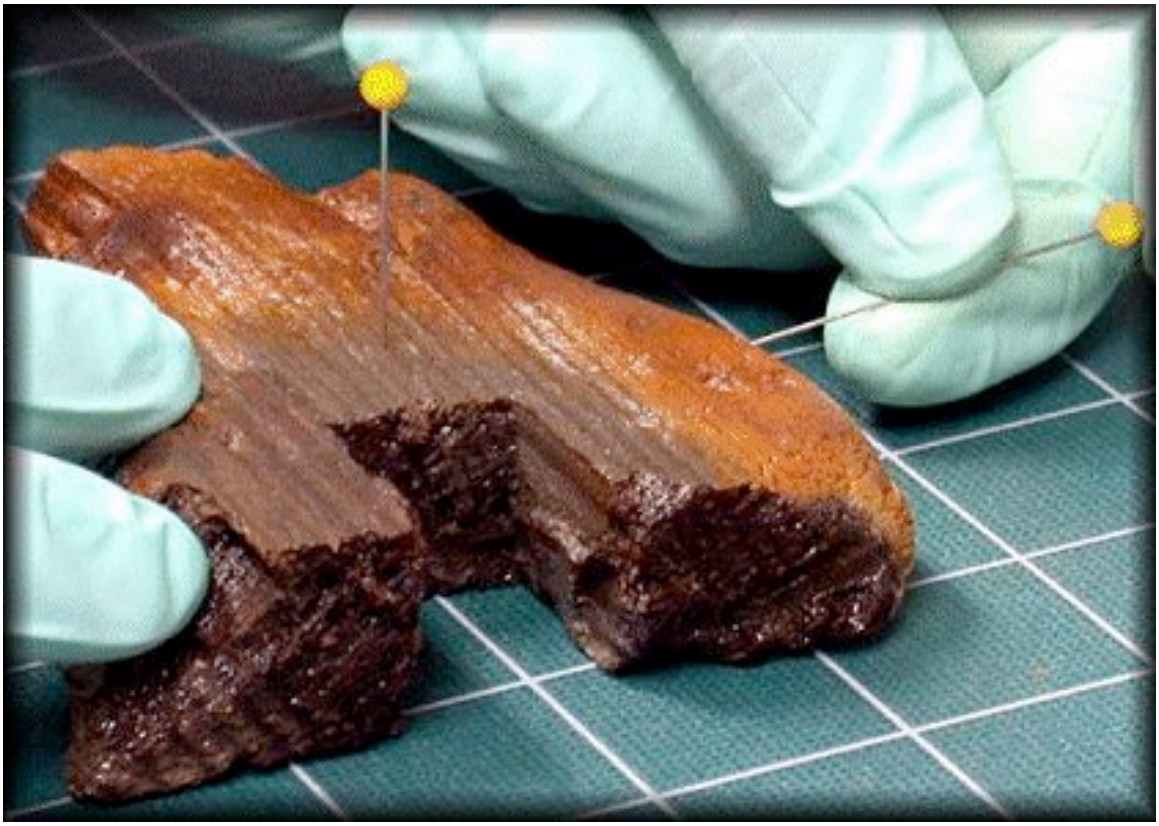


Figure 11. An image provided by The Queen Anne's Revenge Conservation Laboratory located at East Carolina University of the pin test being performed by a conservator.

A quantitative method that is nondestructive, quick, requires minimal sample preparation and sample size, would be ideal for conservators working with waterlogged wood. A prospective alternative that would be practical for a conservation lab is the use of Fourier transform infrared spectroscopy with the attenuated total reflectance accessory (ATR-FTIR). ATR-FTIR spectroscopy is a convenient technique for studying the chemistry of the decay of archeological wood in a rapid manner, since minimal sample preparation and small quantities of wood would be necessary to obtain detailed structural information. Most importantly, spectroscopy can be used as a nondestructive method to chemically characterize pre and post treatment of degradation in waterlogged wood. Also, there are portable and handheld options for ATR-FTIR that would be ideal for conservator in the field where objects cannot be moved and sampling is not possible. [19]

The portable ATR-FTIR enables rapid results for determining the level of degradation of the artifact, which in turn affects decision making related to the resources and skills needed to conserve the wood. ATR-FTIR is potentially a powerful tool that has the capability of saving irreplaceable wooden artifacts from being erased from history.

1.6 Project Objective

For this study, lignin is the primary component targeted for analysis. As previously explained, lignin is the last major component within wood to degrade. Determining its concentration will give a more quantitative measure of the degree of degradation. Lignin is the most complex of the three structural components of wood, and complete elucidation of its chemical structure is still ongoing. The difficulty in understanding the chemical structure is due to the fact that the linkages between phenylpropane units in lignin consist of several types of ether and carbon-carbon bonds that have no regularly repeating multi-unit structures in the lignin

molecules. [20,21] The chemical structure of lignin consists of aromatic rings (p-hydroxyphenyl, guaiacyl, and syringyl nuclei), functional groups that are found within the side chains (alcohol, aldehyde, carbonyl, and double bond groups), and linkages between the monomer units. [21] It is thought that the majority of structural features of lignin are known, but due to the heterogeneous nature of lignin many analytical methods are required to completely characterize lignin.

Gravimetric analysis was used as a standard method to determine the amount of Klason lignin in each sample. Klason lignin refers to extractive free and acid insoluble lignin. Insufficient removal of extractives will give a false Klason lignin weight. This technique was used to obtain reference data for the ATR-FTIR so that known quantities of Klason lignin were compared and correlated to the spectra obtained.

After the methodology has been validated, untreated samples of Black Beard's ship, the Queen Anne's Revenge (QAR), may be analyzed using the ATR-FTIR to determine the degree of chemical degradation of the wood.

1.7 Project Design

Quantitative analysis of complex materials, such as wood, with a small sample size is extremely difficult without the proper equipment or appropriate sample preparation. To create repeatability and accurate results, white birch wood tongue depressors were used as the model, since tongue depressors vary little in weight and dimensions from one another. [22] Tongue depressors are widely favored throughout conservation studies since they decompose easily, and studies are easily duplicated. [22]

To prepare the samples for gravimetric analysis, ten tongue depressors were milled using a spice grinder until the entire sample has gone through a sieve (ASTM E11) to obtain particles

of 500 microns or less sample size. In attempt to make a very complex and heterogeneous model more controlled, random sampling methods were used to account for any variability there might be between tongue depressors. Also, to avoid error associated with small sample size, a larger sample size was taken from the bulk milled and sieved wood for gravimetric analysis.

After preparation of the wood, gravimetric analysis was performed. The gravimetric analysis included a series of extractions to remove different components of the wood. The first three extractions, cyclohexane/ethanol, ethanol, and boiling distilled water, were to remove any lipophilic and hydrophilic extractives. These extractives are non-cell wall components, such as oils and resins, and are relatively small molecules ($<C_{40}$). Wood extractives usually comprise 4-10% of the dry weight of wood, but can vary tremendously by environment, within species, between species, and within trees. [9,23] Two types of extraction, sonication and Soxhlet apparatus, were evaluated and compared for the first two extractions in the gravimetric methodology. The last extraction, sulfuric acid, removes any hemicellulose and cellulose remaining in the sample. After the sample had all extractives, hemicellulose, and cellulose removed, and completely dried, the weight was determined and the % Klason lignin was calculated. Klason lignin refers to extractive free and acid insoluble lignin.

The first goal of this project is to determine the ideal extraction method to obtain accurate and reliable Klason lignin values by gravimetric analysis. First, a gravimetric technique shared by a reliable source was tailored to the lab and resources provided at the time. [24] This method implemented sonication to remove extractives. The sonication method provided too much variation within the data and did not match expected results found in literature. Therefore, the sonication method was compared to another modified method. The new method is a combination of methods reviewed in literature and adapted to the resources available. [10,20,24-27]

The methods were compared and the new method implementing Soxhlet extraction obtained more reliable results within the range of component values found in birch wood.

The second goal of this project was to verify that the more degraded waterlogged wood becomes, the higher the concentration of lignin in comparison to hemicellulose and cellulose. This was verified by using the information obtained from gravimetric studies as training data for the attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) so that known quantities of lignin can be compared and correlated to the spectra obtained. Unfortunately, due to time limitations, the correlation between the methods could not be determined, but the data showed clear evidence of a trend between degradation time and the structural components. The gravimetric data using the modified Soxhlet method and the ATR-FTIR data both show evidence that the more degraded the sample, the higher the concentration of lignin in comparison to hemicellulose and cellulose concentrations.

Chapter 2: Instrumental and Analytical Methods

2.1 Attenuated Total Reflectance

The attenuated total reflectance accessory (ATR) for the FTIR was used to collect spectral data pertaining to the different structural components of wood. ATR takes advantage of the optical properties of high refractive index (n) materials. As Snell's law describes, light bends or refracts when moving from one medium to another based on the refractive indices of the material. From the diagram below (Figure 12), the blue ray depicts refraction described by Snell's law.

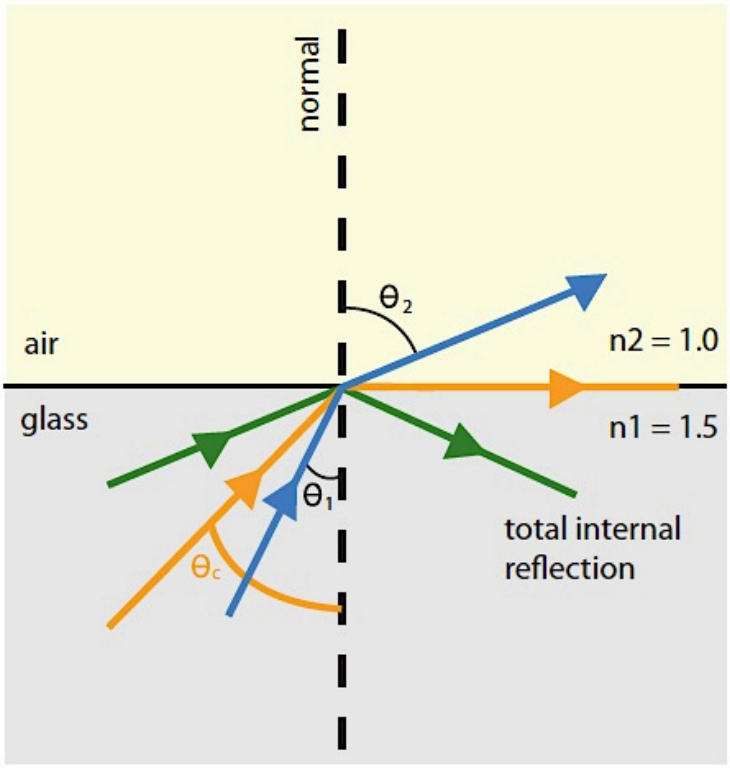


Figure 12. A diagram depicting Snell's law.

The angle of refraction is dependent upon the angle of incidence, θ_1. At the critical angle (the yellow ray) something unusual happens. Once the critical angle is reached, light will not pass into the second medium. Rather, the light travels along the boundary between the two media. The size of the critical angle is dependent on the relative refractive indices of the two mediums and the relationship is shown in the equation, equation 1, below: [28]

$$\sin\theta_c = \frac{n_2}{n_1} \qquad \textbf{Eq. 1}$$

At any angle greater than the critical angle, all light reflects back into the original medium. This is shown as the green ray in Figure 12 and is also called 'total internal reflection', which is occurring within the ATR cell. [28]

A very simple diagram of the ATR method is shown below in Figure 13. An infrared beam is directed into an optically dense crystal and strikes the boundary between the crystal and sample at an angle greater than the critical angle. [28] This internal reflectance creates an evanescent wave that extends beyond the surface of the crystal into the sample held in contact with the crystal.

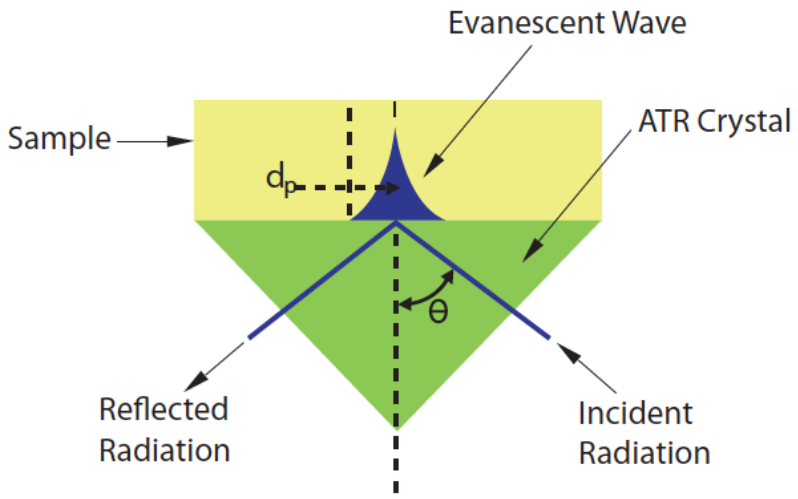


Figure 13. A simple diagram of attenuated total reflectance accessory for FTIR.

The evanescent wave persists only a few microns (0.5-5μ) beyond the crystal surface and into the sample. The distance the evanescent wave extends into the sample at each reflection point is the depth of penetration, equation 2. The depth of penetration is the distance at which the intensity of the radiation within the material falls approximately 37% of its original value just below the surface. [28]

$$d_p = \frac{\lambda}{2\pi(n_1^2 sin^2\theta - n_2^2)^{\frac{1}{2}}} \qquad \textbf{Eq. 2}$$

In regions of the infrared spectrum where the sample absorbs energy, the evanescent wave is attenuated or altered. The attenuated energy from each evanescent wave is passed back to the IR beam, which then exits the opposite end of the crystal and is passed to the detector in

the IR spectrometer. The detector records the attenuated IR beam as an interferogram signal, which was used to generate an infrared spectrum.

2.2 Gravimetric Analysis

Gravimetric analysis quantitatively measures the difference in the mass of a sample before and after a chemical process. The analyte can be removed by precipitation or vaporization. In this study, the analyte was removed by precipitation. The sample was extracted to remove any nonstructural components then hydrolyzed with sulfuric acid to obtain Klason lignin as the end product. The precipitate after each extraction was collected by filtration, washed, and dried to a constant weight. The amount of analyte from the original sample was calculated from the mass of the precipitate. The ideal product of a gravimetric analysis should be pure, insoluble and easily filterable, and should possess a known composition. [29]

When followed correctly, gravimetric analysis can provide exceptionally precise results with high accuracy. For example, gravimetric analysis was used to determine the atomic masses of many elements to six-figure accuracy. [29] Gravimetric analysis has also been used to calibrate instruments instead of using reference standards. The downside of this method is that the process is very lengthy, time dependent, and one mistake within the procedure will more than likely require the analysis to be restarted.

2.3 Soxhlet Extraction

The most commonly used method of extraction for wood components has traditionally utilized liquid-solid extraction via Soxhlet extraction. Essentially, the Soxhlet solid-liquid extraction is used to extract soluble compounds from a solid matrix. The attractive feature of

Soxhlet extraction is that the sample is constantly being eluted with fresh solvent and an establishment of solution equilibrium is not a concern.

2.3.1 The Soxhlet Apparatus Set-up and Process

A Soxhlet apparatus consists of a Soxhlet extractor and a reflux condenser, which is placed on top of the extractor and mounted on top of a round bottom-distilling flask (Figure 14). Once the apparatus is set up, the sample is weighed into a porous thimble, which is then placed into the Soxhlet extractor. The round bottom flask is filled with the solvent of choice and placed inside a silicon oil bath. The chosen solvent, will selectively dissolve the target substance, but leave behind the undesired insoluble solid. The solvent is then heated to reflux after two rubber hoses are attached to the condenser, to achieve a constant flow of cold running water through the condenser.

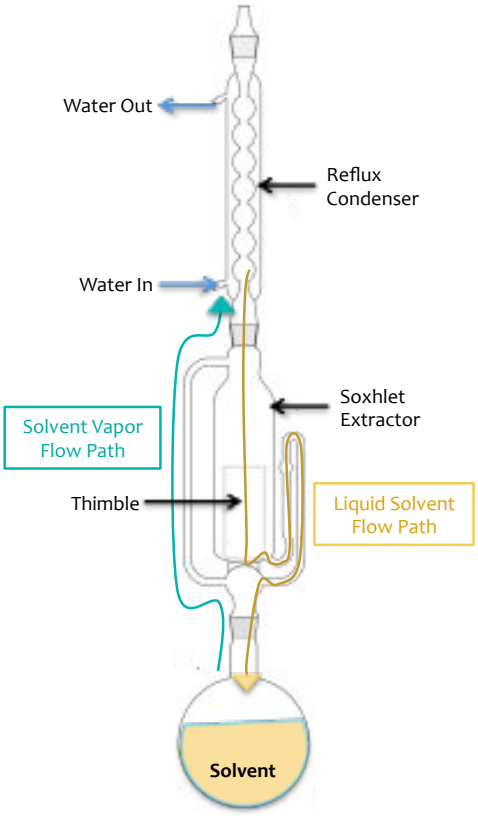


Figure 14. A schematic representation of a Soxhlet extraction apparatus.

Once reflux is attained, vapors begin to rise through the larger sidearm into the condenser where it liquefies and the condensate drips down into the thimble and extracts the desired compound from the sample. As the thimble becomes full of the hot solvent, the smaller sidearm acts as a siphon, and drains the solvent containing the dissolved compound back into the distillation flask.[30] This cycle can be repeated over hours, days, or until the operator believes the extractives have been completely removed from the sample. Once the extraction is completed, the sample will have all extractives dissolved and accumulated in the distillation flask. This accumulation is due to the extractives having a higher boiling point than the solvent.

Chapter 3: Gravimetric Analysis Utilizing Sonication and Comparison with IR Trends

3.1 Introduction

To determine the degradation of wood quantitatively by spectroscopy several preliminary tests were established to develop the baseline for this method. The specimens for this thesis were medical grade Northern Maine White birch tongue depressors. The tongue depressors are unique as a medium due to that they are consistent in dimension and vary little in weight. The tongue depressors were degraded by a 1% sodium hydroxide solution for varying increments of time. This method was chosen based on James Parrent's success in degrading tongue depressors using a 1% sodium hydroxide solution for determining the efficiency of sucrose for conservation treatment. [22] After degrading the tongue depressors, a known and well established method for determining the concentration of Klason lignin in samples by gravimetric analysis was used. It is important to keep in mind that birch species typically consists of 17-21% Klason lignin and a small quantity of acid soluble lignin. [9,11-15] Acid soluble lignin levels can be determined by using UV. [9] For the purpose of this thesis determining acid soluble lignin levels was not necessary, since acid soluble lignin would be removed more quickly than acid insoluble lignin in waterlogged archaeological wood. At this point, FTIR was used to establish whether degraded structural components could be detected, and then compared to the gravimetric analysis data to determine if IR could quantitate the level of degradation.

3.2 Experimental

3.2.1 Chemical Degradation of Wood

A batch of medical Northern Maine White birch wood tongue depressors (Puritan®) were soaked in a 1% sodium hydroxide solution made with 18 MΩ·cm deionized water (pH ~13.5) without agitation. Two depressors were removed at various increments of time ranging from 10 minutes to 33 weeks. After removal from the sodium hydroxide solution, the depressors were copiously washed with 18 MΩ·cm deionized water. To ensure that all sodium hydroxide was removed, the wood was rinsed with water until the wash water had a litmus paper reading of neutral (pH ≈ 7). Each washed tongue depressor was lyophilized (Kinetics FTS systems, 200 mTorr) overnight, then stored in a sealed falcon tube in a refrigerator at 4 °C until further use.

3.2.2 Sample Preparation

The prewashed chemically degraded tongue depressors were milled using a spice grinder and sieved (ASTM E11) to obtain particles of 500 microns or less sample size. The milled sample was then re-lyophilized overnight and stored in the fridge at 4 °C in an airtight container until further use.

3.2.3 Klason Lignin Analysis

3.2.3.1 Original Sonication Extraction Method: Method A

Extraction 1: Cyclohexane & Ethanol (2:1)

Prior to analysis, a set of medium porosity (10 – 15 μm pore) glass filters (F_i) and glass test tubes (tt_i) were dried at 105 ± 3 °C in a furnace, until a constant weight was obtained. Approximately 0.5000 g of the finely ground milled wood was accurately weighed using an analytical balance and added to a dry pre-weighed test tube (tt_{iCE}). The sample of milled wood was extracted with 5 mL of cyclohexane/ethanol (2:1 v/v) by sonication for 6 hrs. After sonication the suspension was filtered using a dry, pre-weighed glass filter (medium porosity). The filtrate was collected and placed in a labeled 20 mL scintillation vial. The test tube was washed with cyclohexane/ethanol (2:1 v/v) and the washes were filtered through the same filter to ensure complete transfer of all wood particles remaining in the test tube. The filter containing the wood and test tube with any wood particles remaining ($tt_{residue}$) were dried in a vacuum oven (0.1644 atm) at 40 °C overnight. After drying, the weight of dry wood was recorded.

$$\mathbf{TSL_{CE} = tt_{CEresidue} - tt_{iCE}} \qquad \textbf{Eq. 3}$$

To account for any loss of sample during transfer (TSL_{CE}), the test tube with residue weight ($tt_{CEresidue}$) was subtracted to the initial test tube weight (tt_{iCE}) as shown in equation 3. The loss of sample during transfer (TSL_{CE}) was added to the wood weight after the first extraction (WWt_{ACE}) to obtain the total wood weight after the first extraction ($TWWt_{ACE}$).

$$\mathbf{TWWt_{ACE} = WWt_{ACE} + TSL_{CE}} \qquad \textbf{Eq. 4}$$

After determining the total sample weight after the first extraction *(*$TWWt_{ACE}$), the dry weight of extractives removed from the cyclohexane and ethanol extraction was determined (E_{CE}). The weight of extractives removed (E_{CE}), was determined by subtracting the sample weight before the extraction (WWt_{BCE}) from the total sample weight after the first extraction ($TWWt_{ACE}$).

$$\mathbf{E_{CE} = WWt_{BCE} - TWWt_{ACE}} \qquad \textbf{Eq. 5}$$

Once the extractive weight (E_{CE}) was determined, it was divided by the initial weight before the cyclohexane and ethanol extraction (WWt_{BCE}) and multiplied by 100 to obtain the percent extractive weight for the cyclohexane and ethanol extraction ($\%E_{CE}$).

$$\mathbf{\%E_{CE} = (E_{CE} \div WWt_{BCE}) \times 100} \qquad \textbf{Eq. 6}$$

Extraction 2: Ethanol

After drying, the weight of the dry sample was recorded. This sample was then extracted with 5 mL of ethanol (95% v/v) in the same manner as previously described in extraction 1. After filtering and drying overnight (as previously described) the sample weight was measured. The same process of math from the previous section was also used to account for any loss of

sample during transfer (TSL_{EE}), equation 7, so that an accurate total wood weight after the second extraction ($TWWt_{AEE}$), equation 8, could be obtained.

$$\mathbf{TSL_{EE} = tt_{EEresidue} - tt_{iEE}} \qquad \textbf{Eq. 7}$$

$$\mathbf{TWWt_{AEE} = WWt_{AEE} + TSL_{EE}} \qquad \textbf{Eq. 8}$$

After determining the total sample weight after the second extraction *(*$TWWt_{AEE}$), the amount of dry weight of extractives removed from the cyclohexane and ethanol extraction was determined (E_{EE}). The weight of extractives removed (E_{EE}), was determined by subtracting the sample weight before the extraction (WWt_{BEE}) to the total sample weight after the second extraction ($TWWt_{AEE}$).

$$\mathbf{E_{EE} = WWt_{BEE} - TWWt_{AEE}} \qquad \textbf{Eq. 9}$$

Once the extractive weight (E_{EE}) was determined, it was divided by the initial weight before the ethanol extraction (WWt_{BEE}) and multiplied by 100 to obtain the percent extractive weight for the ethanol extraction ($\%E_{EE}$).

$$\mathbf{\%E_{EE} = (E_{EE} \div WWt_{BEE}) \times 100} \qquad \textbf{Eq. 10}$$

Extraction 3: Boiling Water

The sample was then placed into a pre-weighed and dried test tube *(*tt_{iW}), containing a stir bar and extracted with 5 mL of boiling distilled water on a hotplate for an hour. After boiling, the sample was filtered using the same process mentioned before with dry pre-weighed medium porous filters and washed with distilled water (~10-30 mL). The sample particles within the filter and test tubes were air-dried for three days, followed by overnight drying in a vacuum oven (0.1644 atm) at 40°C to obtain extractive-free wood.

The math from the previous extractions was also followed for the boiling water extraction. The loss of sample during transfer (TSL_W) was taken into consideration by subtracting the test tube with residue weight ($tt_{Wresidue}$) to the initial test tube weight (tt_{iW}) as shown in equation 11.

$$\mathbf{TSL_W = tt_{Wresidue} - tt_{iW}} \qquad \textbf{Eq. 11}$$

Then the loss of sample during transfer (TSL_W) was subtracted by the initial sample weight after the third extraction *(*WWt_{AW}) to obtain an accurate total sample weight after the third extraction ($TWWt_{AW}$).

$$\mathbf{TWWt_{AW} = WWt_{AW} + TSL_W} \qquad \textbf{Eq. 12}$$

After determining the total sample weight after the third extraction *(*$TWWt_{AW}$), the dry weight of extractives removed from the boiling water was determined (E_W). The weight of

extractives removed (E_W), was determined by subtracting the sample weight before the extraction (WWt_{BW}) from the total sample weight after the first extraction ($TWWt_{AW}$).

$$\mathbf{E_W = WWt_{BW} - TWWt_{AW}} \qquad \textbf{Eq. 13}$$

Once the extractive weight (E_w) was determined, it was divided by the initial weight before the boiling water extraction (WWt_{BW}) and multiplied by 100 to obtain the percent extractive weight for the boiling water extraction ($\%E_W$).

$$\mathbf{\%E_W = (E_W \div WWt_{BW}) \times 100} \qquad \textbf{Eq. 14}$$

Extraction 4: Klason Lignin

Extractive free sample was weighed (WWt_{BKLE}) and placed in a clean dry beaker. The beaker was placed in an ice bath and 1.5 mL of 72% H_2SO_4 was added drop wise over 4 minutes. The sample was carefully macerated with a glass rod while the sulfuric acid was added. After the addition of acid, the beaker was placed in a water bath at 20° C for 2 hours with frequent stirring. The acid and extractive free sample was poured into 30 mL of room temperature distilled water. The beaker was rinsed with additional distilled water to obtain a total volume of approximately 57 mL in the new flask, for a final acid concentration of 3% H_2SO_4. The suspension was heated to boiling for 4 hours with continuous stirring. After 4 hours, the suspension was clamped and positioned at an incline overnight to allow the particles to settle to make the filtering process more efficient. The following day the sample was filtered through a dry pre-weighed medium porous filter (F_{iKLE}) and rinsed with boiling water (~300 mL). The filter with sample was placed

in an oven and dried at 105±3° C overnight to obtain a constant weight (F_{fKLE}). After drying, the weight of dry Klason lignin (WWt_{AKLE}) was recorded and the sample stored in a dry pre-weighed 20 mL scintillation vial.

$$\mathbf{WWt_{AKLE}=F_{fKLE}-F_{iKLE}} \qquad \textbf{Eq. 15}$$

To find the percent extractives from the Klason lignin extraction, the weight of dry Klason lignin (WWt_{AKLE}) was divided by the extractive free wood (WWt_{BKLE}) then multiplied by 100.

$$\mathbf{\%E_{KLE}=(WWt_{AKLE}\div WWt_{BKLE})\times 100} \qquad \textbf{Eq. 16}$$

To determine the percent Klason lignin of the sample, 100 was subtracted by the sum of percent extractives from each extraction.

$$\mathbf{\%KL=100-(\%E_{CE}+\%E_{EE}+\%E_{W}+\%E_{KLE})} \qquad \textbf{Eq. 17}$$

3.2.4 Infrared Spectroscopy

Approximately 50 mg of prepared finely ground sample was weighed and then pressed (10 tons) into a pellet. The pressed sample pellet was lyophilized for 24 hours to remove any moisture and analyzed using infrared spectroscopy.

Infrared spectra were collected on a Nicolet 6700 Fourier transform infrared spectrometer (Thermo Nicolet, Madison, WI), equipped with a DTGS detector and a golden gate single bounce diamond ATR (45˚ incidence angle) crystal. Individual spectra (64 scans) were recorded

under ambient conditions with a spectral window of 4000 cm^{-1} to 800 cm^{-1}, at a resolution of 4 cm^{-1}. A total of two spectra, one on each side of the pellet, were recorded for each sample.

After obtaining spectra for each sample, spectra were normalized by Dr. Paul Gemperline using the multiplicative scatter correction (MSC) method. Normalization was necessary to account for the deviation from scaling or gain effects of the variables measured from their true value by a multiplicative factor. Scaling differences generally arise from instrumental sensitivity effects, possible source or detector variations, or path-length effects and scattering effects. [31] MSC is a method used to correct differences in baseline offsets and path length due to differences in particle-size distributions in infrared reflectance spectra of powdered samples. [32] A simple linear regression of each measured spectrum were regressed against a reference spectrum, and then corrected by using the slope or intercept of this fit to the measured spectrum to achieve MSC. [32]

3.3 Results and Discussion

3.3.1 IR Analysis of Degraded Wood Samples

Spectra of tongue depressors that have been soaked from 0 days to 33 weeks were analyzed using FTIR-ATR. Figures 15 and 17 show unprocessed IR spectra in the fingerprint region between 1800 and 900 cm^{-1} for tongue depressors degraded at different periods of time. Each time point has an average of at least four spectra. The assignments of characteristic IR bands related to structural components of wood are summarized in Table 2.

Table 2. Overview of the main peak assignments examined in the IR spectra.

Wave number (cm-1)	Assignment
1750	Hemiacetal
1650	C=O conjugated aldehyde and ketone of Lignin Oxidation Products
1370	Symmetric C-H deformation and bending of carbohydrate
1230	Syringyl ring of lignin, C-C, C-O, C-H and C = O stretch of lignin & xylan

The degraded samples gave an immediate indication of the aggressiveness of the caustic environment on wood polymers. After the first day, peaks associated with hemicellulose (1740, 1230 cm^{-1}) had disappeared or dramatically decreased.

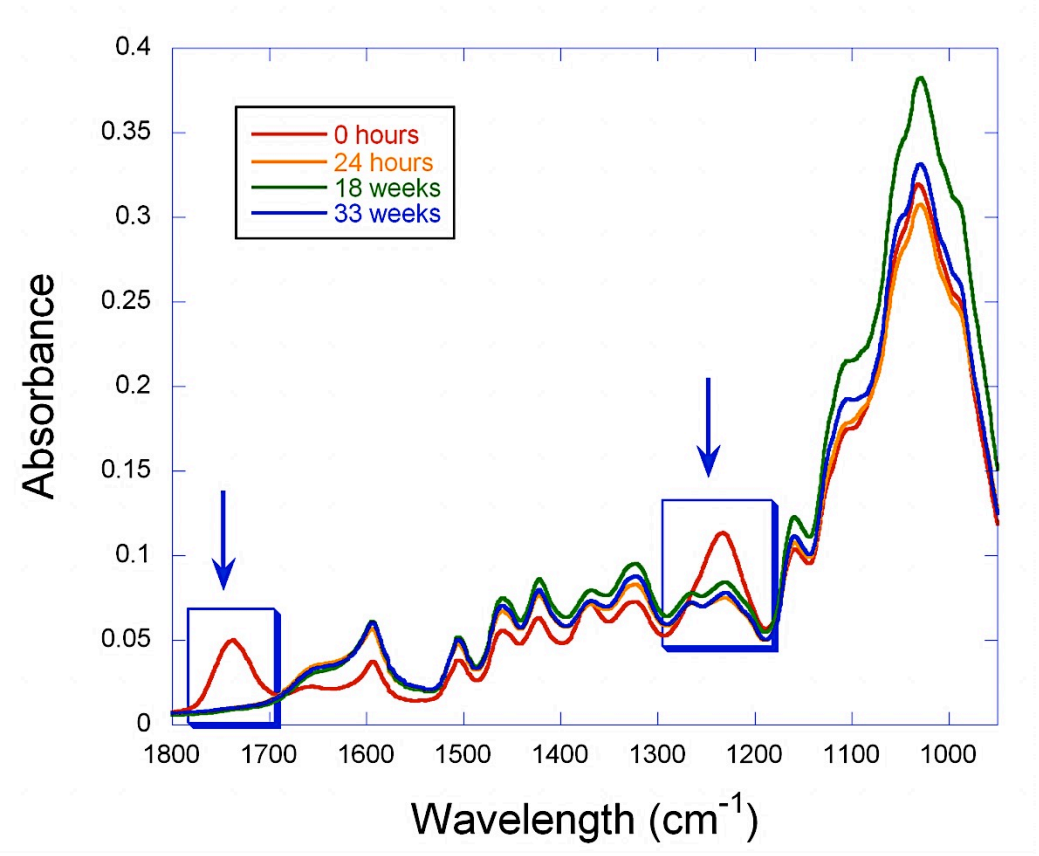
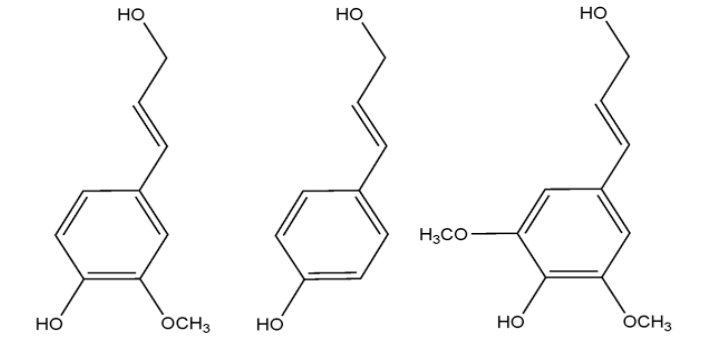


Figure 15. Representative IR Spectra (finger print region) of the degraded tongue depressor samples at the following time points: 0 hours —, 24 hours —, 18 weeks —, and 33 weeks —. Each spectrum is an average of several runs.

The peak at 1740 cm^{-1}, associated with hemiacetal, completely disappeared. [33-35] While the peak at 1230 cm^{-1} had resolved to show an additional peaks: at 1260 cm^{-1} (due to guaiacyl ring of lignin and C=O stretch) and at 1230 cm^{-1}, which is associated with sugar C-H and C-O bonds within the ring, and the syringyl ring of lignin. [33,34,36-38]



guaiacyl (g) p-hydoxyphenyl (p) syringyl (s)

Figure 16. Lignin incorporates aromatic compounds, which differ by the number of methoxy groups attached to the aromatic ring.

These findings suggested that the chemical degradation rate was much quicker than expected. Therefore, another batch of tongue depressors were degraded over a period of 24 hours since the IR spectra indicate that hemicellulose and cellulose are degraded completely within the first day. More analysis would be necessary to confirm that the disappearance or decrease of peaks were not due to part of the structure giving rise to the IR signal, chromophore, being chemically altered.

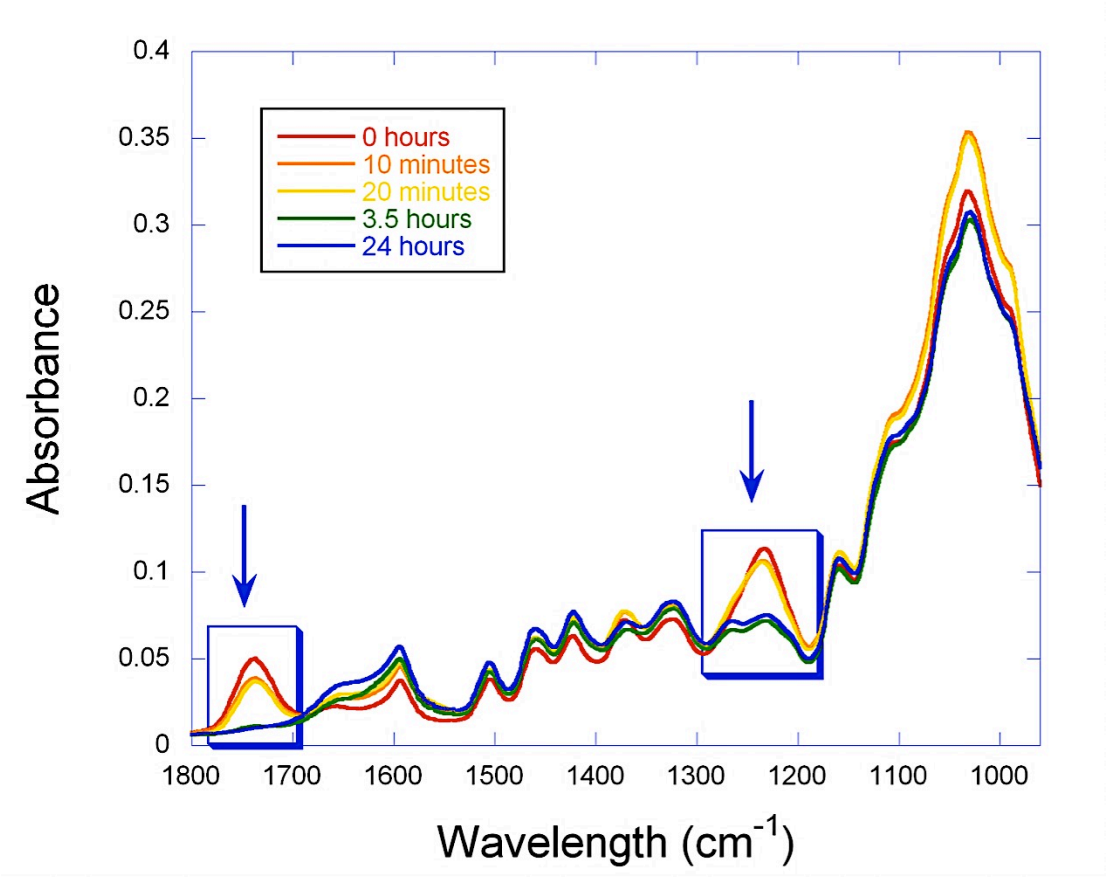


Figure 17. Representative IR Spectra (finger print region) of the degraded tongue depressor samples at the following time points: 0 hours —, 10 minutes hours —, 20 minutes hours —, 3.5 hours —, and 24 hours —. Each spectrum is an average of several runs.

In samples degraded within 4 hours using infrared analysis (Figure 17) shows similar results to those obtained from 0 days to 33 weeks. That is, the same peaks were affected, but at a more gradual rate. It was determined that the process of degradation occurs within the first 4 hours of soaking.

Although, the goal is to quantitatively determine the degradation of the three main wood components, the spectra suggest that the degradation of cellulose, hemicellulose, and lignin occurs within the first 4 hours. The spectra were further analyzed and corrected (Figure 18), to obtain more quantitative observations about the structural changes from the rate of degradation.

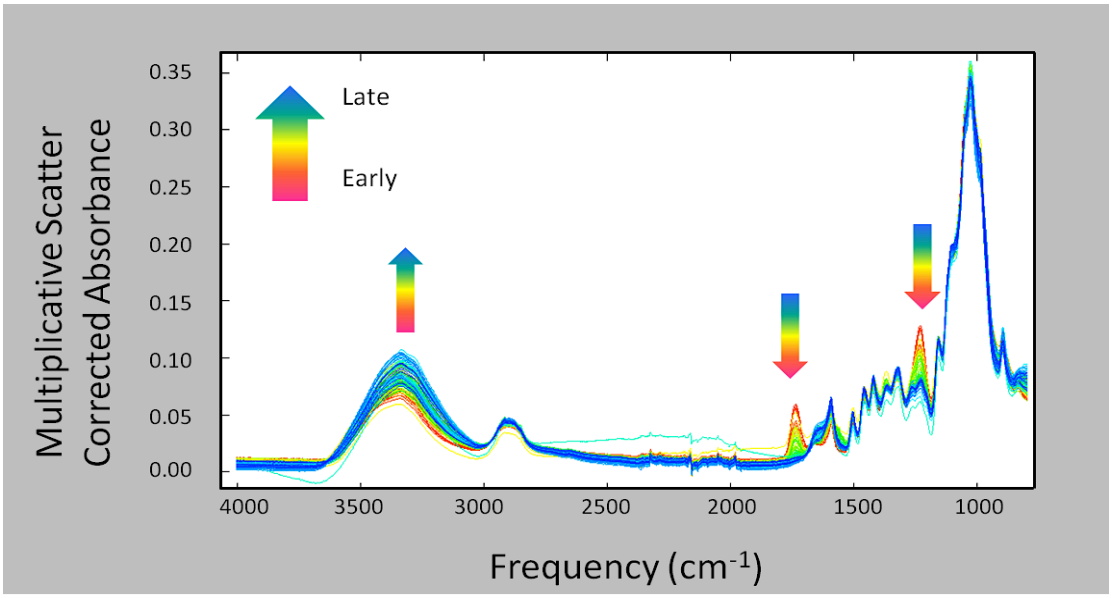


Figure 18. Multiplicative scatter corrected absorbance plot of IR spectra obtained from all wood samples degraded for 0 hours to 33 weeks. IR spectra are color coded according to time degraded such that wood samples degraded for shorter periods of time appear red, and wood samples degraded for longer periods of time appear blue.

Examination of all spectra for each degraded tongue depressor reveals interesting trends. In the plot above (Figure 18), spectra were examined from 4,000 to 800 cm^{-1} then normalized using the multiplicative scatter correction method. Multiplicative scatter correction, explained previously, essentially is taking into consideration the potential scattering due to the powdered

sample. The spectra are color coded according to time such that spectra recorded earlier in time appears in red, and spectra recorded late in time appear blue. A second plot, Figure 19, is included that shows a blow up of the region from 1200 to 1800 wavenumbers.

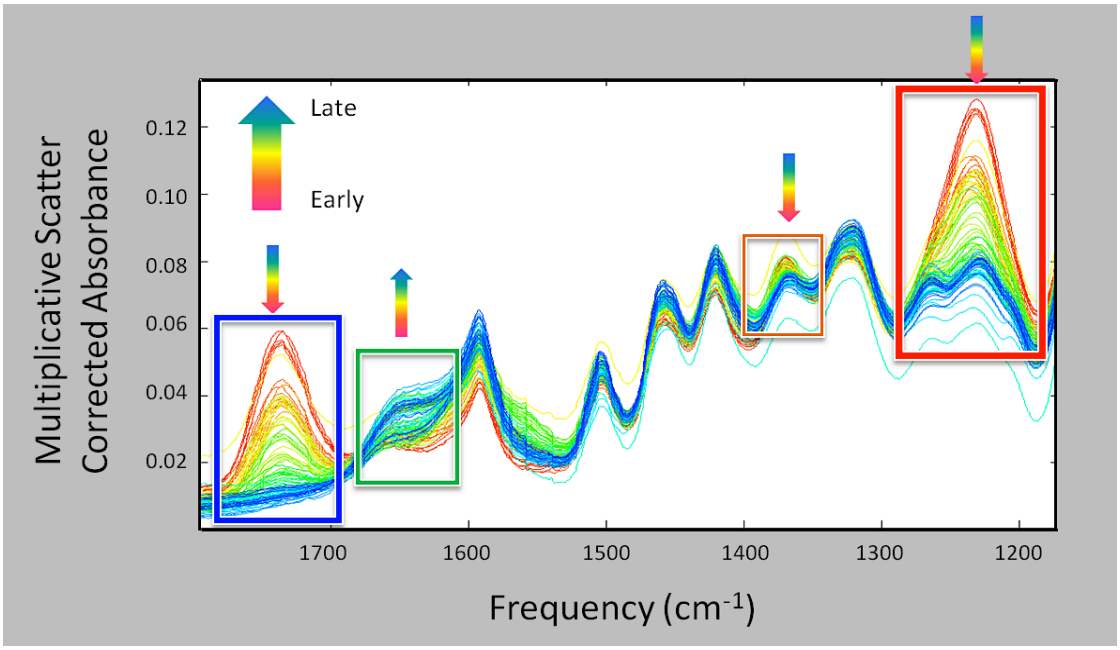


Figure 19. Multiplicative scatter corrected absorbance plot of fingerprint region focusing on specific peak changes. The boxed regions represent peaks associated with hemicellulose, cellulose, and lignin.

Figure 19 shows some interesting and strong trends of structural degradation. As seen before in the previous plots, the peak at 1230 cm^{-1} and 1740 cm^{-1} are decreasing while an additional peak at 1260 cm^{-1} is resolved, which indicates that cellulose and hemicellulose are degrading allowing a peak associated with lignin to resolve as degradation time increases. Several new peaks, not clearly defined in the previous Figures 18 and 19, also support the trend of hemicellulose and cellulose degrading as degradation time increases. The peak at 1370 cm^{-1},

assigned to symmetric C-H and C-O in plane bending assignments of carbohydrates, is decreasing.[33-35] The increasing peak at 1650 cm^{-1} represents carbonyl conjugated aldehyde and ketone assignments to lignin.[33,34,39] As degradation time increases; the peak at 1650 cm^{-1} assigned to lignin increases. The several peaks within the 1500-1300 range that are not changing, are assigned to aromatics associated to lignin and should not change.[33,40]

Peaks at 1740 cm^{-1}, 1370 cm^{-1}, and 1230 cm^{-1} all decrease, while the peak at 1650 cm^{-1} increases over time of degradation implying that hemicellulose and cellulose have completely degraded and only lignin remains. As hemicellulose and cellulose degrade, it is thought that the concentration of lignin will increase, therefore causing the increase in absorbance at 1650 cm^{-1}.

3.3.2 Gravimetric Analysis

Gravimetric analysis was used to quantitatively determine the amount of insoluble (Klason) lignin within the degraded tongue depressors. Equations used to obtain gravimetric data were discussed in section 3.2.3 Klason Lignin Analysis. The results obtained from the gravimetric analysis were compared to the IR data to determine whether IR can quantitatively determine the degradation of wood.

The results found from the gravimetric analysis are located in Appendix A. Klason lignin values of all degraded tongue depressors observed are in the plot found in Figure 20.

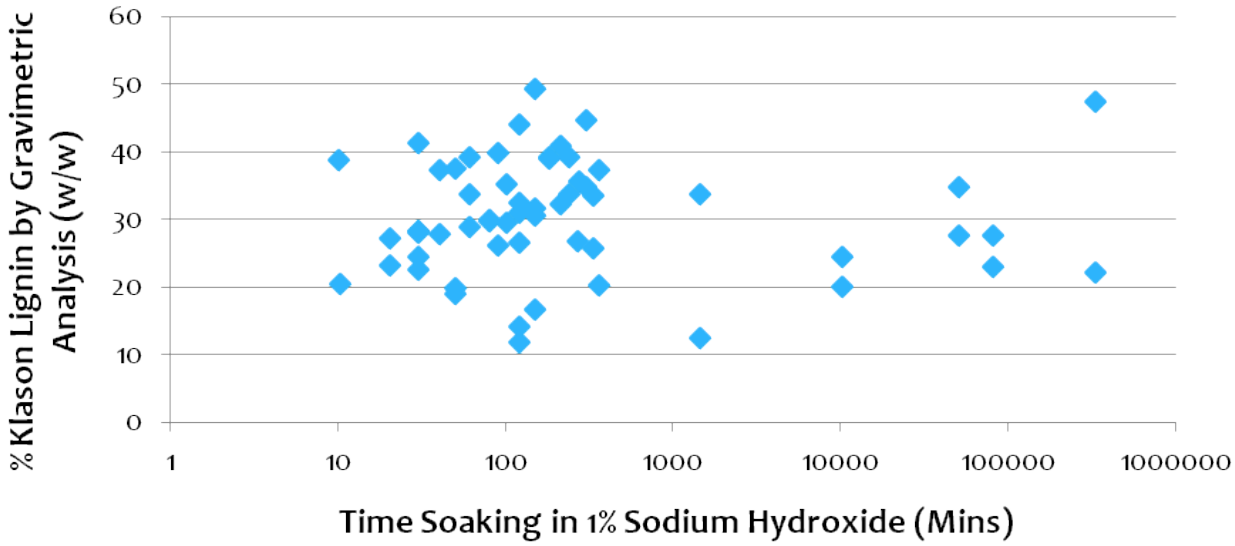


Figure 20. Gravimetric analysis of Klason Lignin Content data for all degraded tongue depressors from day 0 to week 33.

There is no clear trend observed from the Klason lignin content of the chemically degraded tongue depressors. As mentioned in Chapter 1, section 1.4, the expected structural component percent of Klason lignin in fresh birch wood is 17-21%. Ideally, since lignin is the last structural component to degrade, a trend of Klason lignin percent would increase over the time of degradation, due to the hemicellulose and cellulose degrading at a more rapid rate than lignin. Therefore, day 0, which was not chemically degraded, should have Klason lignin values of 17-21%. As mentioned previously, birch is a hardwood, therefore during sulfuric acid hydrolysis the lignin breaks down differently than softwood creating acid soluble lignin. As shown in appendix A, the triplicate values of Klason lignin % for day 0 are 25.2%, 25.1%, and 24.2%. The average of these values is 25.9% Klason lignin, which is much higher than expected from literature of lignin values from 17-21%. Unfortunately, as shown from the scatter plot

(Figure 20), the Klason lignin values do not consistently increase then level out or begin to decrease due to the lignin finally becoming degraded. Instead, the plot shows the variability of degradation at each time point. Therefore, these high values of lignin and variability must be due to the high degree of error within the gravimetric analysis. For example, the duplicate run at degradation time 10 minutes, has % Klason lignin values of 20.6% and 38.9%. The degree of variability is clearly too high in the Klason lignin values to obtain any useful information from inconsistent replicates of gravimetric analysis for each time point.

3.4 Conclusions

Infrared analysis has shown to be an efficient method to observe structural component features of wood. Analysis by IR has revealed interesting trends associated to the degradation of the structural components of wood, as well as confirm that as wood degrades hemicellulose and cellulose disappear, while lignin is the last to remain. Further analysis would be necessary to confirm that the disappearance or decrease of peaks were not due to part of the structure giving rise to the IR signal, chromophore, being chemically altered. Although, examination of the spectra reveals very interesting trends, there was no correlation between the spectra and the Klason lignin values obtained through gravimetric analysis. Degradation was found to be much quicker than expected, since spectra suggest hemicellulose and cellulose completely degrade within four hours. Therefore the gravimetric method was reexamined and focused on earlier time points within 6 hours.

Sample size and extraction method were the two key components of % Klason lignin variability. To obtain reliable % Klason lignin results with minimal error, the sample preparation and size must be modified as well as more replicates for each time point must be done. This

method will be modified and compared to the original gravimetric analysis method. These solutions are further discussed in Chapter 4.

Chapter 4: Comparison of Sonication and Soxhlet Extraction Method

4.1 Introduction

The original method of extraction for gravimetric analysis and the sample size has potentially contributed to Klason lignin variation in degraded tongue depressor samples. To test these parameters as the potential sources of Klason lignin variation, the sample size was changed and the extraction by sonication method was compared to Soxhlet extraction.

The sample size was changed to create more homogeneity within a heterogeneous sample. In the original method, one tongue depressor was ground until enough sample was passed through a 500 micron particle size sieve. This clearly does not represent the sample as a whole, and one tongue depressor cannot represent the variability in structural component concentration throughout a tree and its species. Therefore, the method was modified to mill ten tongue depressors completely to create a very fine powder so that the entire sample could pass through the 500 micron sieve. This represents variability of lignin within a tree and its species, as well as creates a more homogenous sampling of the complex heterogeneous matrix of wood.

To obtain more quantitatively consistent results, the original sample weight used in the gravimetric analysis was changed from approximately 0.5000 g to 5.0000 g. After the first three extractions, 0.5 g of the extractive free wood was used for the Klason lignin extraction using sulfuric acid. Due to the miniscule weight of extractives being removed, obtaining quantitatively accurate results of these weights requires a larger sample size.

Lastly, the sonication extraction method was tested for its efficiency by comparing it to the Soxhlet extraction. Soxhlet extractions are an old and very well known method within the pulping industry. The sonication method of extraction was thought to be ideal because multiple samples could be placed into the sonication bath for long periods of time allowing the operator to

leave and then return when the extraction was over and ready to filter. It was not thought of at the time, due to extractives being no more than 10% of fresh wood weight, that these samples would be reaching solution equilibrium. The solvent could only diffuse out as much of the extractives as it could hold and since the solvent was not repeatedly being refreshed, this potentially contributing to the wide range of Klason lignin values and each sample potentially reaching a different equilibrium point. If not all extractives are removed from the wood before Klason lignin analysis, than the extractives contribute to the Klason lignin weight, therefore giving inaccurate results. This issue was resolved by using the Soxhlet extraction method; since ultimately, several extractions would yield greater recovery than a single extraction, therefore solution equilibrium would no longer be a concern. The Soxhlet extraction apparatus is a simple setup that improves the consistency and overall extraction efficiency, therefore making it an ideal method to perform a large series of frequent extractions.

By using Soxhlet and modifying these potential causes of Klason lignin variation from the previous method and comparing it to the original method, the results will confirm the more efficient and consistent method. It is also important to keep in mind, that wood is a complex mixture of hydrophilic extractives, which are being extracted in the first three extractions. Insufficient extraction of the extractives will give a false % Klason lignin value. The last extraction, Klason lignin method, uses sulfuric acid to remove all cellulose, hemicellulose, and soluble lignin. This method is proficient in determining the percent lignin in softwoods, but hardwood lignin is somewhat soluble in 72% sulfuric acid, potentially creating an acid soluble portion amounting from 3-20% of the total lignin content. [9]

4.2 Sample Preparation

Twenty tongue depressors were milled using a spice grinder and completely sieved (ASTM E11) to obtain particles of 500 microns or less sample size. This was done to ensure that the sample was representative of the entire lot of material being tested. The milled sample was then lyophilized overnight and stored in a fridge at 4 °C in an airtight falcon tube until further use.

4.3 Sonication vs. Soxhlet Method of Extraction

4.3.1 Sonication Extraction Method

The three trials of time zero using the sonication extraction method are the samples discussed in Chapter 3.

4.3.2 Soxhlet Extraction Method

A set of medium porosity glass thimbles (10 – 15 μm pore) were dried at 105 ± 3 °C in a furnace, until constant weight was obtained. Approximately 5.0000 g of the finely ground milled wood was weighed using an analytical balance and added to a dry pre-weighed thimble (tw_i). The thimble containing the milled wood was inserted into the Soxhlet apparatus with at least 150 mL of cyclohexane/ethanol (2:1 v/v) in the collection round bottom flask. The apparatus was heated to reflux and run for 6 hours at a reflux rate of twelve to fifteen solvent exchanges per hour in the Soxhlet thimble. After the extraction time was complete, the thimble was removed carefully washed thoroughly with 95% ethanol then dried in a vacuum oven (0.1644 atm) at 105 °C overnight. After drying, the weight of dry sample and thimble was recorded (tw_{ACE}). To find

the weight of the dry sample only the initial dry pre-weighed thimble, tw_i, was subtracted to the weight of dry sample within the thimble, tw_{ACE}, to obtain the actual sample weight after the extraction $WWt_{ACE.}$

$$\mathbf{WWt_{ACE}=tw_{ACE}-tw_i} \qquad \textbf{Eq. 18}$$

After determining the sample weight after the extraction (WWt_{ACE}), the weight of extractives (E_{CE}) were found by subtracting the wood weight before the cyclohexane and ethanol (2:1) extraction (WWt_{BCE}) to the sample weight after the extraction (WWt_{ACE}).

$$\mathbf{E_{CE}=WWt_{BCE}-WWt_{ACE}} \qquad \textbf{Eq. 19}$$

The weight of extractives, E_{CE}, divided by the sample weight before the extraction WWt_{BCE} was multiplied by 100 to obtain the percent extractives removed from the wood sample.

$$\mathbf{\%E_{CE}=(E_{CE}\div WWt_{BCE})\times 100} \qquad \textbf{Eq. 20}$$

The sample was then extracted with 150 mL of ethanol (95% v/v) in the same manner as before. After filtration and drying overnight (as previously described) the wood weight with thimble (tw_{AEE}) was accurately weighed. To obtain the total amount of sample weight after the extraction (WWt_{AEE}), the wood weight with thimble after extraction (tw_{AEE}) was subtracted by the thimble weight tw_i.

$$WWt_{AEE} = tw_{AEE} - tw_i \quad \text{Eq. 21}$$

The weight of extractives for the second extraction (E_{EE}) using ethanol was determined the same as the previous extraction. The sample weight after the first extraction (WWt_{ACE}) using cyclohexane and ethanol (2:1) was subtracted to the sample weight after the second extraction (WWt_{AEE}) to obtain the weight of extractives removed from ethanol (E_{EE}).

$$E_E = WWt_{ACE} - WWt_{AEE} \quad \text{Eq. 22}$$

To determine the percent extractives removed by the second extraction the weight of extractives from ethanol (E_{EE}) was divided by the sample weight after the first extraction (WWt_{ACE}) and multiplying by 100.

$$\%E_{EE} = (E_{EE} \div WWt_{ACE}) \times 100 \quad \text{Eq. 23}$$

The sample was then removed from the thimble into a pre-weighed and dried 500 mL Pyrex bottle (P_{BW}) containing a stir bar and extracted with 400 mL of 18 MΩ·cm deionized water in a hot water bath for an hour. After boiling, the wood was filtered using dried and pre-weighed medium porous filters (F_{iW}) and washed with 18 MΩ·cm deionized water (~1 L). The sample with filter (F_{fW}) was placed in a vacuum oven (as previously described) to obtain extractive-free wood weight (WWt_{AW}). The thimble with sample remains (tw_{BW}) and the pyrex with sample remains (P_{AW}) were placed in the vacuum oven and allowed to cool to obtain accurate total weights to account for any sample loss due to transfer. The total sample loss (TSL_W) was

determined by finding the weight of residual sample within the thimble (tw_L) and pyrex container (P_L). To find the amount of sample loss from the transfer of sample out of the thimble (Tw_L) was determined by subtracting the thimble with residual weight (tw_{BW}) by the original weight of the thimble (tw_i).

$$\mathbf{Tw_L = tw_{BW} - tw_i} \qquad \textbf{Eq. 24}$$

Next, the amount of sample loss from the pyrex (P_L) to the pre-weighed filter (F_{iW}) was found. This loss was determined by taking the weight of the vacuum oven dried pyrex container after the hot water bath extraction (P_{AW}) and subtracting it by the initial weight of the pyrex container (P_{BW}).

$$\mathbf{P_L = P_{AW} - P_{BW}} \qquad \textbf{Eq. 25}$$

After determining the amount of sample loss associated with the thimble (Tw_L) and the pyrex container (P_L), the total sample loss due to transfer was found (TSL_W).

$$\mathbf{TSL_W = tw_L - P_L} \qquad \textbf{Eq. 26}$$

To obtain the true sample weight before the third extraction (WWt_{BW}), the sample weight after the second extraction (WWt_{ACE}) was subtracted by the total sample loss (TSL_W).

$$\mathbf{WWt_{BW}=WWt_{ACE}-TSL_W} \qquad \textbf{Eq. 27}$$

To determine the weight of extractives removed from the boiling water extraction (E_W), the sample weight after the extraction (WWt_{AW}) was found by subtracting the filter with sample (F_{fW}) to the initial filter weight (F_{iW}).

$$\mathbf{WWt_{AW}=F_{fW}-F_{iW}} \qquad \textbf{Eq. 28}$$

The weight of extractives removed from the extraction (E_W) was determined by subtracting the sample weight before the third extraction (WWt_{BW}) to the sample weight after the third extraction (WWt_{AW}).

$$\mathbf{E_W= WWt_{BW}-WWt_{AW}} \qquad \textbf{Eq. 29}$$

After determining the weight of extractives removed from the boiling water bath (E_W), the percent extractives removed could be determined. This was done by dividing the weight of extractives (E_W) by the sample weight before the extraction (WWt_{BW}) then multiplying by 100.

$$\mathbf{\%E_W=(E_W \div WWt_{BW})\times 100} \qquad \textbf{Eq. 30}$$

4.2.3 Klason Lignin Analysis

Extractive free wood was weighed to approximately 0.5000 grams and placed in a clean dry beaker. The beaker was placed in an ice bath and 7.5 mL of 72% H_2SO_4 was added drop wise over 4 minutes. The wood was carefully macerated with a glass rod while the sulfuric acid was added. After the addition of acid, the beaker was placed in a water bath at 21° C for 2 hours with frequent stirring. The acid and extractive free wood was poured into a pre-weighed 500 mL flask filled with 150 mL of room temperature 18 MΩ·cm deionized water. The beaker was rinsed with additional distilled water so that the total weight of the new flask with water and extractive free wood was approximately 465.0 g, for a dilute acid concentration of 3% H_2SO_4. The suspension was heated to boiling for 4 hours with continuous stirring. After 4 hours, the suspension was clamped, capped, and positioned at an incline overnight to allow the particles to settle. The following day the sample was filtered through a dry pre-weighed medium porous filter (F_{iKLE}) and rinsed with hot water (~1 L). The filter with sample was placed in an oven and dried at 105±3^{o} C overnight to obtain a constant weight (F_{fKLE}). After drying, the weight of dry Klason lignin was determined and stored in a dry pre-weighed 20 mL scintillation vial. The weight of Klason lignin (WWt_{AKLE}) was determined by subtracting the dried filter with sample (F_{fKLE}) to the initial dried filter (F_{iKLE}).

$$\mathbf{WWt_{AKLE}=F_{fKLE}-F_{iKLE}} \qquad \textbf{Eq. 31}$$

The weight of extractives removed by the sulfuric acid hydrolysis (E_{KLE}) was determined by subtracting the extractive free sample weight (WWt_{BKLE}) to the Klason lignin weight (WWt_{AKLE}).

$$\mathbf{E_{KLE}=WWt_{BKLE}-WWt_{AKLE}} \qquad \textbf{Eq. 32}$$

The percent extractives removed by the Klason lignin method was determined after dividing the weight of extractives (E_{KLE}) to the original sample weight before the sulfuric acid extraction (WWt_{BKLE}) and multiplying by 100.

$$\mathbf{\%KL=(WWt_{AKLE}\div WWt_{BKLE})\times 100} \qquad \textbf{Eq. 34}$$

$$\mathbf{\%E_{KLE}=(E_{KLE}\div WWt_{BKLE})\times 100} \qquad \textbf{Eq. 33}$$

To obtain the percent Klason lignin (%KL) in the sample the Klason lignin weight (WWt_{AKLE}) was divided by the extractive free wood weight (WWt_{BKLE}) then multiplied by 100.

The percent Klason lignin equation was confirmed by following another set of equations that take into consideration of the difference from the beginning of the gravimetric analysis with roughly 5.000 g (WWt_{BCE}) to the fourth extraction starting sample weight of 0.5000 g (WWt_{BKLE}). A factor was determined to account for the modification between these weights by dividing the wood weight before the cyclohexane and ethanol (2:1) extraction (WWt_{BCE}) to the extractive free sample weight before the Klason lignin extraction (WWt_{BKLE}).

$$\mathbf{Factor = WWt_{BCE} \div WWt_{BKLE}} \qquad \textbf{Eq. 35}$$

The factor was multiplied by the weight of Klason lignin found after the last extraction (WWt_{AKLE}) to determine the scaled up Klason lignin weight (KL_{wt}).

$$\mathbf{KL_{wt} = WWt_{AKLE} \times Factor} \qquad \textbf{Eq. 36}$$

After determining the scaled up Klason lignin weight, the percent Klason lignin could be found. This was done by dividing the scaled up Klason lignin weight by the starting wood weight before the first extraction (WWt_{BCE}) then multiplying by 100. This gives the same value as the percent Klason lignin found in equation 34.

$$\mathbf{\%KL = (KL_{wt} \div WWt_{BCE}) \times 100} \qquad \textbf{Eq. 37}$$

4.3 Discussion

4.3.1 Comparison of Extraction Methods

Gravimetric analysis was repeated using the same solvents, order, and time for both extraction methods with a few modifications. Each extraction method was run in triplicate with a starting sample of 5.0000 g of milled tongue depressors. The Soxhlet method modified the boiling water and sulfuric acid extraction as discussed above. Approximately, 0.5000 g of

extractive free wood was extracted using sulfuric acid to obtain Klason lignin then extraction methods were compared. Results are shown below in Tables 3 and 4.

Table 3. Results (± standard error) associated with each extraction for better comparison of the sonication and Soxhlet extraction methods.

Method	% Extractives Removed By Cyclohexane & Ethanol (2:1)	% Extractives Removed By Ethanol	% Extractives Removed By Hot Water	% Carbohydrates Removed By Sulfuric Acid	% Klason Lignin in Sample
Sonication	6.38±0.61	4.23±0.07	0.69±0.24	62.83±0.82	25.88±1.19
Soxhlet	0.98±0.24	3.02±0.12	1.62±0.20	82.56±0.46	17.44±0.46

The % extractives removed for each extraction with the standard error associated are found above in Table 3. The extractives removed from the first and second extraction are much larger using the sonication method than the Soxhlet extraction. This difference is attributed to the mechanical factors of sonication causing further physical breakdown of the sample into smaller particle sizes that can pass through the medium porosity filters. Although higher % extractives for each extraction seems more efficient, in literature these values seem out of proportion for fresh birch wood as shown in the Table 4. [9,11-15] This explains why the percent extractives removed by water and sonication is much smaller.

Table 4. A compilation of chemical composition of U.S. woods. [9,11-15]

Type	Extractives	Carbohydrates (Hemicellulose & Cellulose)	Lignin
Softwood	4-25%	55-71%	9-34%
Hardwood	4-16%	65-85%	16-30%
Birch Species (Yellow, River, & Paper)	5-7%	73-78%	17-21%

Overall, the sonication method has proven to be insufficient compared to the Soxhlet extraction method as highlighted in Table 5. The total percent extractives removed from the

Table 5. Summary of results (± standard error) associated for method comparison between sonication and Soxhlet extraction.

Method	Total % Extractives Removed	Total % Carbohydrates Removed	% Klason Lignin in Sample
Sonication	11.29±0.66	62.83±0.82	25.88±1.19
Soxhlet	5.61±0.74	82.56±0.46	17.44±0.46

sonication method, 11%, is much higher than the 2-4% extractives expected in birch wood. [9] As previously discussed, the high % extractives is an indication that the sonication method is causing the mechanical breakdown of samples to small enough particle sizes to pass through a medium porosity filter. Another interesting observation is that the Klason lignin is much larger using the sonication method than the Soxhlet extraction method. The percent Klason lignin is much higher than the expected values 17-21% found literature for fresh birch samples. [9] These high values indicate that the extractives were not completely removed, therefore contributing to the percent Klason lignin values. [41] Also, the sonication data shows a pattern of higher standard

error values associated at each extraction with exception to the ethanol extraction. This is most likely due to the loss of sample from transfer of glassware between each extraction using the sonication method.

Fortunately, the Soxhlet method obtained much closer data to the expected fresh birch extractives and Klason lignin values with a lower standard error (Table 5). The Soxhlet method was able to obtain more consistent results closer to the expected range of values found in literature of the structural components of Birch wood, 5-7% extractives and 17-21% Klason lignin.[7]

After developing a more quantitative and repeatable method, tongue depressors were degraded and extracted using the modified Soxhlet technique. Only three time points of degradation were investigated due to limited time and were based on the previous Chapter 3 IR results showing that degradation of carbohydrates ended at 6 hours. Ten tongue depressors were degraded at time points 0, 2, and 6 hours and milled as previously described. The gravimetric analysis was run simultaneously and in triplicate. The results are found below in Tables 6 and 7. As degradation time increased, the extractives removed by ethanol and water decreased. This is most likely due to the extractives degrading leaving fewer extractives to be removed. The extractives removed by cyclohexane and ethanol did not follow this trend. Extractives removed by cyclohexane and ethanol decreased at 2 hours of degradation then increased at 6 hours. Ironically, the standard error found for these values are much smaller in comparison to the extractives removed by the remaining extractions.

Table 6. Results (± standard error) associated with each extraction and time period of degradation using Soxhlet.

Time Soaking (mins)	% Extractives Removed By Cyclohexane & Ethanol (2:1) (%)	% Extractives Removed By Ethanol (%)	% Extractives Removed By Water (%)	% Carbohydrates Removed By Sulfuric Acid (%)	% Klason Lignin in Sample
0	0.98±0.24	3.02±0.12	1.62±0.20	82.56±0.46	17.44±0.46
120	0.67±0.03	2.86±0.18	0.90±0.17	82.33±0.44	17.67±0.44
360	0.73±0.02	2.06±0.05	0.74±0.07	81.57±0.84	18.43±0.84

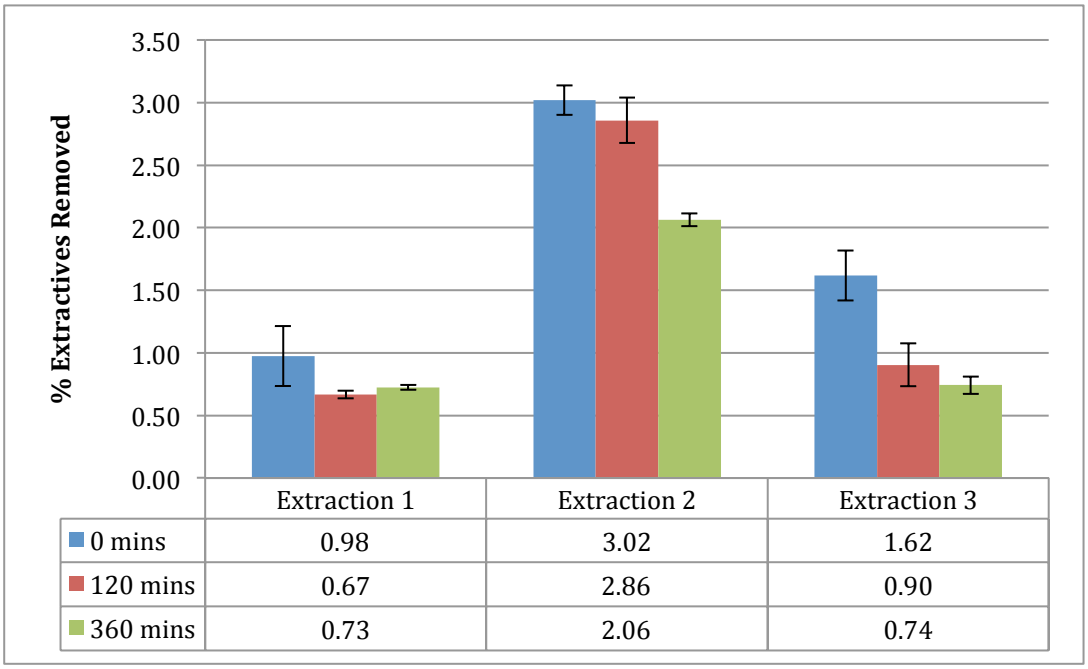
Overall, the total amount of extractives removed over increasing degradation time decreases. The same trend was seen for the sulfuric acid extraction, as degradation time increases the amount of carbohydrates removed decreases and Klason lignin values increase. Unfortunately, the standard error was large due to minimal sampling of each time period, which caused these values to be very close or overlapping.

Table 7. Summary of results (± standard error) for degradation using the Soxhlet method.

Time Soaking (mins)	Total % Extractives Removed	Total % Carbohydrates Removed	% Klason Lignin in Sample
0	5.62±0.34	82.56±0.46	17.44±0.46
120	4.43±0.25	82.33±0.44	17.67±0.44
360	3.53±0.08	81.57±0.84	18.43±0.84

The data obtained shows similar trends as to the previous degradation studies discussed in Chapter 3. The total extractives and carbohydrates removed decrease as degradation time

increases, while the % Klason lignin values increase as degradation time increases. These values are shown within the chart below, Figure 21.



	Extraction 1	Extraction 2	Extraction 3
0 mins	0.98	3.02	1.62
120 mins	0.67	2.86	0.90
360 mins	0.73	2.06	0.74

Figure 21. The % extractives removed from degraded tongue depressors for each extraction. The blue bar represent 0 minutes, the red bar represents 120 minutes, and the green bar represents 360 minutes of degradation time.

Although the gravimetric analysis shows a trend, the true error associated with the method is not known. The standard error of the sampling distribution is high, as shown in Figure 22 of the % Klason lignin for the degraded samples, but could be reduced by increasing the number of replications for each time period. The validity of the method was verified by a two-sample analysis assuming equal variances; a 50% confidence level of correlation was obtained (Figure 24). Clearly, a 95% confidence level of correlation is ideal, but due to time limitations more replications and time points will need to be analyzed to obtain a more quantitative method.

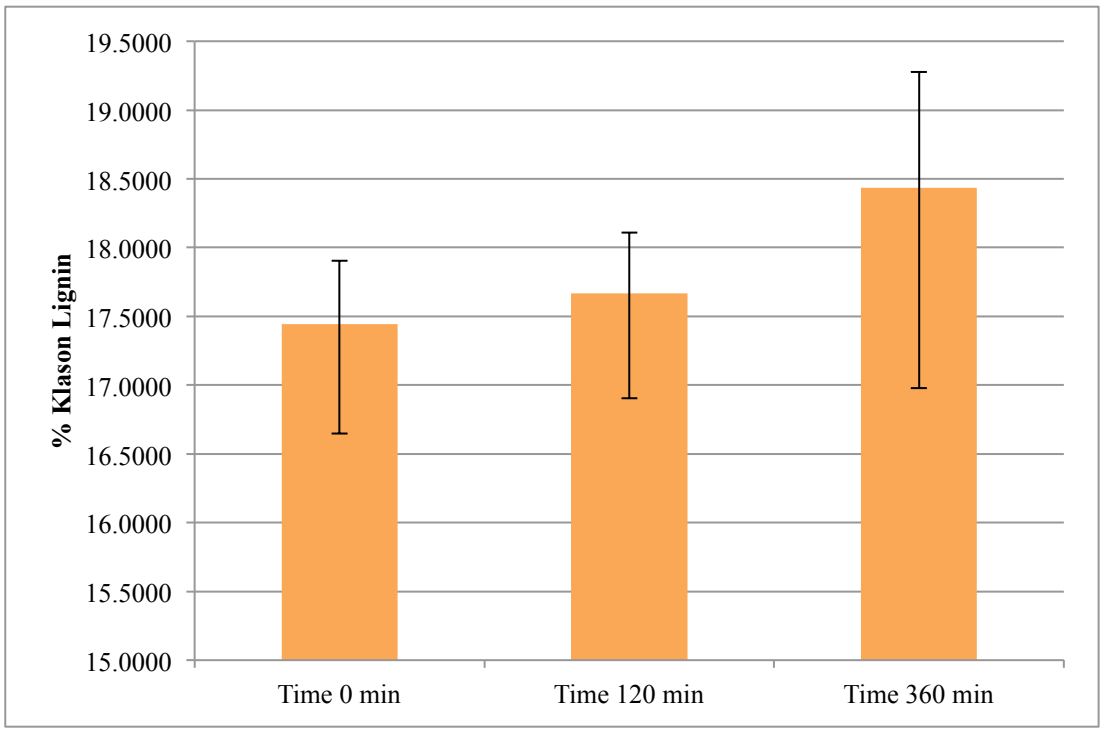


Figure 22. The % Klason lignin for the degraded samples including error bars of standard error.

4.4 Overall Conclusions

The modified Soxhlet extraction method and changes to the sample homogeneity and size contributed to developing a more efficient and reliable method. Results were closer to expected literature values of birch Klason lignin 17-21% and extractives 5-7%. The Soxhlet analysis on the degraded samples still show the trend of hemicellulose and cellulose being removed as lignin concentration increases as degradation time increases.

The IR has shown to be an efficient tool for picking out structural components rapidly. IR has proven to be more sensitive by supplying more detail about the structural components of degraded birch samples. In order to establish the IR as a quantitative method, the number of

replicates for each degradation time point needs to be determined statistically to accurately develop the range of degradation for each structural component of birch wood.

Chapter 5: Future Studies

5.1 Future Research

The first goal of this project was to determine the ideal extraction method to obtain accurate and reliable Klason lignin values by gravimetric analysis. The second goal of this project was to verify that the more degraded waterlogged wood becomes, the higher the concentration of lignin in comparison to hemicellulose and cellulose.

Unfortunately, due to time limitations, the correlation between the methods could not be determined, but the data showed evidence of a trend between degradation time and the structural components. The gravimetric data using the modified Soxhlet method and the ATR-FTIR data both show evidence that the more degraded the sample, the higher the concentration of lignin in comparison to hemicellulose and cellulose concentrations.

Future studies should obtain more degraded time points of gravimetric data within six hours. The more replicates and time points will obtain better standard error as well as give an idea of the range of extractives and Klason lignin values to expect for each time point. The correlation between Klason lignin values from the modified gravimetric analysis and IR data would be verifiable with a larger data set.

Most importantly, more analysis would be necessary to confirm that the disappearance or decrease of peaks were not due to part of the structure giving rise to the IR signal, chromophore, being chemically altered. At this point, multivariate analysis can be determined whether necessary or useful for finding more structural component trends and to validate the method. Ultimately, the future goal of this project would be to create a database of gravimetric values of degraded samples to be used as training data for the ATR-FTIR.

After the methodology has been validated, untreated samples of Black Beard's ship, the Queen Anne's Revenge (QAR), may be analyzed using the ATR-FTIR to determine the degree of degradation of the wood.

Appendix A: Method A Extraction by Sonication

Type	Time Soaking (mins)	Initial Wt. of Test Tube (g)	Initial Wt. of Wood (g)	Wt of Dry Sample After 1st Extraction (Cyclohexane / EtOH) (g)	Final Wt of Test Tube with Sample Remains (g)	Loss (g)	Total Sample Wt. After 1st Extraction (g)	Wt. of Extracted Components for 1st Extraction Only (g)	Percent Solids Extracted in 1st Extraction (%)
Sonication 1	0	51.0979	0.6529	0.6103	51.1046	0.0067	0.6170	0.0359	5.50
Sonication 2	0	50.8771	0.6272	0.5766	50.8803	0.0032	0.5798	0.0474	7.56
Sonication 3	0	51.9000	0.5666	0.5310	51.9012	0.0012	0.5322	0.0344	6.07
AVG								0.0392	6.38
STDDEV								0.0058	0.87
STDER								0.0034	0.50

Type	Time Soaking (mins)	Initial Wt. of Test Tube Prior to 2nd Extraction (EtOH) (g)	Wt. of Sample Prior to 2nd Extraction (g)	Wt. of Dry Sample After 2nd Extraction (EtOH) (g)	Final Wt. of Test Tube with Sample Remains (g)	Loss (g)	Total Sample Wt. After 2nd Extraction (g)	Wt. of Extracted Components for 2nd Extraction Only (g)	Percent Solids Extracted in 2nd Extraction (%)
Sonication 1	0	51.6429	0.6103	0.5837	51.6407	0.0000	0.5837	0.0266	4.36
Sonication 2	0	51.8726	0.5766	0.5526	51.8726	0.0000	0.5526	0.0240	4.16
Sonication 3	0	51.5626	0.5310	0.5089	51.5609	0.0000	0.5089	0.0221	4.16
AVG								0.0242	4.23
STDDEV								0.0018	0.09
STDER								0.0011	0.05

Type	Time Soaking (mins)	Initial Wt. of Test Tube Prior to 3rd Extraction (Hot Water) (g)	Wt. of Sample Prior to 3rd Extraction (g)	Wt. of Dry Sample After 3rd Extraction (Hot Water) (g)	Final Wt. of Test Tube with Sample Remains (g)	Loss (g)	Total Sample Wt. After 3rd Extraction (g)	Wt. of Extracted Components for 3rd Extraction Only (g)	Percent Solids Extracted in 3rd Extraction (%)
Sonication 1	0	52.3148	0.5837	0.5815	52.3156	0.0008	0.5823	0.0014	0.24
Sonication 2	0	50.8797	0.5526	0.5483	50.8790	0.0000	0.5483	0.0043	0.78
Sonication 3	0	51.9243	0.5089	0.5036	51.9235	0.0000	0.5036	0.0053	1.04
AVG								0.0037	0.69
STDDEV								0.0017	0.33
STDER								0.0010	0.19

Type	Time Soaking (mins)	Wt. of Wood prior to sulfuric acid treatment (g)	Wt. of Dry Medium Porosity Filter (g)	Wt of Dry Medium Porosity Filter and Dry Wood After Sulfuric Acid Treatment (g)	Wt. of Remaining Sample Particles (g)	Wt. of Extracted Components for Sulfuric Acid Treatment Only (g)	Percent Solids Extracted by Sulfuric Acid (%)	Percent Lignin in Sample (%)
Sonication 1	0	0.5823	29.9053	30.1283	0.2230	0.3593	61.70	28.20
Sonication 2	0	0.5483	29.8119	30.0183	0.2064	0.3419	62.36	25.15
Sonication 3	0	0.5036	30.2910	30.4701	0.1791	0.3245	64.44	24.29
AVG						0.3419	62.83	25.88
STDDEV						0.0142	1.17	1.68
STDER						0.0082	0.67	0.97

Appendix B: Method B Soxhlet Extraction

	Time Soaking (mins)	Initial Wt. of Wood (g)	Initial Wt. of Thimble (g)	Thimble and Wood Wt. (g)	Wt. of Sample After Extraction #1	Wt. of Extractives Removed in Extraction #1 (g)	% Extractives Removed by Cyclohexane & Ethanol (2:1) (%)
Soxhlet 1	0	5.0245	35.429	40.4006	4.9716	0.0529	1.05
Soxhlet 2	0	5.1426	35.0592	40.1321	5.0729	0.0697	1.36
Soxhlet 3	0	5.0756	35.2067	40.256	5.0493	0.0263	0.52
AVG						0.0496	0.98
STDDEV						0.0179	0.35
STDER						0.0103	0.20

	Time Soaking (mins)	Thimble And Sample Wt. After Extraction #2 (g)	Sample Wt. After Extraction #2 (g)	Wt. of Extractives Removed in Extraction #2 (g)	% Extractives Removed by Ethanol (%)
Soxhlet 1	0	40.2503	4.8213	0.1503	3.02
Soxhlet 2	0	39.9895	4.9303	0.1426	2.81
Soxhlet 3	0	40.0931	4.8864	0.1629	3.23
AVG				0.1519	3.02
STDDEV				0.0084	0.17
STDER				0.0048	0.10

	Time Soaking (mins)	500 mL Pyrex Wt. (g)	Thimble And Sample Remains (g)	Sample Loss from Thimble (g)	Sample Wt. Before Extraction #3 (g)	Filter Wt. (g)	Pyrex and Sample Remains (g)
Soxhlet 1	0	355.6	35.4409	0.0119	4.8213	126.5182	355.6
Soxhlet 2	0	358.1	35.0678	0.0086	4.9303	130.5062	358.1
Soxhlet 3	0	375.4	35.2079	0.0012	4.8864	53.1156	375.4
AVG							
STDDEV							
STDER							

	Time Soaking (mins)	Sample Loss from Pyrex (g)	Loss (g)	Sample Wt. Before Extraction #3 Considering Sample Loss (g)	Filter and Sample Wt. (g)	Sample Wt. After Extraction #3 (g)	Wt. of Extractives Removed in Extraction #3 (Considering Sample Loss) (g)	% Extractives Removed by Water (%)
Soxhlet 1	0	0.0	0.0119	4.8094	131.2522	4.7340	0.0754	1.57
Soxhlet 2	0	0.0	0.0086	4.9217	135.3639	4.8577	0.0640	1.30
Soxhlet 3	0	0.0	0.0012	4.8852	57.9037	4.7881	0.0971	1.99
AVG						4.7933	0.0788	1.62
STDDEV						0.0506	0.0137	0.28
STDER						0.0292	0.0079	0.16

	Time Soaking (mins)	Wt. of Sample Before Extraction #4 (g)	Extraction #4 Filter Wt. (g)	Filter and Klason Lignin Wt. (g)	Total Klason Lignin Wt. (g)	Weight of Extractives Removed in Extraction #4 (g)	% Extractives Removed by Sulfuric Acid (%)	% Klason Lignin in Sample
Soxhlet 1	0	0.5436	60.1069	60.2004	0.0935	0.4501	82.80	17.20
Soxhlet 2	0	0.5514	60.1372	60.2383	0.1011	0.4503	81.66	18.34
Soxhlet 3	0	0.5411	55.2244	55.3153	0.0909	0.4502	83.20	16.80
AVG					0.0952	0.4502	82.56	17.44
STDDEV					0.0043	0.0001	0.65	0.65
STDER					0.0025	0.0000	0.38	0.38

Appendix C: Degradation Analysis

	Time Soaking (mins)	Initial Weight of Wood (g)	Initial Wt. of Thimble(g)	Wt. of Thimble and Wood (g)	Wt. of Sample After Extraction #1	Wt. of Extractives Removed in Extraction #1 (g)	% Extractives Removed by Cyclohexane & Ethanol (2:1) (%)
Soxhlet 1	0	5.0245	35.429	40.4006	4.9716	0.0529	1.0528
Soxhlet 2	0	5.1426	35.0592	40.1321	5.0729	0.0697	1.3553
Soxhlet 3	0	5.0756	35.2067	40.256	5.0493	0.0263	0.5182
AVG						0.0496	0.9755
STDDEV						0.0179	0.3461
STDER						0.0103	0.1998
Soxhlet E	120	5.0438	52.0995	57.1125	5.013	0.0308	0.6107
Soxhlet I	120	5.0978	35.3389	40.4022	5.0633	0.0345	0.6768
Soxhlet K	120	5.0024	52.3144	57.2811	4.9667	0.0357	0.7137
AVG						0.0337	0.6670
STDDEV						0.0021	0.0426
STDER						0.0012	0.0246
Soxhlet F	360	5.0842	35.3672	40.4158	5.0486	0.0356	0.7002
Soxhlet G	360	5.0729	34.9906	40.0248	5.0342	0.0387	0.7629
Soxhlet J	360	5.1155	35.1776	40.2566	5.0790	0.0365	0.7135
AVG						0.0369	0.7255
STDDEV						0.0013	0.0270
STDER						0.0008	0.0156

	Time Soaking (mins)	Wt. of Thimble and Sample After Extraction #2 (g)	Sampe Wt. After Extraction #2 (g)	Wt. of Extractives Removed in Extraction #2 (g)	% Extractives Removed by Ethanol (%)	500 mL Pyrex Wt. (g)	Thimble And Sample Remains (g)
Soxhlet 1	0	40.2503	4.8213	0.1503	3.0232	355.6000	35.4409
Soxhlet 2	0	39.9895	4.9303	0.1426	2.8110	358.1000	35.0678
Soxhlet 3	0	40.0931	4.8864	0.1629	3.2262	375.4000	35.2079
AVG				0.1519	3.0201		
STDDEV				0.0084	0.1695		
STDER				0.0048	0.0979		
Soxhlet E	120	56.9802	4.8807	0.1323	2.6391	374.8000	52.1124
Soxhlet I	120	40.2640	4.9251	0.1382	2.7294	373.8000	35.3491
Soxhlet K	120	57.1219	4.8075	0.1592	3.2053	370.4000	52.3189
AVG				0.1432	2.8580		
STDDEV				0.0115	0.2484		
STDER				0.0067	0.1434		
Soxhlet F	360	40.3073	4.9401	0.1085	2.1491	374.9000	35.3702
Soxhlet G	360	39.9245	4.9339	0.1003	1.9924	353.6000	34.9945
Soxhlet J	360	40.1526	4.9750	0.1040	2.0476	375.7000	35.1798
AVG				0.1043	2.0630		
STDDEV				0.0034	0.0649		
STDER				0.0019	0.0375		

	Time Soaking (mins)	Sample Wt. Before Extraction #3 Considering Sample Loss (g)	Filter and Sample Wt. (g)	Sample Wt. After Extraction #3 (g)	Wt. of Extractives Removed in Extraction #3 (Considering Sample Loss) (g)	% Extractives Removed by Water (%)
Soxhlet 1	0	4.8094	131.2522	4.7340	0.0754	1.5678
Soxhlet 2	0	4.9217	135.3639	4.8577	0.0640	1.3004
Soxhlet 3	0	4.8852	57.9037	4.7881	0.0971	1.9876
AVG				4.7933	0.0788	1.6186
STDDEV				0.0506	0.0137	0.2829
STDER				0.0292	0.0079	0.1633
Soxhlet E	120	4.8678	131.2992	4.8118	0.0560	1.1504
Soxhlet I	120	4.9149	40.2054	4.8665	0.0484	0.9848
Soxhlet K	120	4.8030	57.0897	4.7753	0.0277	0.5767
AVG				4.8179	0.0440	0.9040
STDDEV				0.0375	0.0120	0.2411
STDER				0.0216	0.0069	0.1392
Soxhlet F	360	4.9371	135.3786	4.9021	0.0350	0.7089
Soxhlet G	360	4.9300	39.8887	4.8981	0.0319	0.6471
Soxhlet J	360	4.9728	40.1072	4.9296	0.0432	0.8687
AVG				4.9099	0.0367	0.7416
STDDEV				0.0140	0.0048	0.0934
STDER				0.0081	0.0028	0.0539

	Time Soaking (mins)	Sample Loss from Thimble (g)	Sample Wt. Before Extraction #3 (g)	Filter Wt. (g)	Pyrex and Sample Remains (g)	Sample Loss from Pyrex (g)	Loss (g)
Soxhlet 1	0	0.0119	4.8213	126.5182	355.6000	0.0000	0.0119
Soxhlet 2	0	0.0086	4.9303	130.5062	358.1000	0.0000	0.0086
Soxhlet 3	0	0.0012	4.8864	53.1156	375.4000	0.0000	0.0012
AVG							
STDDEV							
STDER							
Soxhlet E	120	0.0129	4.8807	126.4874	374.8000	0.0000	0.0129
Soxhlet I	120	0.0102	4.9251	35.3389	373.8000	0.0000	0.0102
Soxhlet K	120	0.0045	4.8075	52.3144	370.4000	0.0000	0.0045
AVG							
STDDEV							
STDER							
Soxhlet F	360	0.0030	4.9401	130.4765	374.9000	0.0000	0.0030
Soxhlet G	360	0.0039	4.9339	34.9906	353.6000	0.0000	0.0039
Soxhlet J	360	0.0022	4.9750	35.1776	375.7000	0.0000	0.0022
AVG							
STDDEV							
STDER							

	Time Soaking (mins)	Wt. of Sample Before Extraction #4 (g)	Extraction #4 Filter Wt. (g)	Filter and Klason Lignin Wt. (g)	Total Klason Lignin Wt. (g)	Weight of Extractives Removed in Extraction #4 (g)	% Extractives Removed by Sulfuric Acid (%)
Soxhlet 1	0	0.5436	60.1069	60.2004	0.0935	0.4501	82.7999
Soxhlet 2	0	0.5514	60.1372	60.2383	0.1011	0.4503	81.6649
Soxhlet 3	0	0.5411	55.2244	55.3153	0.0909	0.4502	83.2009
AVG					0.0952	0.4502	82.5552
STDDEV					0.0043	0.0001	0.6505
STDER					0.0025	0.0000	0.3756
Soxhlet E	120	0.5066	35.4114	35.5027	0.0913	0.4153	81.9779
Soxhlet I	120	0.5056	59.9286	60.0135	0.0849	0.4207	83.2081
Soxhlet K	120	0.5288	35.1863	35.2825	0.0962	0.4326	81.8079
AVG					0.0908	0.4229	82.3313
STDDEV					0.0046	0.0072	0.6239
STDER					0.0027	0.0042	0.3602
Soxhlet F	360	0.5043	53.1103	53.1970	0.0867	0.4176	82.8079
Soxhlet G	360	0.5328	134.4041	134.5004	0.0963	0.4365	81.9257
Soxhlet J	360	0.5295	35.0421	35.1482	0.1061	0.4234	79.9622
AVG					0.0964	0.4258	81.5653
STDDEV					0.0079	0.0079	1.1893
STDER					0.0046	0.0046	0.6867

	Time Soaking (mins)	% Klason Lignin in Sample	(KL wt/extraction #4 sample wt)*100	Factor	Klason Lignin Wt. of Starting Wood Sample	% Klason Lignin
Soxhlet 1	0	11.5564	17.2001	9.2430	0.8642	17.2001
Soxhlet 2	0	12.8684	18.3351	9.3264	0.9429	18.3351
Soxhlet 3	0	11.0671	16.7991	9.3802	0.8527	16.7991
AVG		11.8306	17.4448			17.4448
STDDEV		0.7605	0.6505			0.6505
STDER		0.4391	0.3756			0.3756
Soxhlet E	120	13.6219	18.0221	9.9562	0.9090	18.0221
Soxhlet I	120	12.4010	16.7919	10.0827	0.8560	16.7919
Soxhlet K	120	13.6964	18.1921	9.4599	0.9100	18.1921
AVG		13.2398	17.6687			17.6687
STDDEV		0.5939	0.6239			0.6239
STDER		0.3429	0.3602			0.3602
Soxhlet F	360	13.6339	17.1921	10.0817	0.8741	17.1921
Soxhlet G	360	14.6720	18.0743	9.5212	0.9169	18.0743
Soxhlet J	360	16.4079	20.0378	9.6610	1.0250	20.0378
AVG		14.9046	18.4347			18.4347
STDDEV		1.1443	1.1893			1.1893
STDER		0.6607	0.6867			0.6867

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