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I am submitting herewith a thesis written by Clinton Thomas Sattler entitled, "Value Prior to Processing of Oriented Strand Board Flakes Through Hot Water Extraction." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Chemical Engineering.

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Value Prior to Processing of Oriented Strand Board Flakes Through Hot Water Extraction of Hemicellulose

A Thesis presented for the Master of Science Degree

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Abstract

Research has already shown that the extraction of a valuable hemicellulose-rich stream is a viable option for revenue generation in the pulp and paper industries. Applying the value prior to pulping concept to the composite panel industry is a natural extension. If a hemicellulose extraction is accomplished under the right conditions, a non-trivial amount of fine chemicals can be generated, while leaving the woody substrate structurally intact for production to traditional products, such as oriented strand board (OSB). According to literature, the removal of hemicellulose can increase the dimensional stability while decreasing the degradability of OSB panels. This research studied the effects of hemicellulose removal by hot water extraction on softwood OSB wood flakes. Three reaction hold temperatures (120, 140, 160 deg C) and three isothermal hold times (20, 40, 60 min) were investigated. This research focuses on the changes that occurred in the physical and chemical properties of the wood flakes after extraction at each condition, while characterizing and classifying changes that occur in the liquid phase hydrolysates. Results indicated the extraction of hemicellulose in quantifiable levels begins at 120 deg. C and 40 mins and cellulose extraction begins at 140 deg C, 40 mins. The level of extraction of lignocellulosic materials, the decrease of wood flake thickness, and the acidity of the recovered hydrolysates all increase with an increase in extraction severity. The most promising results in regards to industrial implementation of OSB flake extraction occur at an extraction temperature of 140 deg C and 20 mins, coupled with hydrolysate conversion to high value chemicals.

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1. Justification and Objectives

There are currently many factors driving the utilization of renewable biomass for energy, fine chemical and polymeric applications. These include economics, environmental concerns, and practical interests.

Economically, the North American forest products industry is in desperate need of value added product lines. Profit margins have been slashed in recent years as a result of global competition that utilizes low-cost labor, quicker growing equatorial biomass, and government subsidized large-scale manufacturing facilities [Canadian Forestry Innovation Council (2005)]. Despite these economic woes, forest industries provide important contributions to the economies of many countries, including those countries in North America. Increasing efficiencies and revenues are vital to the health of these important industries, as they will continue to provide jobs and contribute to the gross national products of their respective countries [Duff (1996)].

Environmentally, governments are investing heavily in the search for "green" sources of fuel and chemical feedstocks [Canettieri (2007)]. Lignocellulosic materials can meet this demand in a carbon neutral manner, helping to relieve the carbon dioxide burden in the atmosphere [Amidon (2006)]. Interest is growing regarding the use of natural biomass-derived polymers to replace the petroleum-derived polymers that are commonplace today [Methacanon (2003)]. According to Patel, "Approximately 107 billion pounds of plastics were produced from petroleum in North America during 2003. Worldwide, less than 1% of this amount was produced from renewable, bio-based feedstocks [Patel (2005)]." Using lignocellulosic biomass, rather than starch based biomass, can provide the appropriate

chemical precursors for polymer production without competing with food and animal feed markets. [Parajo (1996), Schmidt (1997)].

Governments around the globe are mandating stringent standards on the levels of volatile organic compounds (VOC's) that wood product industries can release. Since these compounds are found naturally in wood, processing must be done to limit VOC emissions [Ingram (2000)]. Many processes that extract chemicals from biomass also extract VOC's into the chosen solvent [Speaks (1999), Roffael (2006)]. Shifting VOC's from flue gases into easier to clean solvents can save on the capital expenditures used currently for flue gas scrubbing, and allow for the recovery of valuable organic compounds [Seider (1998)].

Regarding practical interests, Keenan et al. suggest that "the hemicellulose fraction of woody biomass, typically 20%-35% on the dry weight mass is a currently underutilized resource, potentially utilized for bio-based fuels, chemicals and polymer material [Keenan (2004)]." Utilizing this "wasted" lignocellulosic material efficiently can produce revenue-generating streams for forest product manufacturing facilities [Duff (1996)]. For example, most biomass waste streams from processes are sent to generators, boilers or hog fuel units. Since the hemicellulose fraction of biomass has poor heating value, an economical advantage would be obtained from diverting hemicellulose into a higher return product stream [White (1987), Friedl (2005)]. Example high return products include furans and various organic acids. These chemicals have been targeted by the United States Department of Energy as the most important building block chemicals derived from sugar sources [Werpy (2004)]. Literature suggests that composite panel manufacturers may benefit from the extraction of hemicellulose from their raw material. Hemicellulose is an amorphous, hygroscopic polymer [Sjostrom (1993)]. The extraction of hemicellulose from raw material should provide greater

dimensional stability in the final product, as in the Masonite process in the fiberboard industry [Mosier (2005)]. Removal of the hemicellulose polymer will lessen the number of sites for free moisture to bond to the wood particles and therefore lessen the shrinking and swelling associated with moisture bonding. Because of the amorphous nature of the hemicellulose, careful extraction should have negligible effect on the strength of the wood material [Sjostrom (1993)]. Research has also indicated that extraction of hemicellulose increases the durability of woody substrates by decreasing the susceptibility of the substrate to mold biodegradation [Ye (2006)]. However, because mass is being removed from the flake, a decrease in the density of the flake is expected.

Utilizing biomass for energy and chemical applications is nothing new, and has been carried out on a variety of substrates. Great emphasis has already been placed on the recovery and synthesis of chemicals from biomass chips that will be pulped for use in the paper industry [Syverud (2002), Canadian Forest Innovation Council (2005), American Forest and Paper Association (2006)]. Research has already shown that the extraction of value from hemicellulose is a viable option for the pulp and paper industries [van Heiningen (2006)]. Research is focusing on utilizing waste streams from these industries as raw materials for integrated bio-refineries [Magdzinski (2006), van Heiningen (2006)]. Applying this concept to the composite panel industry is a natural extension.

Studies have been completed regarding the derivation of sugar-derived chemicals from such diverse lignocellulosic materials as sugar cane bagasse, corn cobs, and rice straw, all of which have been milled or ground [Sun (2002), Sun (2004a)]. However, the goal of past research has been to maximize the yield of sugars, acids, or other valuable chemicals from lignocellulosic materials with no regard to the structural alterations incurred by the

woody substrate [Canettieri (2007)]. The natural extension of previous research would be to apply a carefully performed extraction to flaked raw material, which would remove non-crystalline hemicellulose from the material while leaving the structure bearing elements of the wood (the lignin matrix and cellulose) intact. This is possible because the amorphous hemicellulose polymer will be extracted under milder conditions than are necessary for the extraction of either the lignin or crystalline cellulose polymers [Eseghlalian (1997), Xiang (2003)]. The result of this extraction would be revenue-producing chemical streams derived from hemicellulose, operating concurrently with the production of standard engineered forest products such as oriented strand board (OSB).

The specific goals of the research are as follows: extract hemicellulose under a variety of conditions, characterize and quantify the products (both sugar and co-products) released into the liquid phase hydrolysates during the reaction, characterize and quantify any chemical and physical changes that have occurred in the wood flake, develop rapid analysis techniques to aid future research in this area, perform a rough economic analysis to determine if the extraction process has potential to be a value added process, and recommend the optimal extraction condition for future, larger scale studies.

Experimentation will define the conditions (i.e. temperature and reaction time) that will maximize the level of hemicellulose extraction from *Pinus taeda* (loblolly pine) flakes, while seeking to leave the cellulose and lignin polymers largely intact. Quantification of extract products will be carried out by high performance liquid chromatography (HPLC). Testing will be done to quantify any changes in the physical dimensions of the flake that may be detrimental to industrial implementation in the future. Spectroscopy will be done to correlate time consuming analytical data to rapidly available infrared spectra.

2. Introduction

A. Wood Components

Woody biomass is a collection of cellulose, hemicellulose, and lignin polymers collectively known as lignocellulosic materials [Alen (2000)]. The amount of these polymers covers a wide range, dependent upon species environmental factors, and even location within the same tree [McMillin (1968), Sjostrom (1993), Jones (2006)].

Cellulose is the main polysaccharide in wood generally making up 33% to 42% of softwood species such as *Pinus taeda* on a dry mass basis [Sjostrom (1993)]. Cellulose is found most abundantly in the secondary cell wall. The chemical composition is known, but structure (size and molecular weight) in both crystalline and fibril forms remain a debated topic [Laine (2005)]. Intermolecular hydrogen bonding forms bundles of cellulose which are known as microfibrils. In these microfibrils, regions of highly ordered densely packed strands of cellulose give rise to crystallinity, while in other regions the cellulose is less ordered and gives rise to amorphous regions. Microfibrils aggregate to form fibrils, which in turn aggregate to form bulk cellulose fibers [Sjostrom (1993)]. The degree of crystallinity varies for a variety of reasons, but the bulk of cellulose is generally crystalline in nature [Laine (2005)].

The repeating unit of cellulose is cellobiose with each repeated unit 180° from the previous, forming a chain lattice. Cellobiose is a disaccharide composed of D-glucose units linked through β(1-4) glycosidic bonding [Sjostrom (1993)] (Figure 1). Research indicates that cellulose from wood sources contains about 10,000 glucose units [Laine (2005)]. Co-planar chains of cellulose are held together by a network of hydrogen bonds. Parallel planes of chains are held together with Van der Waals forces which form a weaker layer lattice

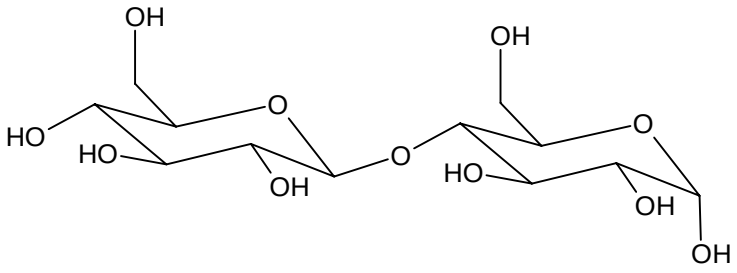

Figure 1: Cellobiose

[Bjorkman (2002)]. The strength derived from such long range order provides much of the mechanical strength of the cell wall while also making the cellulose molecule resistant to hydrolysis until harsh conditions are encountered [Xiang (2003)].

Hemicellulose generally composes 20-30% of the dry mass of wood and is also a constituent of the cell wall [Sjostrom (1993)]. Hemicellulose, unlike cellulose, is an amorphous polymer. Lacking the crystalline structure of cellulose, hemicellulose is much easier to hydrolyze and provides relatively little strength and structure to the wood [Toget (1996), Sun (2005)]. Hemicellulose is a hygroscopic polymer as the non-structured (amorphous) OH groups present in the hemicellulose polymers provide hydrogen bonding sites for water [U.S. Department of Agriculture (1974)]. Although removal of any lignocellulosic component will result in a decrease in the amount of moisture that will be absorbed at saturation, loss of hemicellulose has the most dramatic affect on this quantity [Sun (2005)]. Theoretically, removal of hemicellulose from woody substrates is desirable because removal should decrease the equilibrium moisture content (fiber saturation point), which increases the dimensional stability of the wood, while having little impact on the strength of the material [Sjostrom (1993), Mosier (2005)].

Hemicellulose, along with lignin, does form a protective "wrapper" around cellulose molecules, and the removal of hemicellulose will make the cellulose more susceptible to hydrolysis [Hsu (1996), Sun (2005)]. Unlike cellulose, hemicellulose is composed of more than one type of saccharide. Decomposition of the hemicellulose polymer yields monomeric components including D-glucose, D-mannose, D-galactose, D-xylose and L-arabinose [Sjostrom (1993)]. Hemicellulose is generally synthesized from 200 monomer units, making hemicellulose much shorter than cellulose [Sun (2004a)].

Softwood hemicelluloses are predominantly galactoglucomannans (commonly referred to as glucomannans, Figure 2). The backbone of this hemicellulose is composed of linked β-D-glucopyranose and β-D-mannopyranose units. From this backbone, α-D-galactopyranose molecules are linked as single units. Galactoglucomannans are easily depolymerized by acids, with the bond between galactose and the polymer backbone being the most susceptible to chemical attack [Shimizu (2001), Laine (2005)].

Arabinoglucuronoxylan (commonly referred to as xylan) is another constituent of hemicellulose. The backbone of this constituent is made up of linked β-D-xylopyranose

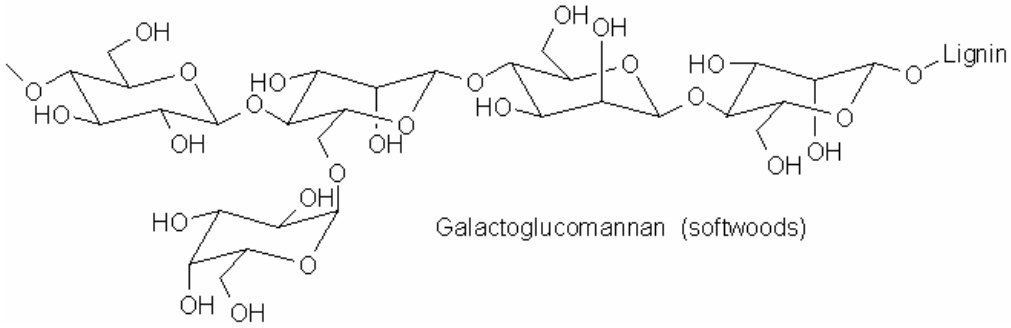

Figure 2: Galactoglucomannan [Laine (2005)]

units, with branching of 4-O-methyl-α-D-glucuronic acid groups and arabinofuranose units. These later side chains are easily hydrolyzed by acids to yield arabinose. Acetyl groups are also found as lateral chains on this polymer [Shimizu (2001), Laine (2005)].

A minor constituent of hemicellulose in most softwood species is arabinogalactan. The backbone of this polymer is made up of β-D-galactopyranose units, with other β-D-galactopyranose and L-arabinose units branched from virtually every monomer [Shimizu (2001), Laine (2005)].

Lignin content generally varies between 26-40% on a dry mass basis (with the higher value corresponding to lignin content in compression wood) and is the second most abundant natural polymer on earth [Bjorkman (2002)]. Lignin is partially dissolved during acid hydrolysis and the degrees of dissolution can be quantified using direct ultraviolet spectrophotometric methods [Sluiter (2007)]. The exact structure of lignin remains uncertain, although several have been suggested [Ralph (2005)]. Efforts to study the structure generally result in extensive alterations to the original lignin structure [Adler (1977), Mao (2006)]. Despite this, the major structural elements of lignin have been determined (Figure 3). Lignin is a polymer found in the secondary cell wall and is built up with phenylpropane units joined by ether linkages, with coniferyl alcohol being the main precursor of lignin. From this polymer backbone, methoxyl, phenolic hydroxyl and terminal aldehyde groups extend as side chains, with the phenolic hydroxyl groups functioning to link neighboring phenylpropane backbones [Alder (1977), Sjostrom (1993)]. The relative composition of these side chain functional groups varies widely, but the lignin matrix helps provide structure to the cell wall [Bjorkman (2002)]. The molecular weight of lignin can vary from 3,000 to 20,000 [Glasser (1993)].

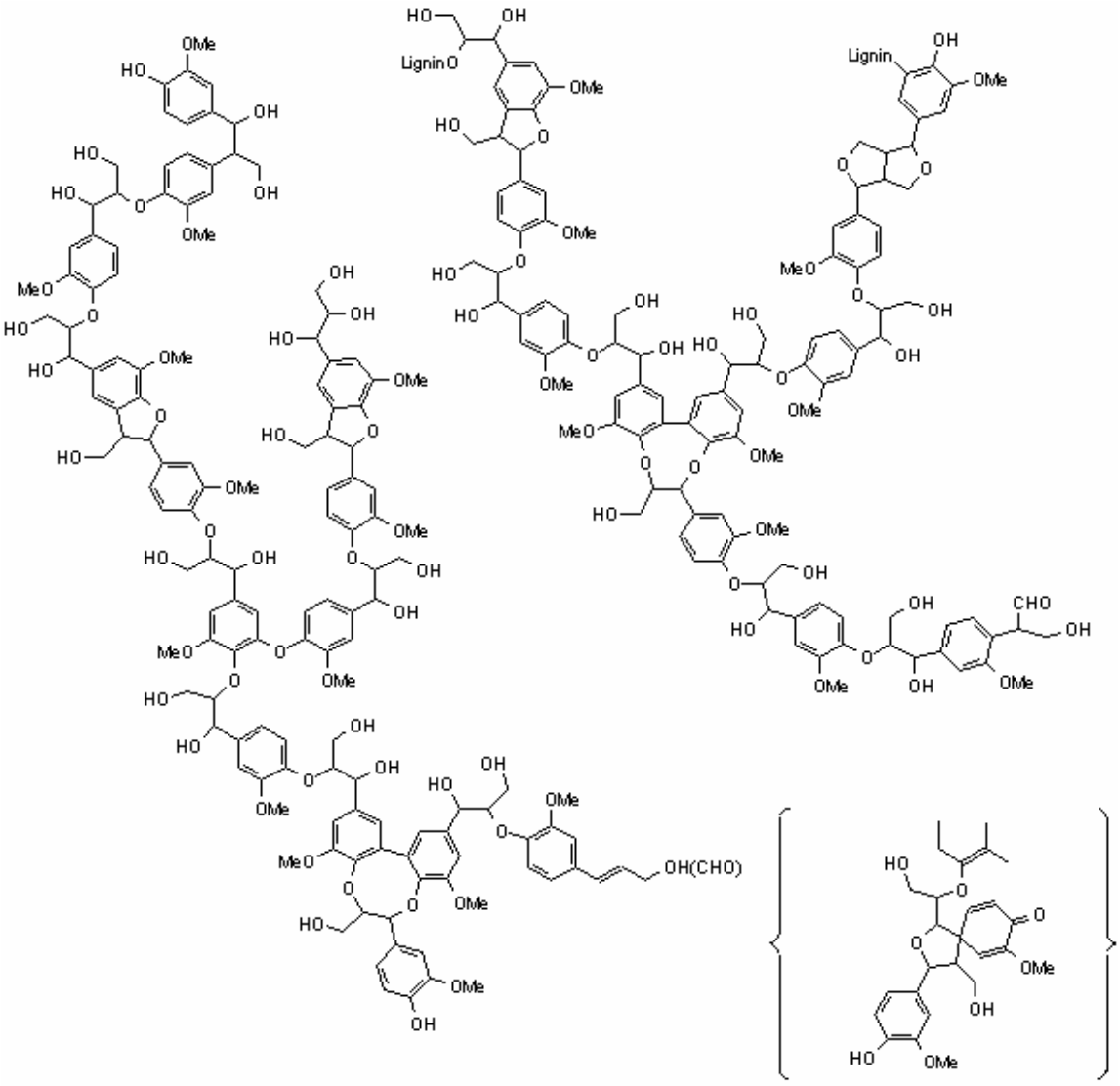

Figure 3: Lignin [Brunow (1998)]

Although presently debated, it is generally accepted that lignin forms chemical bonds with the polysaccharide polymers of wood [Sjostrom (1993)]. Lignin forms definite bonds between each of the hemicellulose constituents through ester and ether linkages [Sun (2004b)]. Some research indicates that glycosidic bonding also bridges lignin and hemicellulose, as well as indicating that there is some bonding between lignin and cellulose [Laine (2005)]. When hydrolyzed, lignin produces a variety of compounds including phenols, alcohols, and aldehydes through various bond cleavage reactions [Mao (2006)].

B. Extraction Techniques

There are five main chemical methods used to extract lignocellulosic materials from biomass: acid hydrolysis, alkali hydrolysis, enzymatic hydrolysis, steam explosion, and hot water extraction [Fang (1999), Badger (2002), Methacanon (2003), Mosier (2005), Amidon (2006), Mishima (2006)].

Acid hydrolysis is an attractive process because of its high product yields at relatively low reactor residence times [Xiang (2003), Mosier (2005)]. The glycosidic bond in both hemiceullulose and cellulose are cleaved, and the resulting oligomers are quickly depolymerized to carbohydrates [Saeman (1954)]. Near complete conversion of lignocellulosic materials have been reported for a variety of agricultural products [Tsao (1982)] but degradation of monosaccharides to aldehydes is also high [Garrote (2001)]. Acid hydrolysis is already a commercially viable process as acid catalyzed conversion of lignocellulosic materials to furfural has been done on an industrial scale for years [Sjostrom (1993)]. However, in regards to the goals of this research, acid hydrolysis is a poor choice for extraction. Acid treatment, even in dilute quantities, solubilizes lignin and hemicellulose easily, leaving the cellulosic polymer susceptible to hydrolysis [Toget (1996)]. Lignin and

cellulose will need to remain intact to provide strength and structure to the cell wall of the extracted wood flake. Acid hydrolysis is a capital intensive process [Schell (2004)] which is a serious concern in a cash-strapped industry. The corrosive nature of the acid solution requires expensive materials of construction. Required acid neutralization steps or acid scrubbing processes add capital and utility costs to acid catalyzed hydrolysis. There are also increased safety risks to personnel whenever acids are used in large quantities [Seider (1998)].

Alkali treatment has been used for years by the pulp and paper industry, as this method is very effective in delignifying woody material, as well as solubilizing some hemicelluloses [Pan (1998), Fang (1999)]. Cellulose will not be dissolved by alkali; however alkali can cause significant swelling of cellulose fibers [Sjostrom (1993)]. Under alkali conditions, side groups of the hemicellulose polymers are also cleaved, yielding a structure more susceptible to hydrolysis on the polymer backbones in some hemicelluloses [Chang (2000)]. However, the xylan backbone is stabilized against alkali catalyzed degradation, and the cleavage of glycosidic bonds in susceptible hemicelluloses is extremely slow [Sjostrom (1993)]. Large amounts of lignin can be dissolved in alkali solutions at temperatures as low as 170ºC, so appreciable amounts of lignin decomposition could be expected in the range of temperatures under investigation in this project [Sjostrom (1993)]. Many factors make alkali catalyzed hydrolysis unacceptable for this study. The destruction of the lignin in the lignocellulosic matrix will result in the loss of strength in the cell wall [Olsson (1992)]. Steric changes in crystalline cellulose as a result of swelling will lead to a destabilized lattice, thus further decreasing the strength of the cell wall [Xiang (2003)]. Since xylan is a major component of the softwood species under investigation in this study, yields using alkali

catalyzed hydrolysis would be very low, and the time frame required for hydrolysis with alkali solvents is longer than that of other processes. Whereas acid catalyzed hydrolysis occurs on the order of minutes, alkali catalyzed hydrolysis occurs on the order of hours [Mosier (2005)].

Enzymatic hydrolysis has emerged as an exciting method for hydrolyzing lignocellulosic material, as conversion efficiencies are high and controllable, and the mild process does not require expensive construction [Keller (2003)]. Enzymes such as hemicellulases have high selectivity to depolymerize hemicellulose into monosaccharides [Mosier (2005)]. However, enzymes have their downsides, too. Enzymatic hydrolysis removes hemicellulose as oligomers. Conversion to feedstocks such as monosaccharides requires further hydrolysate processesing, which increases the complexity of processing [Seider (1999), Palmqvist (2000), Mosier (2005)]. Although enzyme costs are rapidly decreasing, at present direct enzymatic hydrolysis to products such as ethanol is still prohibitively expensive for industrial implementation [Nagle (2002)]. Mass transfer limitations must also be taken into account. Enzymatic conversion generally only occurs in an absorbed monolayer. Because this study utilizes wood flakes, rather than ground or milled substrate, the ratio of surface area to volume is relatively low, and the level of conversion achieved in a reasonable time frame is severely limited [Chang (2000), Mosier (2005)]. Limitations of enzymatic conversion also occur because of the internal structure of woody substrates. According to Badger, "for the enzymes to work, they must obtain access to the molecules to be hydrolyzed. For enzymatic processes to be effective, some kind of pretreatment process is thus needed to break the crystalline structure of the lignocellulose and remove the lignin to expose the cellulose and hemicellulose molecules [Badger (2002)]."

Because of the low conversion of chip-like lignocellulosic material to valuable chemicals without destruction of the either the OSB flake macrostructure or the internal lignocellulosic matrix, enzyme hydrolysis is unacceptable in the current study.

Steam explosion is commonly used as a pretreatment method for the extraction of hemicellulose from biomass [Hsu (1996), Mosier (2005)]. Steam is applied to a sealed vessel containing biomass and after an appropriate isothermal hold time the pressure is rapidly relieved [Hsu (1996)]. This process is used commercially in the manufacture of fiberboard to hydrolyze the hemicellulose fraction of woody raw material [Mosier (2005)]. At high temperature, steam acts as a weak acid and catalyzes the depolymerization of hemicellulose. Cleavage of acetyl side groups further catalyzes the conversion of hemicellulose into monosaccharides [Sjostrom (1993), Nabarlatz (2004)]. Extraction of hemicellulose with steam limits the amount of monosaccharide dilution, which can limit downstream energy requirements in product concentrating processes especially when compared to liquid phase extractions [Heitz (1991), Mosier (2005)]. While this method is effective in extracting hemicellulose, the rapid decompression used to terminate the reaction can disrupt the internal structure of the biomass while causing large scale breakage of solid substrate [Brownell (1986)]. This research seeks to limit the disruption and destruction of the lignocellulosic matrix with the removal of hemicellulose and therefore, steam explosion is not a suitable extraction method for this study.

Hot water extraction methods are similar to steam treatment methods, but the pressure under which hemicellulose is extracted is raised to keep the water in the liquid phase [Mosier (2005)]. The extraction process is known by a variety of terms including hot compressed water, uncatalyzed solvolysis, hydrothermolysis, and aquasolv [Bobleter (1981), Mok (1992),

Bouchard (2005)]. Research has indicated at high severity conditions (200°C, 15 minutes residence time), significant amounts of lignocellulosic material is extracted (up to 22% available cellulose, 30% of lignin and all of the hemicellulose fraction) [Mok (1992)]. At low severity conditions, hot water extraction will not degrade lignin and cellulose as quickly as an acid or alkali treatment. However, for conditions to be harsh enough for hydrolysis to occur in a reasonable length of time, the extraction usually occurs under heat and pressure [Mok (1992), Ohgren (2004)], which can lead to increased capital expenditures (thick walled vessels, heat exchangers, etc.) [Seider (1998)]. According to Amidon, water is an ideal extraction solvent because water is non-toxic, abundant, and technology is already in place to clean water of possible contaminants or impurities [Amidon (2006)]. Autocatalysis is more attractive than acid and alkali catalysis because water methods reduce the required chemical loads on solvents for neutralization prior to discharge. However, valuable hydrolysis products are also diluted and therefore product concentration is generally required [Weil (1997)]. Research has indicated that product separation can be completed by membrane separation because of the chemically benign nature of the water solvent. Utilizing membranes for separation can significantly lower energy costs over more conventional separation and concentration technologies [Amidon (2006)]. The advantages of this method make it the extraction method of choice for this study.

C. Reaction Chemistry

Research indicates that the extraction of lignocellulosic materials via hot water extraction is initiated by hydronium ions formed by water disassociation [Nabarlatz (2004)]. The pKa of water has been demonstrated to reach a value of 5.0 at a temperature of 200°C [Weil (1998)]. Autohydrolysis commences when these hydronium ions promote cleavage

and conversion of acetyl and uronic side chain groups in hemicellulose to uronic and acetic acids [Maloney (1984), Li (2005)]. The concentration of these acids in the aqueous phase promotes acid catalyzed degradation of the lignocellulosic polymers [Mosier (2002)]. Hemicellulose is much more susceptible to chemical attack than cellulose because of the amorphous nature of the hemicellulose polymer [Sjostrom (1993), Sun (2004b)]. Glycosidic bonds come under attack and the hemicellulose polymers fragment into soluble oligomers in the aqueous phase [Kumar (2008)]. Once these oligomers are solubilized, depolymerization occurs rapidly to yield the aldopentoses D-xylose and L-arabinose, the aldohexoses D-mannose and D-galactose and small amounts of glucose [Sundqvist (2006)] (Figure 4). These liberated carbohydrates can be further broken down into a variety of other chemicals such as carboxylic acids and glycerol (Figure 5) depending on the severity of reaction conditions [Oefner (1992), Crabtree (2006)]. Under harsher conditions the β(1-4) glycosidic bond between glucose molecules are cleaved as cellulose is attacked [Xiang (2003)]. This cleavage results in the depolymerization of cellulose into glucose and cellobiose units [Saeman (1954)]. The amorphous regions of hemicellulose and cellulose are most prone to this attack, though if harsh enough conditions are encountered, crystalline cellulose could also be attacked [Mosier (2002)]. Most studies indicate that this de-polymerization is a first order Arrhenius reaction [Maloney (1985), Xiang (2003), Li (2005), Kumar (2008)].

The monosaccharides in the aqueous phase degrade under heat and acidic conditions. Xylose and arabinose are dehydrated to form furfural, and the six member sugars are dehydrated to form hydroxymethylfufral [Oefner, (1992), Montane (2002)] (Figure 5). If conditions in the reactor are severe enough furfural and HMF are further degraded into levulinic and formic acids [Sjostrom (1993), Palmqvist (2000)]. Glycerol and carboxylic acid production can be

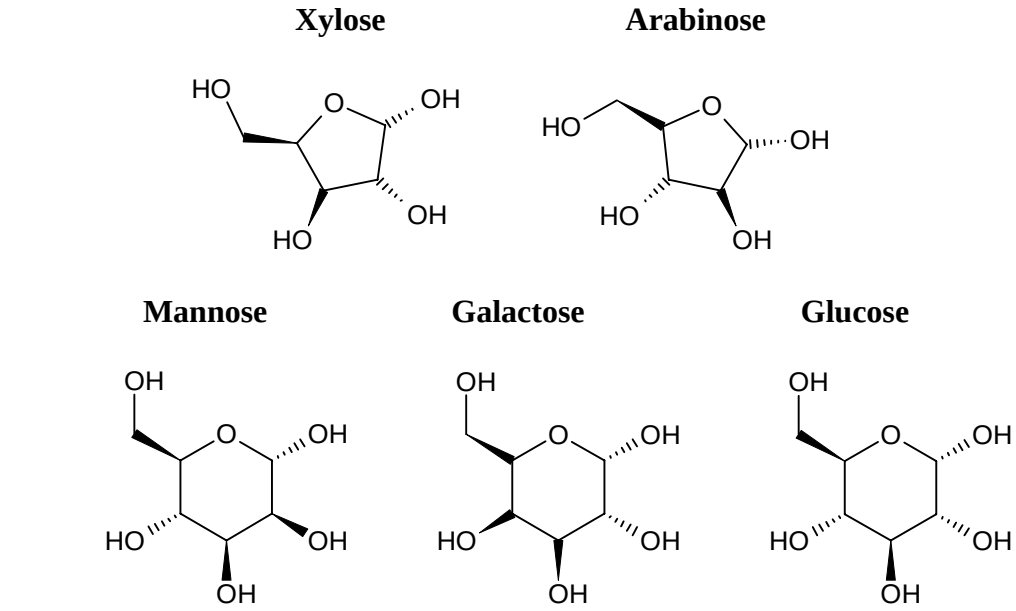

Figure 4: Monosaccharides From Lignocellulosic Material

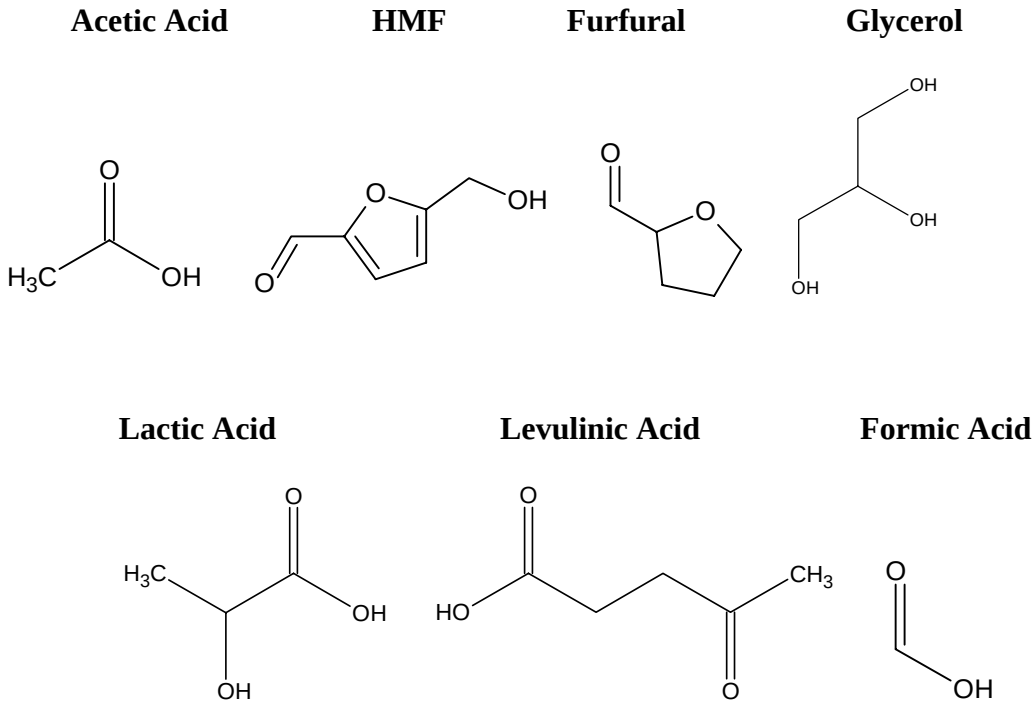

Figure 5: Carbohydrate Co-products

accomplished by cleavage of ring carbon bonds once monosaccharides have been liberated into solution. Yields are generally low until extraction temperatures near 200°C [Oefner (1992)].

Lignin degradation is dominated by condensation reactions, freeing side chain groups, and cleavage of the ether bonds. This cleavage fragments the lignin polymer [Laine (2005)]. The breaking of ether bond will increase the hydrophobicity of the lignin because of the loss of phenolic hydroxyl groups [Sjostrom (1993)]. Lignin conversion should be relatively low, as there is no strong nucleophile in the reaction solution [Sjostrom (1993)].

D. Analytical Methods

Near infrared (NIR) spectroscopy will be used to monitor chemical changes in the wood flake itself, as past research has shown spectroscopy to be a reliable source of information on the chemical make-up of wood and agriculture products [Garbutt (1992), Michell (1995), Sanderson (1996), Shenk (2001), Kelley (2004a)]. According to Kelley and collaborators, "correlations between NIR spectra and cellulose (composition) were generally very good, with $r>0.90$" [Kelley (2004b)]. Literature has shown that variations in wet chemistry results from laboratory to laboratory are usually greater than the error of NIR analysis [McLellan (1991)]. Furthermore, research has indicated that NIR technology can find use an industrial setting [Workman (2001)]. Near infrared spectroscopy utilizes the electromagnetic spectrum which lies between the wavelengths of 750 and 2500 nm [Workman (2001)]. This is the range between the visible range of light and the mid infrared range. Absorption bands (spectral peaks) in the NIR spectrum occur because of the energy absorption required to produce a quantum change in any of three modes of chemical bond vibration; symmetric bond stretching, asymmetric bond stretching and bond bending.

Plotting the energy absorbed (or transmitted) at various frequencies produces the NIR spectra of a sample [Workman (1991)]. For C-H and O-H bonds, overtone and combination bands make up the bulk of the NIR spectra. These are the bonds most prevalent in wood samples [Ciurczak (1991)]. Unfortunately, the super positioning of many different overtones in the NIR region means structural determination solely from NIR spectra is more difficult than when mid infrared and Raman spectroscopy are used [McCarthy (1991)]. However, rapid Raman and mid infrared techniques can only collect up to 10 samples per hour [Meder (1999)], while NIR techniques offer a ten-fold increase to 100 samples per hour [Kelley (2004b)]. For this reason, NIR lends itself more readily to industrial type applications, where fast measurement is required. Vital to the accuracy of Raman and mid infrared spectroscopy is the homogeneity of the sample, which usually requires milling or grinding. By contrast, NIR techniques can be used with whole samples of wood, such as chips and flakes [Raymond (2001)]. Because some chemical species are derived specifically from wood components, prediction of these species concentrations in liquid phase hydrolysates is possible from wood flake NIR spectra [Boysworth (2001), Kelley (2004a)]. NIR is the spectroscopy method of choice for solid phase analysis in this study for these reasons.

Literature has shown that high performance liquid chromatography (HPLC) is a relatively fast and useful method for characterization of hydrolysates [Luzbetak (1982), Boussaid (2001), Agblevor (2004), Griffin (2004), Griffin (2004)]. Using the laboratory analytical procedure produced by the National Renewable Energy Laboratory (NREL) of Golden Colorado, "Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples," researchers can identify and quantify a variety of carbohydrates (cellobiose, glucose, xylose, galactose, arabinose, and mannose) as well as

products arising from carbohydrate degradation (xylitol, succinic acid, lactic acid, glycerol, acetic acid, HMF and furfural) [Sluiter (2005)]. Literature has shown the NREL procedure to be an effective HPLC method [Xiang (2003), Canettieri (2007)]. However, one must take care to recognize the limitations of HPLC characterizations. When a refractive index (RI) detector is used, HPLC is not very sensitive to low carbohydrate concentrations and gradient elutions can not be used to enhance species separation [Ohgren (2007)]. Literature also indicates that baseline separation of HPLC chromatogram carbohydrate peaks is not achieved with virtually all currently used HPLC methods [Agblevor (2004)].

E. Multivariate Methods

In this study, multivariate analysis (MVA) is the preferred method of statistical analysis. Chemical data gathered through NIR spectroscopy is especially suited for analysis through MVA techniques [Martens (1989), Boysworth (2001), Rodrigues (2001), Kelley (2004a, 2004b)]. Because the NIR spectrum is composed of overlapping and overtone bands, no single wavelength is sufficient for prediction or classification of a substance [Ciurczak (2001)]. MVA is a robust method that uses data from the entire NIR spectrum rather than peaks chosen *a priori*. Using the entire NIR spectrum is beneficial to the researcher because the MVA results can be directly applied to the entire chemistry of the sample [Martens (1989)]. Coupling MVA with NIR can provide a much more rapid method of chemical analysis than the more time consuming HPLC method, and is similar to techniques which have already been reported in the literature [Kelley (2004a, 2004b), Jones (2006)].

This research uses two MVA techniques in order to classify and predict the level of lignocellulosic extraction achieved. Principal Component Analysis (PCA) is useful for data classification when large numbers of x variables are used to define the data. Partial Least

Squares Regression (PLS) is used for prediction of a response from a large number of *x* variables [Martens (1989), Beebe (1998)]. Both of these methods are linear methods.

The following is an introduction to PCA and PLS. For a complete description of MVA techniques refer to Martens et al. and Beebe et al. [Martens (1989), Beebe (1998)]. It is from these sources that this introduction has been adapted.

Principal component analysis is a statistical technique that seeks to reduce a large number of independent and dependant variables into a few new variables called principal components (PCs). The goal of doing so is to represent variation in data points (or rather inter-point distances in terms of the original variables) in as few parameters (PCs) as possible. These principal components are linear combinations of the original variables of study. The recasting of the original variables in terms of PCs results in significant data compression.

PCs are ranked in order of the amount of variation in the data set they explain, with PC 1 containing the greatest amount of variation. This occurs because PC 1 is chosen to maximize the covariance between all *x* variables. Commercially available software will generally report what percentage of the total original variation in the data is described by each PC. One must be careful in selecting the number of PC's to use to describe data variation. As the number of the PC increases, the signal to noise ratio decreases. Very high PC's may describe nothing but random noise in the data. When using PC's for data analysis, it is important to ensure that the generated PC's describe real variation in the data rather than noise. Analysis of the loadings plot will help indicate if a PC is describing real variation or simply noise in the data.

The loadings plot of a PC indicates which of the original variables are important in characterizing the variation in the data captured by that PC. Loadings are generally plotted against the original variables of the data set. Loadings approaching 0 are unimportant in the construction of that PC, while loadings near 1 or -1 are most important in the construction of that PC because loadings are equal to the cosine of the angle created by the PC and the original variable axis. In models with multiple PCs, the most important of the original variables will have large loadings in low numbered PCs. If the loading of a PC is structured with characteristic and expected peaks, it is most likely a significant PC, assuming addition of that PC significantly increases the amount of described data variation. However, if the loading of a PC resembles random noise, that PC is most likely not significant in describing real variation. Analysis of the loading values of NIR spectra indicate which absorption bands are most important in the chemical variation of the samples. The signs of these loadings have physical meaning. If a loading in a particular PC has a positive value, samples that have a positive score in that PC space have higher than average values for that variable.

Before recasting the data along principal component axes the data points are defined by coordinates in an n dimensional coordinate system, where n equals the number of x variables in the original data. After recasting, the data points will have coordinates in a new coordinate system with the dimensionality of the new coordinate system equal to the number of significant PCs. The new coordinate of each data point in principal component space is called the score. Plotting data point scores reveal how samples are related and can be used to determine characteristic grouping. If samples are close on the scores plot, there is little variation that exists between them. Scores are plotted against their values in the PCs which

are chosen to create the axes. For NIR data, samples that cluster closely together exhibit similar absorption bands and thus similar chemistry.

To protect against the skewing of PCA results by outliers, cross validation of the PCA model was implemented. In cross validation, samples are removed, one by one, from the original data set (of *n* samples) until all the samples have been removed once. PCs are constructed and residuals are calculated at each step with *n*-1 samples. The residual variance (variance not described by the PCs) for the omitted sample is then calculated. If this data point is an outlier, the residual variance for that point will be inflated and will be more readily detectable in a plot of the residuals.

Partial Least Squares Regression (PLS) is a relative of PCA. PLS is the regression of a response vector (or matrix) onto a few linear combinations of the *x* variables (PCs) that can be used to capture significant variation in the response (*y*) variables. PLS is unique in that the PLS method uses information in both the *x* and *y* variable matrices to construct PCs (referred to as loading weights in PLS). The advantage of this technique is that large variation in *x* variables that have no impact on response variables are ignored during modeling, and the resulting PCs will describe variation more relevant to response variable changes.

The loading weight vector is calculated as the vector that maximizes the sum of the covariance between both *x* and *y* variables simultaneously. In this manner, the loading weight vector is similar to the PC of PCA.

The projection of the *x* variables onto the loading weight vector produces scores, and the regression of the original *x* variables onto these scores produce regression coefficients. The regression coefficients indicate which of the original *x* variables were important in

construction of the loading weight vector. Thus, the regression coefficient vector is similar to the loading vector of PCA. New loading weight vectors are added until the addition of more loading weights does not decrease the residual variance of the data. PLS uses a certain portion of the data set, called the calibration data, to perform a least squares regression and estimate two important parameters in the PLS model; both loadings in terms of *x* and *y* variables. Once these parameters are estimated, they are applied to the rest of the data set for validation.

For a model to be valid, the structure of the regression coefficient and loading weight vectors should be similar, the root mean square error (RMSE, a measure of the precision of the model) should be relatively low, and the coefficient of determination (R^2) should be sufficiently high. Care must be taken in model construction to ensure that proper weighting is given to each response variable. Disproportionate amounts of data at any single value for the response will bias results towards the response with the most data.

3. Experimental Methods

A. Biomass Samples

Pinus taeda flakes were obtained from The Georgia Pacific Corporation Research and Development Center in Atlanta, Georgia. The flakes were obtained from a pilot scale flaker, providing more uniform dimensions than industrial scale flakers. Average flake dimensions are 7.87 cm long, 2.61 cm wide and 0.095 cm thick.

Wood extractives were removed via a 5 hour Soxhlet extraction utilizing toulene and ethanol in a 2:1 ratio (by volume). Extractives free wood flakes were oven dried at 105^{o}C for 24 hours and divided into batches composed of nine flakes each, targeting a total mass of 5 grams on a dry mass basis. Extractives were removed so that all experimentally observed physical and chemical changes were related solely to the removal of lignocellulosic materials.

Chemical composition was determined using the NREL laboratory analytical procedure "Determination of Structural Carbohydrates and Lignin in Biomass" (Table 1) [Sluiter (2007)].

Table 1: *Pinus taeda* composition analysis

Wood Component	% of Dry Mass
Glucose	43.3%
Lignin	26.1%
Mannose	11.9%
Xylose	6.1%
Ash	0.2%

B. Extraction Process

Hemicellulose extractions were carried out at three different hold temperatures (120, 140, 160°C) and three different isothermal hold times (20, 40, 60 mins). Each reaction was replicated 4 times, unless otherwise noted.

Reactions were carried out in a 1 liter Parr series 4523 316 stainless steel reactor equipped with a 600 ml glass liner. The reactor was controlled with a Parr model 4843 controller, which was capable of holding a setpoint temperature to ± 3 degrees Celsius. Heating was accomplished with an internal aluminum block heater executing a linear temperature ramp from room temperature to setpoint temperature at a rate of 1.8°C per minute. The actual required residence time of the substrate in the reactor was much greater than the isothermal hold time because of this rate of heating (Table 2).

Table 2: Average Total Reactor Residence Times

Extraction Conditions			
Temp, °C	Hold Time, Minutes	Total Reactor Residence Time (mins) Avg (*Std Dev*)	N
120	20	65 *(10)*	(4)
120	40	84 *(17)*	(4)
120	60	106 *(4)*	(4)
140	20	82 *(5)*	(4)
140	40	111 *(8)*	(4)
140	60	120 *(14)*	(4)
160	20	92 *(6)*	(4)
160	40	116 *(14)*	(4)
160	60	148 *(17)*	(4)

The relative severity of an aqueous extraction can be quantified according to a severity factor, S_0 [Overend (1987)]. The severity factor is useful in that the factor can be used for comparison of reaction conditions with those of literature [Glasser (1998)]. For aqueous treatments, the severity of the reaction is related to the time and temperature of the extraction [Overend (1987)]. The equation for calculating the severity factor can be found in Appendix B. Because heating is executed as a linear ramp of 1.8ºC, the severity factor can be calculated to include the residence time during the heating cycle in which the contents are greater than 100ºC. The severity factor ranges from a value of 3.20 (120ºC, 20 mins) to 6.27 (160ºC, 60 mins) in this research (Table 3). The dominate factor in determining the severity factor is extraction temperature, so that extractions occurring at 120ºC, 60 mins ($S_0 = 3.68$) are more severe than those occurring at 140ºC, 20 mins ($S_0 = 4.52$).

When the substrate was at the desired reaction temperature, continual agitation was accomplished by controlling an internal blade type impeller to 200 revolutions per minute. Cooling was accomplished via pressure relief after the appropriate residence time has been

Table 3: Severity Factors for Extraction

Extraction Conditions		
Temp, °C	Hold Time, Minutes	S_0, Including Heat-Up
120	20	3.20
120	40	3.49
120	60	3.68
140	20	4.52
140	40	4.88
140	60	4.99
160	20	5.71
160	40	6.03
160	60	6.27

met. During pressure relief cooling water (city supply) flowed through a cooling jacket surrounding the reaction vessel in order to provide additional cooling. The reactor was dismantled once excess pressure had been bled off, and then the wood was separated from the hydrolysate via filtration (P5 qualitative filter paper), and the wood flakes were washed with deionized water to remove deposited products or precipitates. Solid wood flakes and hydrolysates were completely separated on the order of minutes. The pressure relief line was routed through a 500 mL filter flask, which acted as an air cooled condenser. The condensate was collected, combined with the hydrolysate, the entire liquid fraction filtered through a 30-F filter crucible and then frozen at -20°C until liquid phase characterization. Reactor geometry necessitated that a 40:1 ratio of water to dry wood be used (on a mass basis). The percent mass loss of the sample was calculated as the difference in dry mass before and after extraction divided by the dry mass before extraction.

C. Chemical Composition Analysis

A hydrolysate concentration step was necessary before characterization because of the insensitivity of refractive index HPLC detectors and the large amount of water used in this study. Samples were concentrated in a CentriVap® centrifuge heated at 60°C and under 200 millibar of vacuum. 40 mL aliquots of liquid hydrolysate were condensed down to 4 mL aliquots prior to characterization with HPLC. The pH of these concentrated aliquots was recorded. pH data was collected with an accumet® Basic AB15/15+ pH meter. Aliquots were neutralized through the addition of calcium carbonate prior to HPLC analysis as per the NREL procedure, "Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples" [Sluiter (2005)].

HPLC analysis was carried out on a Waters 2695 Alliance Separations Module coupled with a Waters 2414 Refractive Index Detector, with external column heater. Carbohydrate (cellobiose, glucose, xylose, galactose, mannose, and arabinose) characterization utilized a Biorad Aminex HPX-87P column. Co-product (xylitol, succinic acid, acetic acid, lactic acid, glycerol, hydroxymethylfurfural (HMF), and furfural) characterization utilized a Biorad Aminex HPX-87H column. Both columns were equipped with a Biorad H^+/CO_3^- deashing guard column located upstream from the column. The NREL procedure, "Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples" was used as the HPLC protocol [Sluiter (2005)]. Highly convoluted mannose and arabinose peaks prevent quantification of the species that occurs in the lesser concentration (arabinose in the case of this research). Because of non-baseline chromatogram resolution quantification was accomplished using peak intensity (height) rather than the tradition area bound by the detector response curve. HPLC analysis was done in two distinct batches because of sample aging concerns. Four concentrations (calibration points) were used to create calibration curves for each species. Calibration curve details can be found in Appendix A.

Acid soluble lignin content in the liquid fractions was determined by ultraviolet (UV) absorption as per the NREL Laboratory Analytical Procedure, "Determination of Structural Carbohydrates and Lignin in Biomass" [Sluiter (2007)]. Absorption data were collected on 2 mL aliquots at a wavelength of 240 nm to minimize interference between UV absorption by extraction products. UV data were collected in quartz cuvettes in a BioMate 3 spectrometer, with 0.01 normal sulfuric acid as reference (blank). UV absorption data were collected on

unfiltered, unconcentrated aliquots to limit soluble lignin losses that may occur during hydrolysate processing.

D. Wood Flake Analysis

Visible/NIR spectral data were collected on the substrate flakes with a Labspec Pro® fiber optic probe produced by Analytical Spectral Devices, INC., over wavelengths 350 to 2500 nm. Spectra were taken in a darkened room with wood flakes illuminated by a DC light source. The samples were placed in a 10 cm cup and rotated at 45 revolutions per minute to provide sample homogeneity. Eight spectra were collected on each set of nine flakes, with each spectrum being composed of the average of 30 sample scans. For sets of flakes scanned prior to extraction (control samples), the eight spectra collected were averaged to produce one spectrum per sample set in order to prevent statistical biasing issues.

Wood flakes were scanned before and after extraction using an Epson optical scanner to obtai of thickness distributions before extraction n the 2-dimensional projected surface area of the individual flakes. The resulting image files were processed using ImageJ version 1.38x from the National Institute of Health. After setting the appropriate pixel/length ratio, images were converted to binary masks and the surface area of the nine largest flake masks was calculated (Figure 6). Flake thickness measurements were accomplished with TESA digit-cal® digital calipers accurate to 0.01 millimeters. Four thickness measurements were taken per individual wood flake across the axial direction of the flake. Nonparametric (Wilcox Rank- Sum) tests were performed on the samples in order to determine if average values before and after extraction were equal. Analysis of the flake thickness distributions before extraction indicates that the sub-population at each condition are not the same as

Figure 6: Example of Image Processing

the parent population of thicknesses before extraction for all flakes in this study (Table 4, Figure 7). Nonparametric methods were chosen for analysis accordingly. All tests were performed at the 95% confidence level ($\alpha = 0.05$)

Equilibrium moisture content data were measured by equilibrating small samples of flakes for 45 days in closed vessels held at a constant temperature of 22°C at various relative humidities. Mass measurements were recorded at fifteen day intervals to ensure samples reached equilibrium. Each vessel contained different saturated salt solutions to produce environments of varying relative humidities (RH). Water (100% RH), potassium chloride (85% RH), sodium nitrate (65% RH), and lithium chloride (12% RH) were used in this study.

E. Multivariate Analysis

Two multivariate techniques were used in this study. Principal component analysis (PCA) was done in order to determine if chemical changes in the samples could be used to classify specimens according to extraction conditions. PCA treatment was applied to NIR

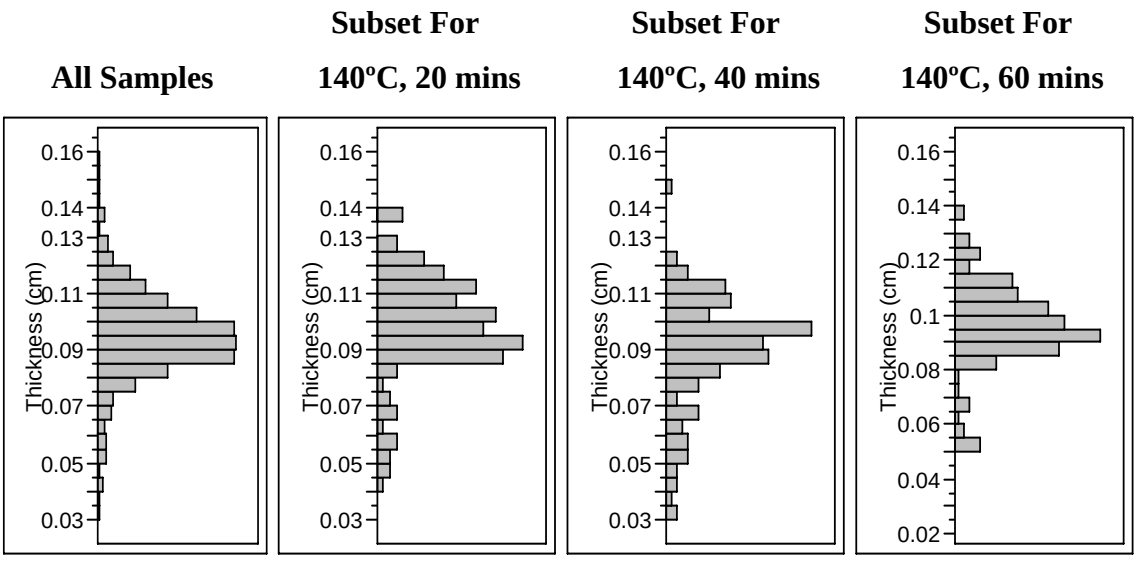

Figure 7: Flake Thickness Distributions Before Extraction

Table 4: Flake Distribution Parameters Before Extraction

Extraction Conditions Sub populations			
Temp, ºC	Hold Time, Minutes	Thickness Before Extraction, cm	
		Avg (*Std Dev*)	N
All Samples, All Conditions		0.094 *(0.016)*	(1152)
140	20	0.099 *(0.018)*	(144)
140	40	0.089 *(0.020)*	(144)
140	60	0.096 *(0.016)*	(144)

spectra collected on the extracted wood flakes, as well as the hydrolysate HPLC chromatograms.

NIR data were preprocessed through conversion from reflectance spectra to absorbance spectra and mean normalized. Data were also corrected for multiplicative scatterusing a full MSC model. The resulting 538 *x* variables were further reduced when only data from 1000 nm to 3500 nm were used, leaving 375 *x* variables with a spectral resolution of 4nm.

PCA on HPLC data was completed only in the residence time range in which quantified chemicals elute for the appropriate separations column. This was done in order to ensure that classification was occurring based on the chemical composition of the hydrolysate only in regards to the species investigated in this study. All principal components were validated using cross validation.

Partial least squares regression (PLS) was completed in order to determine if the observed flake changes and hydrolysate chemical properties could be calculated through coupling rapid NIR (collected on the wood flakes) techniques with predictive models rather than utilizing comparatively time consuming analytical procedure. PLS-1 models (1 independent response variable) were built with the percent change in dry mass after extraction, hydrolysate pH, percent lignin solubilized by extraction and each chemical species concentration as response variables. *X* variables were defined by wood flake NIR data with the proper number of individual samples used in model construction to prevent biasing. If a chemical species was not present in the hydrolysate at a certain extraction condition, that condition was not used in the construction of the model. Two thirds of the appropriate data points were selected at random and used for creation of calibration models,

with the remaining one third of the data points used for model validation. All PLS-1 calibration models were cross validated.

In addition to the PLS-1 model, a PLS-2 model (2 dependant variables) was constructed for prediction of glucose and cellobiose concentrations in the hydrolysate. The PLS-2 model was used for these species as the concentrations of glucose and cellobiose should be related to each other. *X* variables were again composed of wood flake NIR data, with two thirds of the data used for calibration and one third of the data used for validation. The PLS-2 model was also cross validated.

PCA and PLS was carried out on Unscrambler® version 9.0 from CAMO Processes Software of Oslo, Norway.

4. Results and Discussion

A. Solid Phase – Wood Flakes

As the extraction temperature increases, the absolute value of the percent change in dry mass of the samples increases (Table 5). The average percent change in dry mass of the wood flake ranged from -6.74% (120ºC, 20 mins) to -27.29% (160ºC, 60 mins). Within constant temperatures, absolute value of the percent change in dry mass of the samples increases with isothermal hold times. The increase in the absolute value of the percent change in dry mass corresponds directly to an increase in the severity of the reaction. There is considerable overlap in the percent change in dry mass achieved at 120 and 140^{o}C. However, little overlap exists between extractions occurring at 140 and 160^{o}C. This overlap is also visible during principal component analysis of both NIR spectra collected on the wood flakes and on the HPLC chromatography.

The extraction process results in changes in some physical dimensions of the flakes (Table 4). The average thickness of the flakes is decreased after extraction, as is the average flake surface area. Low severity extraction conditions generally produce low percent changes in average thickness (-8.13% at 120^{o}C, 40 mins) while high severity conditions generally produce higher percent changes in average thickness (-14.48% at 160^{o}C, 60 mins), though there are some conditions which violate this trend (-15.25% at 120ºC, 20 mins). The percent change in the average flake surface area ranges from -0.23% (120^{o}C, 40 mins) to -15.44% (160^{o}C, 60 mins). Little correlation exists between the extraction condition and the percent change in surface area. This may occur because the majority of flake damage occurs through random encounters with the mixing apparatus or some other factor that can not be modeled based on the data collected in this study.

Table 5: Properties of Wood Flakes After Extraction

Extraction Conditions					Individual Flake Surface Area (SA), cm^2				Individual Flake Thickness, cm				Equilibrium Moisture Content			
Temp, °C	Hold Time, Minutes	S_0	% Change in Dry Mass Avg (*Std Dev*)	N	Before Extraction Avg (*Std Dev*)	After Extraction Avg (*Std Dev*)	% Change In Avg.SA	N	Before Extraction Avg (*Std Dev*)	After Extraction Avg (*Std Dev*)	% Change In Avg Thkns	N	% Change in mass at Saturation 100% RH	85% RH	65% RH	12% RH
Control		NA	NA		16.15 *(2.12*	NA	NA	(288)	0.094 *(0.016)*	NA	NA	(1152)	23.86%	16.35%	11.64%	3.66%
120	20	3.20	-6.74% *(1.62%)*	(3)	16.26 *(2.12)*	14.52 *(2.71)*	-10.72%, [b]	(27)	0.096 *(0.014)*	0.081 *(0.009)*	-15.25%, [b]	(108)	21.58%	15.57%	12.72%	2.66%
120	40	3.49	-7.68% *(1.49%)*	(3)	15.32 *(2.39)*	15.28 *(1.79)*	-0.23%, [a]	(27)	0.096 *(0.011)*	0.088 *(0.007)*	-8.13%, [b]	(108)	20.21%	14.24%	10.38%	2.89%
120	60	3.68	-10.59% *(3.24%)*	(4)	16.15 *(2.01)*	14.97 *(2.54)*	-7.30%, [b]	(36)	0.094 *(0.015)*	0.083 *(0.010)*	-11.64%, [b]	(144)	19.94%	13.98%	11.37%	3.37%
140	20	4.52	-11.04% *(0.36%)*	(4)	17.32 *(2.40)*	15.38 *(2.71)*	-11.18%, [b]	(36)	0.099 *(0.018)*	0.087 *(0.015)*	-12.27%, [b]	(144)	15.88%	12.66%	10.73%	2.37%
140	40	4.88	-15.51% *(2.03%)*	(4)	15.53 *(1.46)*	14.51 *(1.80)*	-6.57%, [b]	(36)	0.090 *(0.020)*	0.080 *(0.018)*	-10.33%, [b]	(144)	16.75%	13.06%	9.58%	2.98%
140	60	4.99	-16.83% *(2.43%)*	(4)	16.89 *(1.59)*	15.40 *(2.19)*	-8.81%, [b]	(36)	0.096 *(0.016)*	0.083 *(0.015)*	-13.66%, [b]	(144)	16.31%	12.38%	10.60%	2.04%
160	20	5.71	-23.15% *(1.20%)*	(3)	16.45 *(2.09)*	14.89 *(2.47)*	-9.94%, [b]	(27)	0.091 *(0.020)*	0.078 *(0.015)*	-14.39%, [b]	(108)	17.11%	11.93%	7.83%	1.36%
160	40	6.03	-25.03% *(3.11%)*	(3)	15.89 *(2.46)*	14.29 *(2.45)*	-10.12%, [b]	(27)	0.096 *(0.015)*	0.086 *(0.012)*	-10.51%, [b]	(108)	14.68%	12.42%	9.67%	0.92%
160	60	6.27	-27.29% *(2.58%)*	(4)	16.14 *(2.14)*	13.65 *(3.03)*	-15.44%, [b]	(36)	0.094 *(0.012)*	0.080 *(0.008)*	-14.48%, [b]	(144)	16.01%	11.18%	9.72%	0.68%

a – avg. equal before and after extraction
b – avg. not equal before and after extraction

In terms of equilibrium moisture content, the percent change in mass at saturation at 100% relative humidity ranges from a maximum of 23.66% (Control samples) to 14.68% (160ºC, 40 mins) (Table 5). Data indicate a general decrease in the percent change in mass at saturation with an increase in reaction severity. This trend is also visible in data collected at other relative humidities. The decrease in the equilibrium moisture content occurs because of the removal of hygroscopic lignocellulosic material and indicates a greater level of dimensional stability should be observed in the wood flake after extraction [Sjostrom (1993)]. Standard OSB is generally pressed with production line moisture contents near 8% [Wolcott (1994)]. All samples indicate that 8% moisture content is achievable after the extraction process.

The chemical changes that occur to the wood flakes during the extraction process were investigated by NIR coupled with MVA. Typical wood flake NIR spectra at various extraction conditions are presented in Figure 8. Absorption peaks are visible near wavelengths of 1440 (C-H combination band), 1930 (O-H stretch/H-O-H deformation combination band), 2090 (O-H combination band), 2335 (C-H stretch/C-H deformation combination band) and 2352 nm (CH_2 bend, 2^{nd} overtone) and correspond to peaks commonly present in spectra collected on lignocellulosic materials [Shenk (2001)]. All NIR samples are measured by reflection of energy rather than direct measurement of energy absorption. Reflectance measurements penetrate only one to four nanometers into the exposed sample surface [Workman (2001)]. The energy of higher frequency will penetrate more deeply in a sample. At high frequencies, the amount of energy absorbed will therefore be higher. The result of this is that, because of Beer's law (which relates absorbance and the concentration of the absorbing species through a direct proportionality) the baseline in the

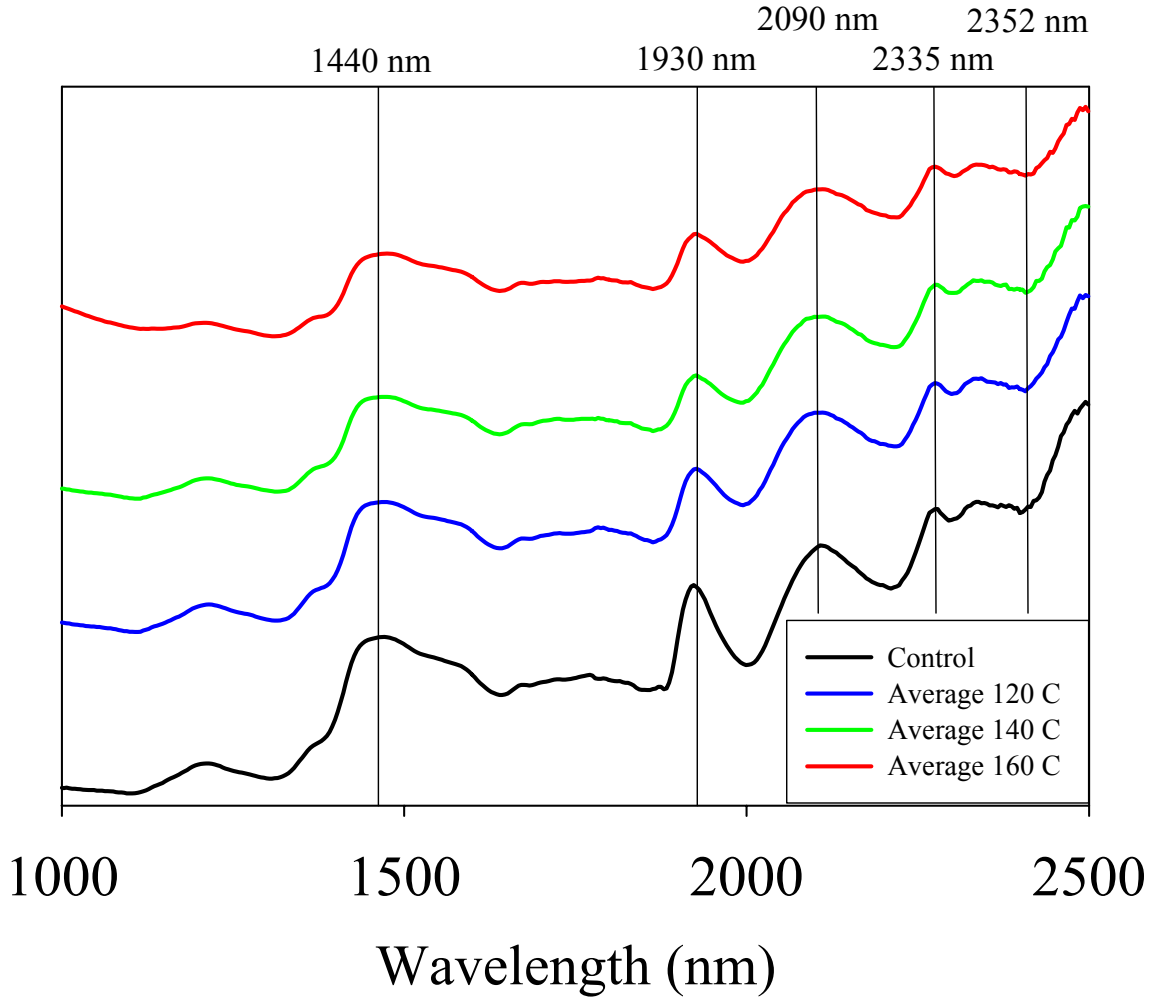

Figure 8: Characteristic NIR Chromatograms of Extracted Wood Flakes

NIR spectra will shift upwards at higher wavelengths. Visible inspection indicates shifts in peak intensities at some characteristic wavelengths, but shifts in peak intensity are more ambiguous for other peaks. PCA will remove this ambiguity.

A decrease in peak intensities at 1440 nm as extraction temperature increases at is visible in the NIR spectra. This change corresponds to a decrease in the amount of C-H bonds present in the solid samples. Decreases in peak intensities with at 1930 nm and 2335 nm is apparent when comparing control (unextracted) samples with samples, but peak intensity changes less evident between the three extracted conditions. Multivariate (PCA) results will indicate if these peak intensities are changing with extraction condition.

Results from the PCA of the wood flake spectra are contained in Figure 9. Figure 9 is a plot of the two principal components of the spectral data. Principal Component 1 contains 96% of the observed variation in the spectra, while Principal Component 2 contains 1% of the observed spectral variation.

It can be observed from the data that the majority of the chemical variation in the wood flakes (PC 1) is related to the extraction condition, while variation observed in PC 2 is related to natural variation in the raw material itself. Clustering of the data is visible in regards to extraction condition, with increasing reaction severity moving generally right to left along the PC 1 axis. This clustering indicates unique chemical identities of the wood flake at each extraction condition. These results agree with literature in that the experimental conditions produce distinct levels of extraction [Eseghlalian (1997)]. As suggested by the mass loss data, there is significant overlap in the chemical identity of wood flakes extracted at the lower temperatures (120 and 140°C) with less data overlap in the 160°C extraction conditions.

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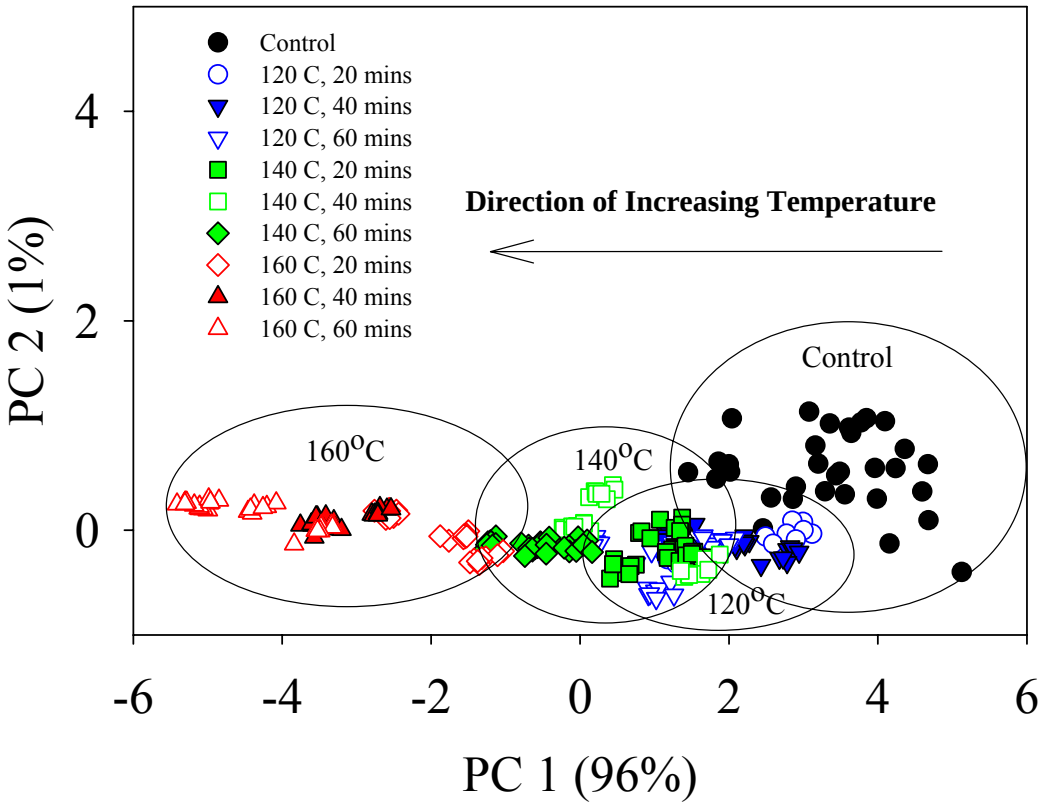

Figure 9: Principal Component Scores for Wood Flake NIR Analysis

The *x*-loading vector indicates which NIR peaks were important for classification (Figure 10). Loading peaks indicate that the important wavelengths for classification occur at 1440 (C-H combination band), 1930 (O-H stretch/HOH deformation combination band), 2090 (O-H combination band), 2335 (C-H stretch/C-H deformation combination band) and 2352 nm (CH_2 bend, 2^{nd} overtone), which are appropriate wavelengths for the substrate under investigation. Interpretation of the positive sign of the loadings, when related to the PCA scores, indicates that samples with negative scores have lower than average values at the important loading peaks. All extractions occurring at 160ºC and the more severe conditions at 140ºC (140ºC, 60 and some at 140ºC, 40 mins) have negative score values on the PC 1 axis (Figure 9). These conditions exhibit the greatest percent change in dry mass, and are therefore expected to contain less than the average amounts of cellulose and hemicellulose.

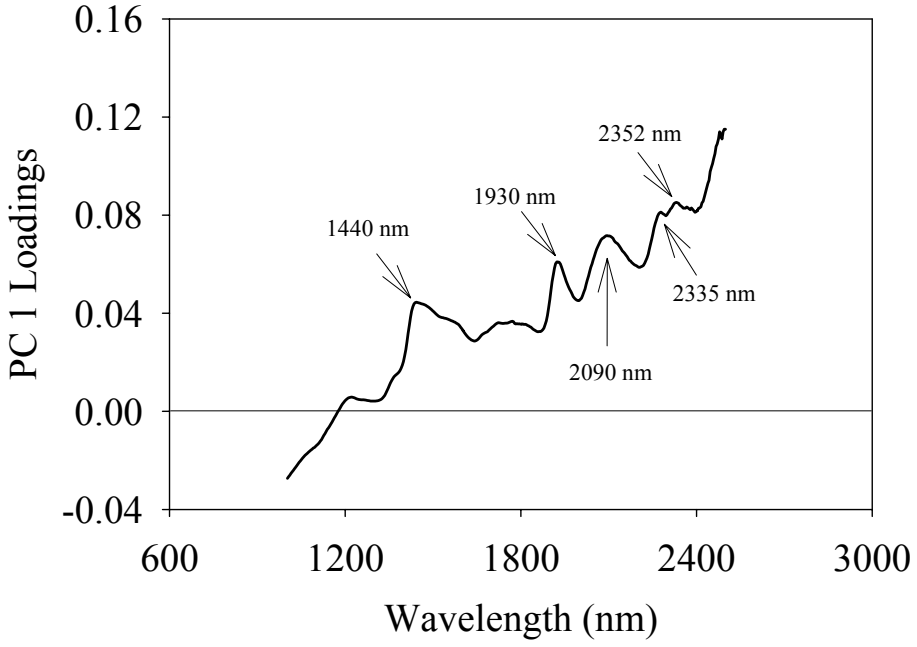

Figure 10: Loading Vector for NIR Wood Flake PCA, PC 1

B. Liquid Phase - Hydrolysates

The product concentrations and hydrolysate pH values are consistent with proposed chemistry and kinetic studies for hot water extraction [Nabarlatz (2004), Li (2005)] (Table 6). Hot water cleaves acetyl groups from hemicellulose side chains to yield acetic and uronic acid. The acid concentrates in the reaction solvent and catalyses the hydrolysis of hemicellulose and, if conditions become harsh enough, cellulose. Carbohydrate degradation to carboxylic acids can also occur as the reaction conditions become more severe [Larsson (1999)]. This concentration of acid is evident in the data with the decrease in pH with increasing reaction severity (Figure 11).

The overall trend in predicting the hydrolysate pH from the percent change in dry mass is appropriate in regards to the known chemistry of the process (Table 6, Figure 11), though the coefficient of determination is relatively low ($R^2 = 0.79$). The maximum hydrolysate pH, with a value of 3.73 corresponds to the least severe extraction conditions (120ºC, 20 mins, Table 6). The minimum hydrolysate pH, with a value of 3.09, corresponds to the most severe extraction conditions studied (160ºC, 60 mins, Figure 11). Further testing should be done to see if the pH of the hydrolysate could be used as an appropriate variable to control the level of extraction in future work.

Literature indicates small amounts of lignin should be hydrolyzed at the studied conditions [Sjostrom (1993)]. This is indeed the case, as the percent solubilized lignin ranges from 0.77% (120ºC, 20 mins) to 1.35% (160ºC, 60 mins) of the total initial lignin content (Table 5). The data indicate an increase in lignin content in the hydrolysate with increasing reaction severity, however the R^2 value.is relatively low ($R^2 = 0.80$, Figure 12).

Table 6: Properties of Recovered Liquid Hydrolysates

Extraction Conditions																	
Temp, ºC	Hold Time, Minutes	S_0	Hydrolysate pH Avg (*Stdev*)	N	% Solubilized Lignin Avg (*Stdev*)	N	Hydrolysate Component Concentrations, grams/Liter										
							Cellobiose Avg (*Stdev*)	Glucose Avg (*Stdev*)	Xylose Avg (*Stdev*)	Galactose Avg (*Stdev*)	Mannose Avg (*Stdev*)	Lactic Acid Avg (*Stdev*)	Glycerol Avg (*Stdev*)	Acetic Acid Avg (*Stdev*)	HMF Avg (*Stdev*)	N
120	20	3.20	3.73 *(0.02)*	(3)	0.78% *-0.04%*	(2)	trace *NA*	trace *NA*	trace *NA*	trace *NA*	trace *NA*	trace *NA*	trace *NA*	trace *NA*	0 *NA*	(3)
120	40	3.49	3.66 *(0.04)*	(3)	0.80% *-0.01%*	(2)	trace *NA*	trace *NA*	trace *NA*	trace *NA*	0.11 *(0.01)*	trace *NA*	trace *NA*	trace *NA*	0 *NA*	(3)
120	60	3.68	3.57 *(0.07)*	(4)	0.88% *-0.02%*	(2)	trace *NA*	trace *NA*	trace *NA*	trace *NA*	0.16 *(0.02)*	trace *NA*	trace *NA*	0.04 *(0.04)*	0 *NA*	(4)
140	20	4.52	3.55 *(0.19)*	(4)	0.87% *-0.07%*	(2)	trace *NA*	trace *NA*	trace *NA*	trace *NA*	0.22 *(0.04)*	trace *NA*	trace *NA*	0.03 *(0.04)*	0 *NA*	(4)
140	40	4.88	3.42 *(0.15)*	(4)	0.93% *-0.13%*	(2)	0.18 *(0.11)*	0.16 *(0.11)*	0.07 *(0.05)*	0.07 *(0.05)*	0.27 *(0.05)*	trace *NA*	trace *NA*	0.07 *(0.05)*	0 *NA*	(4)
140	60	4.99	3.32 *(0.18)*	(4)	1.10% *-0.05%*	(2)	0.24 *(0.13)*	0.23 *(0.14)*	0.13 *(0.06)*	0.11 *(0.03)*	0.35 *(0.10)*	trace *NA*	trace *NA*	0.1 *(0.07)*	0 *NA*	(4)
160	20	5.71	3.2 *(0.01)*	(3)	1.25% *-0.11%*	(2)	0.46 *(0.18)*	0.57 *(0.26)*	0.4 *(0.07)*	0.29 *(0.08)*	0.5 *(0.11)*	trace *NA*	0.11 *(0.10)*	0.18 *(0.03)*	0 *NA*	(3)
160	40	6.03	3.2 *(0.05)*	(3)	1.35% *-0.01%*	(2)	0.5 *(0.17)*	0.71 *(0.33)*	0.62 *(0.09)*	0.41 *(0.16)*	0.55 *(0.07)*	trace *NA*	0.08 *(0.07)*	0.21 *(0.04)*	trace *NA*	(3)
160	60	6.27	3.09 *(0.12)*	(4)	1.24% *-0.15%*	(2)	0.43 *(0.11)*	0.69 *(0.27)*	0.82 *(0.24)*	0.54 *(0.29)*	0.63 *(0.12)*	trace *NA*	0.07 *(0.08)*	0.15 *(0.14)*	0.02 *(0.04)*	(4)

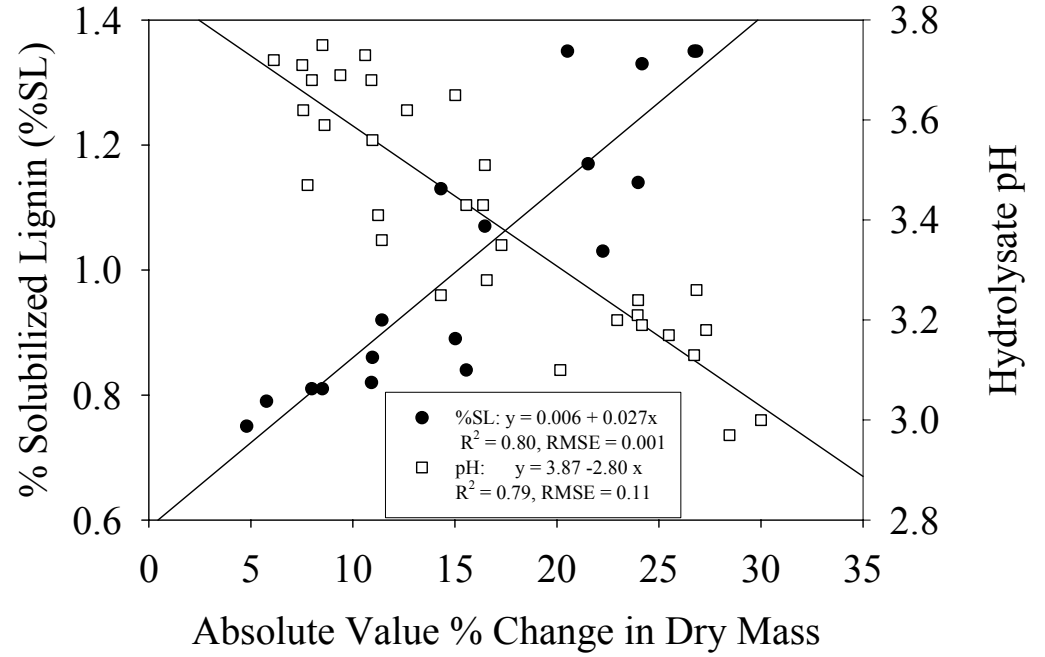

Figure 11: Hydrolysate pH and % Lignin Solubilized versus % Change in Dry Mass

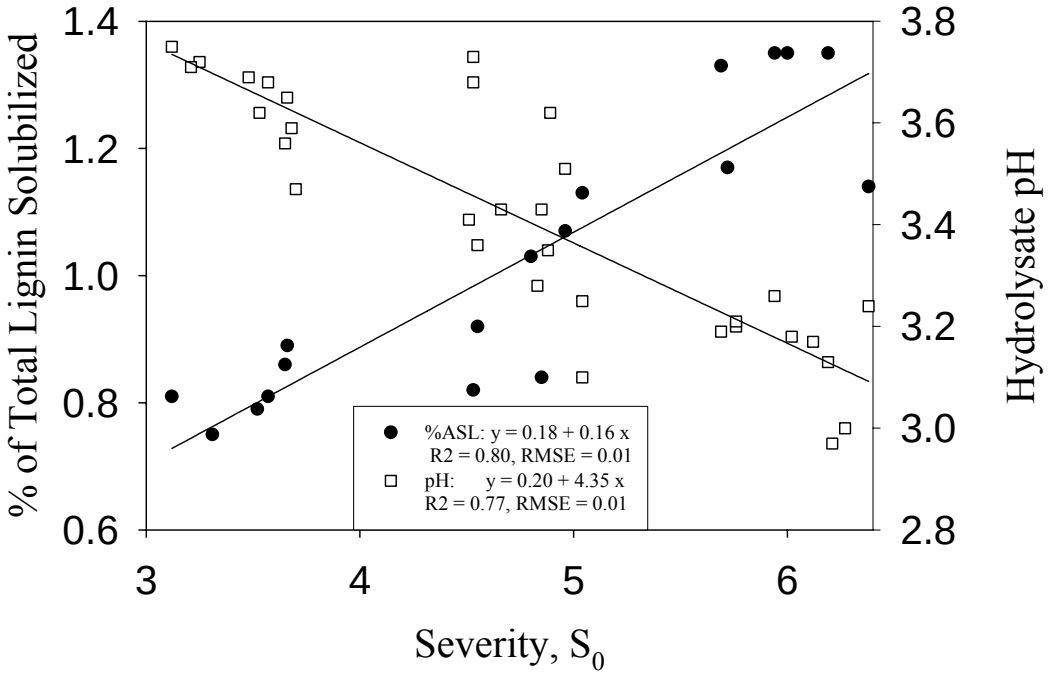

Figure 12: Hydrolysate pH and % Lignin Solubilized Versus Severity

The HPLC hydrolysate chromatograms do exhibit the characteristic peak widening cited in literature (Figure 13). The area under the peaks can not be integrated with substantial data processing to de-convolute the peaks. According to literature, the large peaks occurring with an approximate retention time of 7.15 minutes likely correspond to hemicellulose oligomers [Kumar (2008)]. In hydrolysis reactions oligomeric "chunks" of hemicellulose polymers are solubilzed in the solution before they are depolymerized to monomeric sugars [Sjostrom (1993)]. These oligomeric peaks have greatest intensities at low severity conditions because conditions are not harsh enough to fully depolymerize the oligomers. As reaction severity increases, the peak becomes less distinct, indicating that oligomers are being further broken down into smaller and smaller oligomeric chains [Kumar (1998)]. Conversion of these oligomers to monomeric constituents would be required for quantification of total carbohydrate content in the hydrolysates. Decomposition of the oligomers was not accomplished in this study because the goal of this project was to recover value added products with minimal hydrolysate processing (and thus minimal capital expenditure). The number of chromatogram peaks increases with reaction severity which indicates an increase in reaction severity leads to an increase in the number of carbohydrate species present in the hydrolysate (Table 7).

Extractions at 120ºC and 20 mins produce only trace amounts of carbohydrates which indicate little hydrolysis of the lignocellulosic material is occurring (Table 6). The amorphous nature of hemicellulose leads to this being the first of the lignocellulosic material to be hydrolyzed. Data indicate this is indeed the case as mannose, a monosaccharide derived from hemicellulose, is the first component present in detectable quantities at 120ºC and 40 mins. Mannose is the carbohydrate present at detectable levels at the greatest number

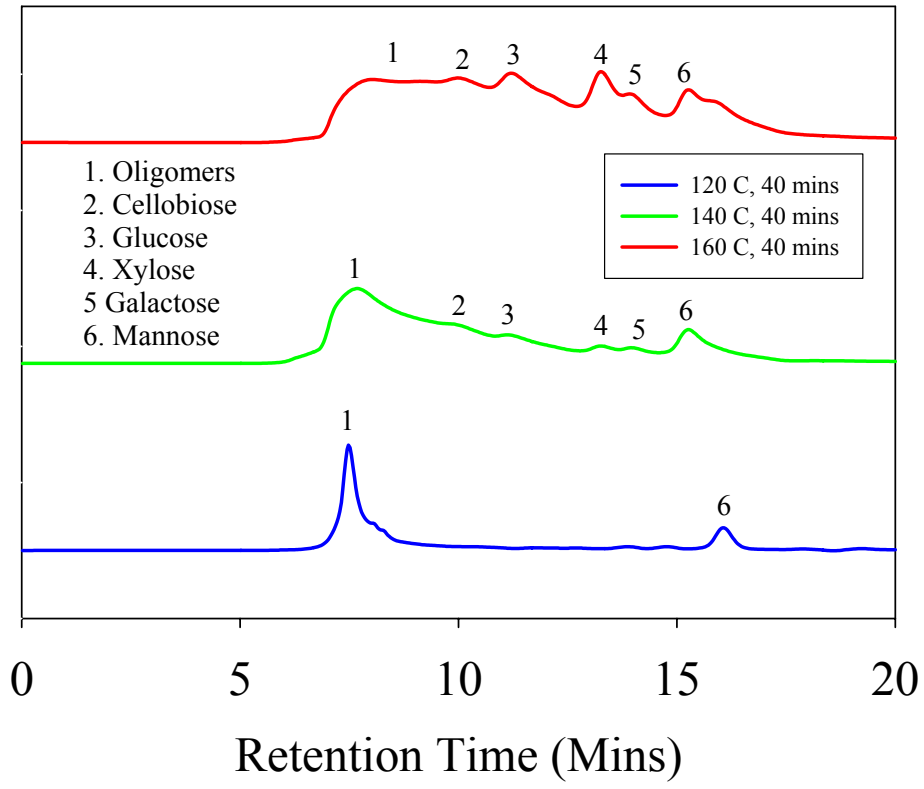

Figure 13: Characteristic Hydrolysate Carbohydrate HPLC Chromatograms

Table 7: Maximum Number of Quantifiable Chemical Species in Hydrolysates

Extraction Conditions			Max. # of Quantifiable Chemical Species in Hydrolysates		
Temp, °C	Hold Time, Minutes	S_0	Carbohydrates	Co-Products	Total
120	20	3.20	0	0	0
120	40	3.49	1	0	1
120	60	3.68	1	1	2
140	20	4.52	1	1	2
140	40	4.88	4	1	5
140	60	4.99	4	1	5
160	20	5.71	4	2	6
160	40	6.03	4	2	6
160	60	6.27	4	3	7

of reaction conditions (from 120ºC, 40 mins to 160ºC, 60 mins), with concentrations ranging from 0.11 g/L (120ºC, 40 mins) to 0.63 g/L (160ºC, 60 mins). This is expected because mannose is the most prevalent hemicellulose carbohydrate (Table 1). The relatively high concentrations of mannose at low severity conditions indicate the hydrolysis of hemicellulose is indeed occurring before that of cellulose. Although mannose is the predominate carbohydrate in hemicellulose, the carbohydrate found in greatest concentration was xylose with a maximum value of 0.82 g/L (160ºC, 60 mins). The concentration of xylose is less than the concentration of mannose until higher severity conditions (160ºC, 40 mins), when HMF is first detected in trace quantities. It is likely that mannose is being converted to HMF, while conditions are not yet severe enough to convert xylose to furfural.

The presence of cellobiose in detectable quantities at 140°C and 20 mins indicates conditions are harsh enough to begin the hydrolysis of cellulose. Conversion of cellulose increases with reaction severity and is indicated by the increase in cellobiose concentration from a minimum value of 0.18 g/L (140°C, 20 mins) to a maximum value of 0.50 g/L (160°C, 40 mins).

As with the carbohydrate HPLC chromatograms, the co-product chromatograms also display characteristic peak spreading (Figure 14) which make calibration based on the area bound by the chromatogram curve without data processing impossible. The large peaks occurring with an approximate residence time of 6.90 minutes correspond to elution of carbohydrates, as verified by laboratory experiments. The negative peak exhibited in the chromatogram corresponds to water, also as verified by laboratory experiments. The water peak is present in the co-product chromatograms because 0.01 normal sulfuric acid is the mobile phase in carbohydrate characterization.

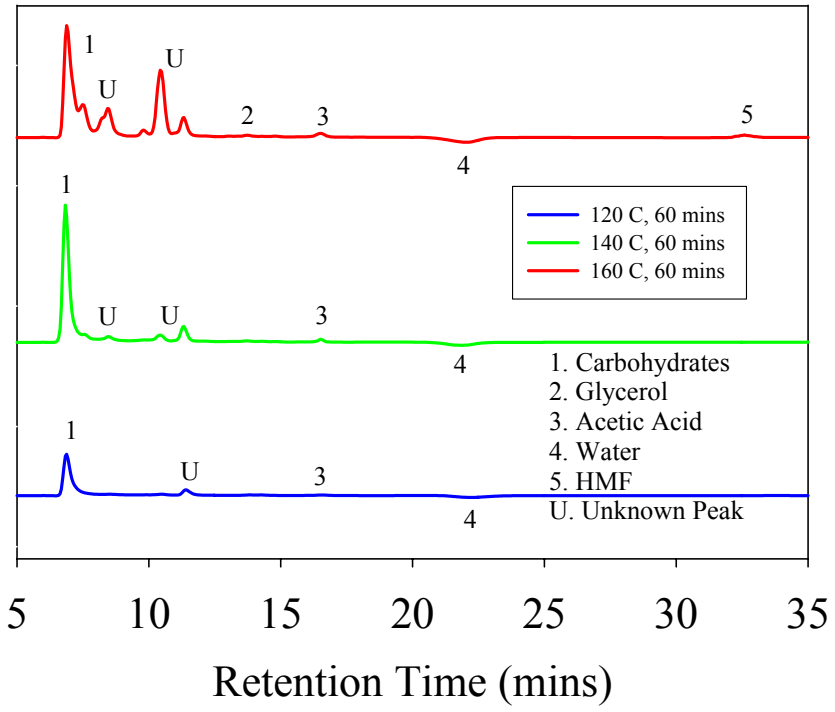

Figure 14: Characteristic Hydrolysate Co-product HPLC Chromatograms

Based on retention times alone, the peak with a retention time 11.5 minutes appears to indicate the presence of xyltiol. Literature indicates that xylitol is produced by the hydrogenation of xylose, but conditions in the reactor do not seem to permit a reduction reaction [Sjostrom (1993)]. Literature makes no mention of the production of xylitol through auto or acid catalyzed hydrolysis, and it seems suspicious that xylitol would be detectable in the hydrolysate before the sugar from which it is derived. Because of this lack of certainty in characterization of this peak, it is conservatively hypothesized that the component eluting from the column at 11.5 minutes is not xylitol. Regardless, the number of co-product species increases with increasing reaction severity, which agrees with literature [Mosier (2005)] (Table 7).

In regards to reaction co-products, acetic acid is the most prevalent. It is found in detectable quantities at most reaction severities, with a minimum concentration of 0.04 g/L (120ºC, 60 mins) and a maximum concentration of 0.22 g/L (160ºC, 40 mins, Table 6). Analysis of the reaction co-products is a bit more difficult, as the variation in the number of observed chemical species at each condition is considerable. This is especially noticeable in the data at 160°C and 60 mins. Of the four replicates characterized at this condition, only two replicates have quantifiable levels of glycerol, and only one replicate has quantifiable levels of HMF (Figure 15).

HMF is first detected in appreciable quantities at 160°C and 60 mins, with a concentration of 0.02 g/L. Reaction conditions are harsh enough to dehydrate hexoses into HMF. Conditions are never severe enough to degrade pentoses to furfural, as furfural is never found at detectable levels. These results coincide with previous studies completed on

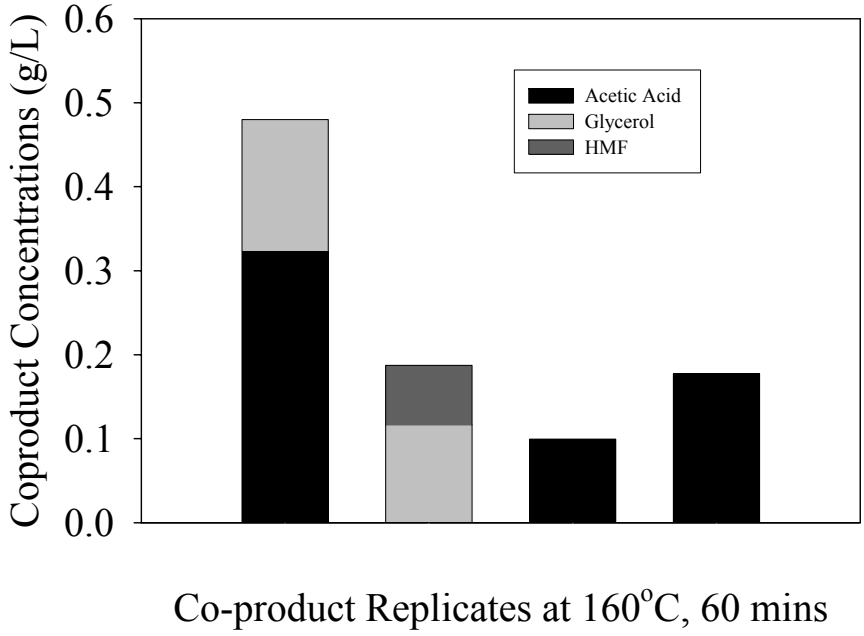

Figure 15: Co-product Concentration Replicates at 160ºC, 60 mins

the required reaction severity for the production of these chemicals [Li (2005), Sundqvist (2006)].

The standard deviation of all products is high and can be attributed to several factors. Natural variability in the woody substrate leads to variation in the composition of the hydrolysate. Previous research has quantified this variation [McMillin (1968), Schimleck (1998), Ingram (2000), Jones (2006)]. This study is especially sensitive to this natural variability because of the relatively small charges of woody substrate (approx. 5 grams) used for each extraction. The variation tends to be highest towards low concentrations of products as the RI detector used for HPLC characterization nears its detection limit. The actual conditions under which extraction occurred are also a source of variation. Residence time in the reactor varied from run to run depending on the mass of wood and water charged to the reactor and the initial temperature of the reactor. The extraction hold temperature could only be held to ± 3ºC. As a result, extractions reported at the same condition could have been extracted at temperatures separated by six degrees Celsius. Because the de-polymerization is an Arrhenius type reaction, this difference in temperature could lead to different levels of extraction and therefore different chemistries of the hydrolysates.

Results from the principal component analysis of the hydrolysate HPLC chromatograms are presented in Figures 16 and 17. These are the PCAs for carbohydrate and co-product chromatograms respectively. Each PCA was accomplished with 17 data points. HPLC data were collected in two separate batches many months apart. This was done in order to protect HPLC samples from aging. In order to remove the variation that occurs because samples were characterized through two separate HPLC runs, data were taken from only one run. Carbohydrate peaks appear between retention times of 9.5 to 15.75 minutes

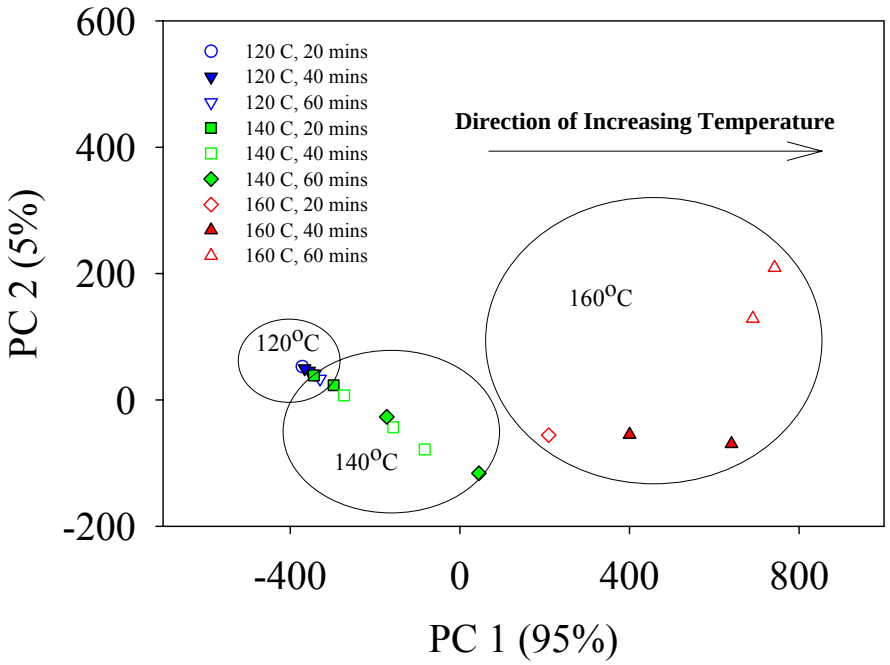

Figure 16: Principal Component Scores for Carbohydrate HPLC Chromatograms

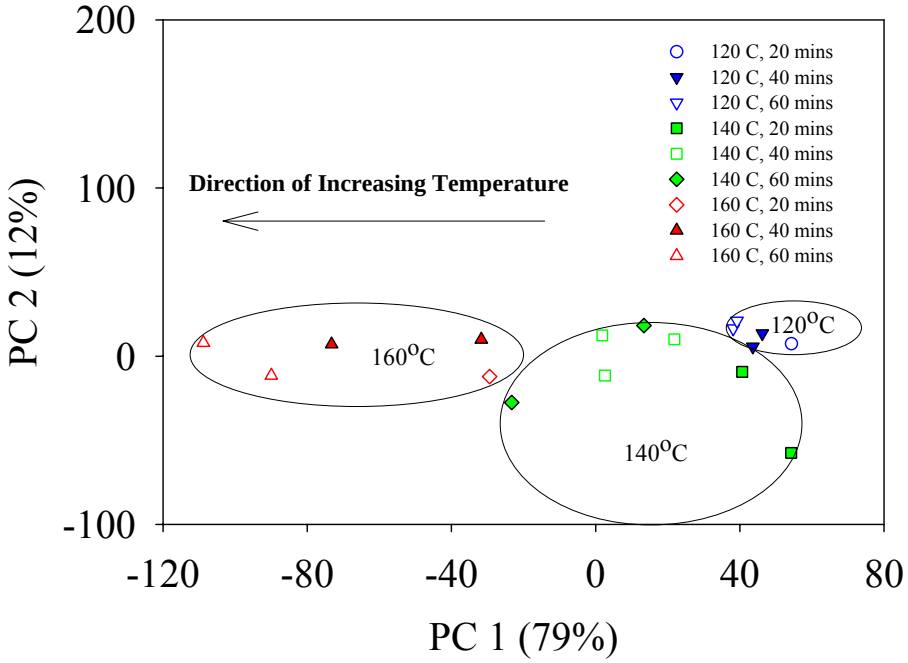

Figure 17: Principal Component Scores for Co-product HPLC Chromatograms

and co-product peaks appear between retention times of 11 to 50 minutes. PCA was accomplished over these respective ranges. Data segregation is observed in both PCAs in regards to extraction condition, indicating the carbohydrate and co-product chemical identities are unique to the extraction condition, and most notably, extraction temperature. This reinforces earlier findings that distinct levels of extraction are achieved at the conditions in this study. Clustering is evident when only reaction temperature is considered, indicating that this is the dominant factor in determining the chemical composition of liquid phase hydrolysates. Data overlap is visible for carbohydrate scores at 120 and 140°C and indicates similar carbohydrate chemistries in hydrolysates at these conditions. This reinforces data previously presented that these conditions produce similar levels of extraction.

Important peaks for hydrolysate characterization occur at 10.02, 11.22, 13.25, 13.92 and 15.25 minutes in the carbohydrate chromatogram (Figure 18). These peaks correspond to cellobiose, glucose, xylose, galactose, and mannose respectively. Important peaks for hydrolysate characterization occur at 13.73, 14.78, 16.53, 22.13 and 32.58 minutes in the co-products chromatogram (Figure 19). These peaks correspond to lactic acid, glycerol, acetic acid, water and HMF respectively. These loadings indicate that characterization of the hydrolysates occurs because of chemical changes appropriate to this study.

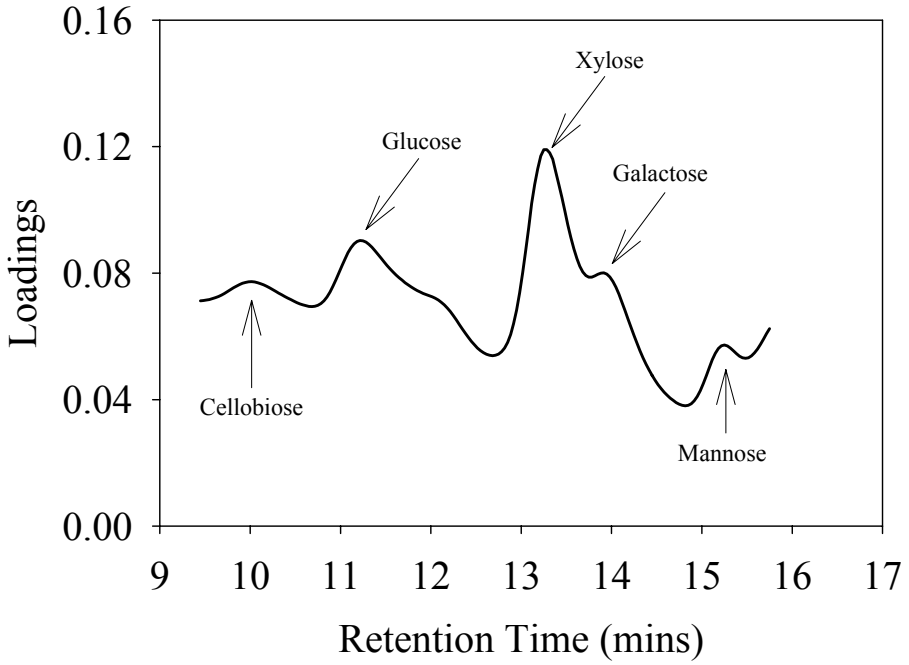

Figure 18: Loading Vector for HPLC Carbohydrate PCA, PC 1

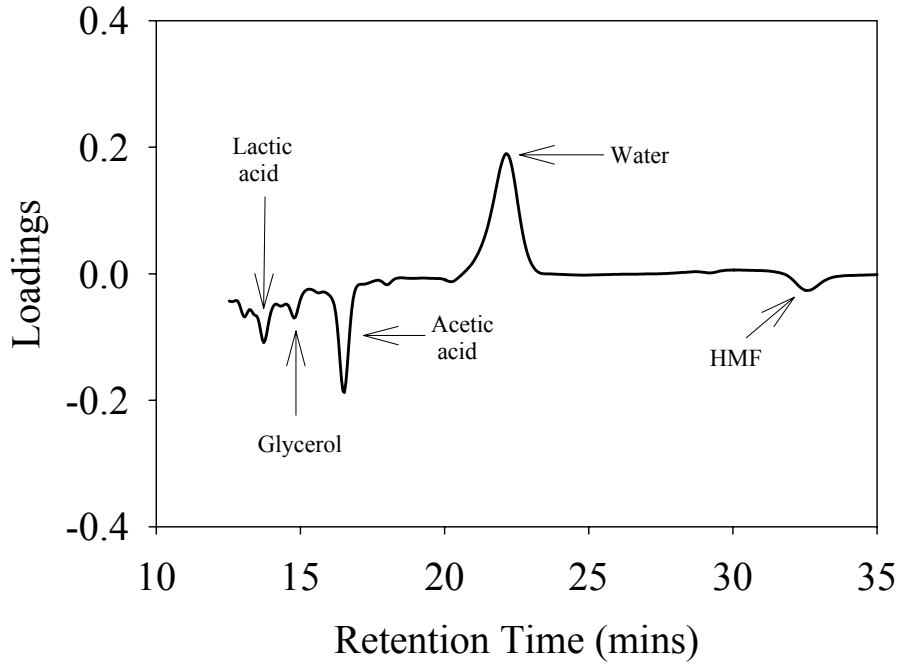

Figure 19: Loading Vector for HPLC Co-products PCA, PC 1

C. Response Prediction

With the industrial scale up of the extraction in mind, it would be beneficial if some of the response variables measured in this study could be predicted by rapid NIR scanning of the extracted flakes. PLS-1 models were generated from NIR wood flake spectra for the percent change in dry mass, percent solubilized lignin, hydrolysate pH, and the chemical concentrations in the hydrolysate. Models could not be generated for glycerol and HMF as not enough samples contained quantifiable levels of these species.

Based on R^2 values, valid NIR correlated models can be formulated for the percent solubilized lignin (R^2=0.97), percent change in dry mass (R^2=0.95), xylose hydrolysate concentration (R^2=0.92), and mannose hydrolysate concentration (R^2=0.91) (Table 8). Models produced for the other response variables have unacceptable coefficients of determination for normal industrial applications (R^2>0.90) [Vining (1998)].

Low R^2 values indicate that valid calibration models cannot be constructed for the hydrolysate pH (R^2 = 0.79) and the hydrolysate concentrations of galactose (R^2 = 0.83), cellobiose (R^2 = 0.83), acetic acid (R^2 = 0.81), and glucose (R^2 = 0.76) (Table 8). The

Table 8: Summary of PLS-1 Models From NIR Wood Flakes

	Calibration				Validation		
Response Variable	R^2	RMSEC	PCS	N	R^2	RMSEV	N
% Solubilized Lignin	0.97	0.0003	2	(9)	0.63	0.002	(5)
% Change in Dry Mass	0.95	0.02	2	(24)	0.90	0.02	(12)
[Xylose]	0.92	0.08	2	(14)	0.86	0.14	(8)
[Mannose]	0.91	0.06	1	(24)	0.70	0.10	(12)
[Galactose]	0.83	0.09	2	(14)	0.80	0.12	(8)
[Cellobiose]	0.83	0.09	2	(14)	0.70	0.11	(7)
[Acetic Acid]	0.81	0.04	2	(20)	0.66	0.05	(10)
Hydrolysate pH	0.79	0.11	1	(22)	0.65	0.13	(10)
[Glucose]	0.76	0.17	1	(14)	0.66	0.22	(8)

reasons for the shortcomings of these models are easily explained. The hydrolysate pH may not exhibit sufficient linearity for a linear model to accurately predict responses. The low R^2 value for the linear model generated to correlate the absolute value in the percent change in mass loss to the hydrolysate pH demonstrates this (Figure 10). Perhaps non-linear methods would produce a more acceptable model. The hydrolysate galactose concentration may be hard to predict from NIR data because of relatively small amounts of galactose in the hemicellulose polymers in solid wood. More large scale changes in the wood chemistry may dominate the variation in the wood flake NIR spectra. Acetic acid is derived from two sources; the cleavage of acetyl side groups, which can be quantified by changes in the wood flake chemistry, and degradation of monosaccharides in the hydrolysate, which would not necessarily have an impact on chemistry in the wood flakes. Models are difficult to generate for cellulose and cellobiose for a similar reason. Both components are derived from cellulose polymers. NIR scans of the wood flake can quantify changes in cellulose, but not necessarily the distribution of products from cellulose degradation, because conversion to glucose from cellobiose can occur in the liquid phase.

Examination of the RMSE of both calibration and validation for the acceptable models indicate the models are appropriate in relation to the natural variability observed in the data. Comparison with the average standard deviation of each variable indicates that the root mean square error of calibration (RMSEC) values generally lie in the observed range of data variation (Table 9). The root mean square error of validation (RMSEV) values tend to be higher than the average standard deviation, but only the RMSEV of the percent solubilized lignin is cause for concern (0.0016 RMSEV versus 0.0007 average value of standard deviation).

Table 9: RMSE and Standard Deviation Comparisons, PLS-1

Response Variable	Avg St Dev	RMSEC	RMSEV
% Solubilized Lignin	0.0007	0.0003	0.0016
% Change in Dry Mass	0.02	0.020	0.024
[Xylose]	0.10	0.081	0.14
[Mannose]	0.07	0.06	0.10

For a PLS model to be validly constructed, the loading weight vector and the regression coefficients should exhibit similar peaks. This is true for the four models that provide acceptable fits to the data. Figures 20 through 23 display the loading weight vectors and regression coefficients for the percent solubilized lignin, percent change in dry mass, hydrolysate xylose concentration, and hydrolysate mannose concentration respectively. Important peaks in PC 1 for the prediction of all response variables can be found at 1443 nm, 1927 nm, 2091 nm, 2331 nm and 2367nm (Figures 18-21). The chemistry related to these peaks has already been illustrated as appropriate to softwood NIR spectra (C-H combination, O-H stretch/H-O-H deformation combination, O-H combination, C-H stretch/C-H deformation combination, and CH_2 bend 2^{nd} overtone respectively). PC 1 describes anywhere from 97% of the variation in the *x* data and 78% of the variation in the *y* data (percent change in dry mass) to 98% of the variation in the *x* data and 78% of the variation in the *y* data (hydrolysate xylose concentration).

PLS-2 models do not provide better predictions of cellobiose and glucose concentrations in the hydrolysate. The R^2 value for both components are unacceptably low ($R^2_{cellobiose} = 0.77$, $R^2_{glucose} = 0.76$, Table 10). Comparing the hydrolysate cellobiose concentration PLS-2 model with the corresponding PLS-1 model indicates that the PLS-1

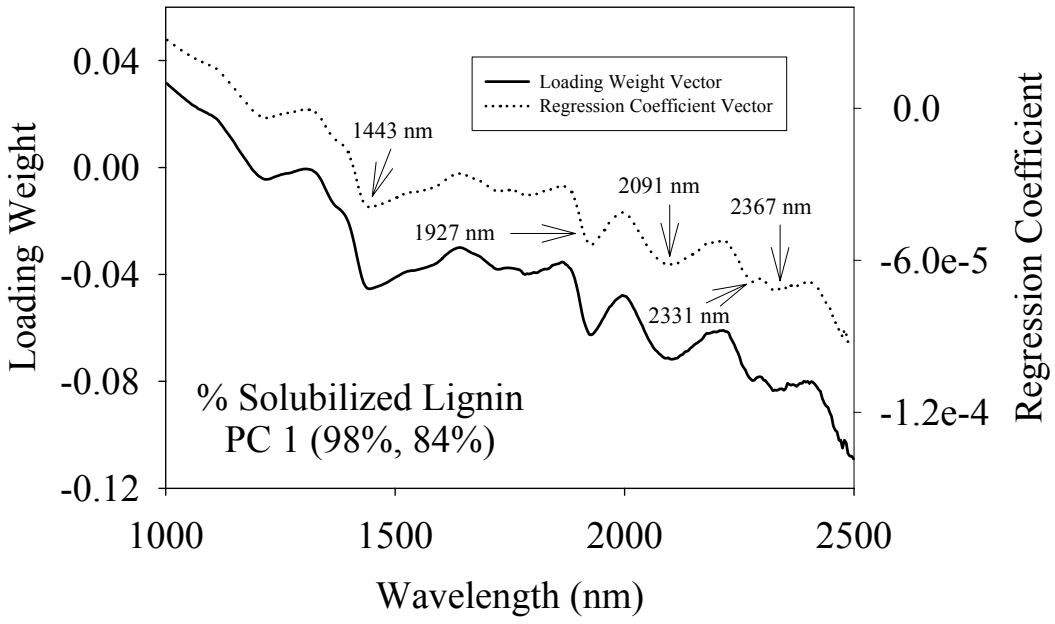

Figure 20: Loading Weight and Regression Coefficient Vectors for % Solubilized Lignin PLS

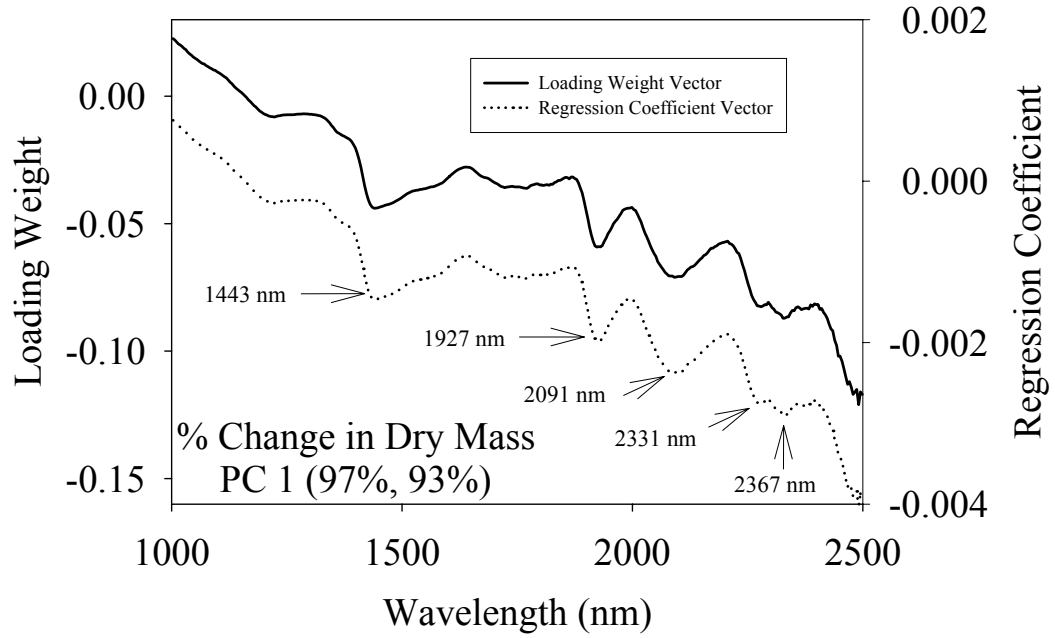

Figure 21: Loading Weight and Regression Coefficient Vectors for Percent Change in Dry Mass PLS

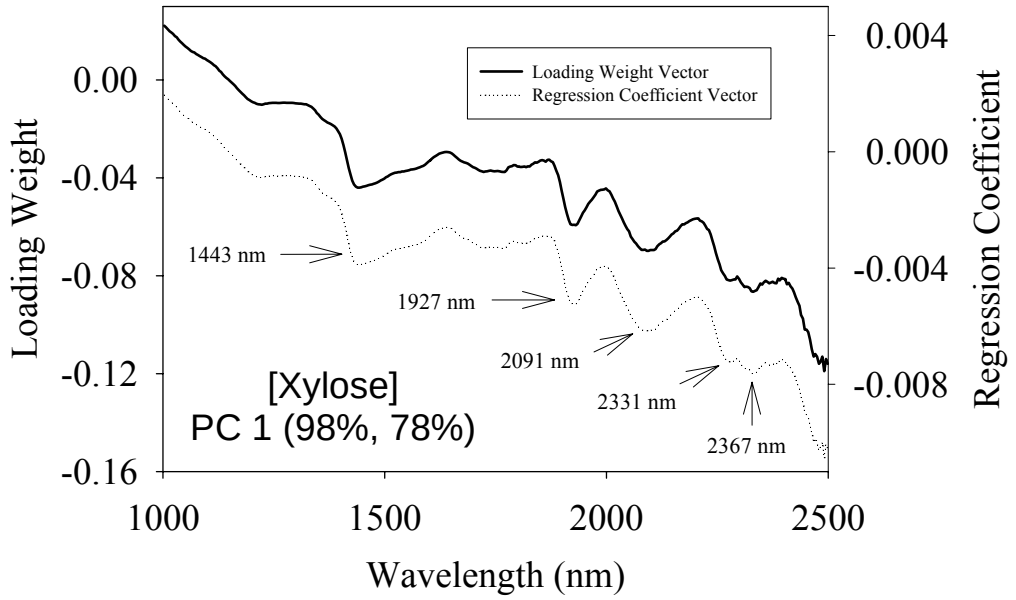

Figure 22: Loading Weight and Regression Coefficient Vectors for Hydrolysate Xylose Concentration PLS

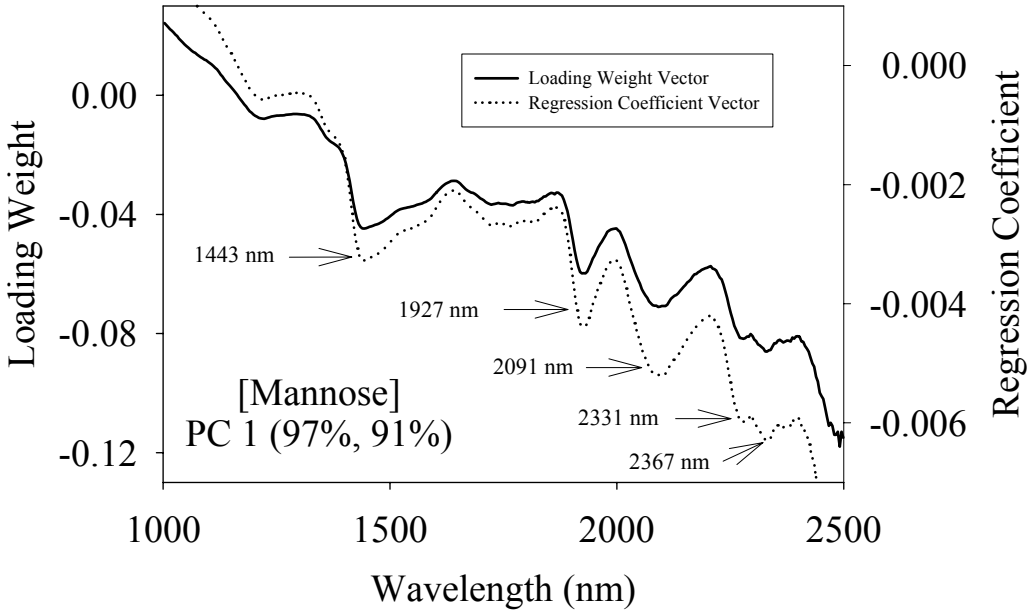

Figure 23: Loading Weight and Regression Coefficient Vectors for Hydrolysate Mannose Concentration PLS

Table 10: Summary of PLS-2 Models from NIR Wood Flakes

	Calibration				Validation		
Response Variable	R^2	RMSEC	PCS	N	R^2	RMSEV	N
[Cellobiose]	0.77	0.11	1	(14)	0.62	0.13	(8)
[Glucose]	0.76	0.17			0.66	0.22	

model has a higher R^2 and a lower RMSE for calibration data (PLS-1; $R^2 = 0.83$ RMSEC = 0.09, PLS-2; $R^2 = 0.77$ RMSEC = 0.11). The R^2 and RMSE values for the calibration data in regards to prediction of glucose concentration in the hydrolysate indicate that the PLS-1 and PLS-2 models are comparable (PLS-1; $R^2 = 0.76$ RMSEC = 0.17, PLS-2; $R^2 = 0.76$ RMSEC = 0.17). Poor PLS-2 modeling may again be attributed to the relatively low number of samples in the analysis. According to ASTM guidelines, calibration should require at least 18 samples [ASTM (2005)]. More data should be collected before the PLS-2 model is considered unacceptable.

Analysis of R^2 and RMSE values of calibration model indicates that the percent solubilized lignin, percent change in dry mass, hydrolysate xylose concentration and hydrolysate mannose composition could be predicted from coupling NIR data collected on extracted flakes with PLS-1 models. Poor validation models suggest otherwise (with the exception of the percent change in dry mass, $R^2 = 0.90$). Literature indicates that more validation data is required for construction of accurate validation models [ASTM (2005)].

D. Economic Analysis

One of the goals of this project was to determine if the extraction process could add a value added chemical stream to an OSB manufacturing facility. As such, a basic economic analysis has been carried out to determine if the hot water extraction process is even

economically viable in the most basic of terms. Is the value of raw material the process consumes greater than the value chemicals produced or of chemicals that can be produced? As the results of this study indicate, the extraction process produced various percent changes in dry mass of the wood flake substrates as well as producing non-trivial amounts of wood flake breakage as indicated by the percent change in flake surface area. Manufacturers would likely be forced to feed more raw wood flakes into their processes to target the same final product density because of these wood losses. Because the change in flake surface area is hypothesized to be a function of random encounters with the mixing apparatus, the average value in the percent change in surface area for all conditions, -8.92%, was used in this analysis and was coupled with the observed percent change in dry mass at each discrete reaction condition. These additional wood requirements (from mass loss and breakage) result in an incremental increase in the wood costs to the OSB manufacturer. Costs based on capital investment and process operation are nearly impossible to estimate at this point in time, therefore in order for the proposed extraction process to be a candidate for further research the revenue generated from the sale of extracted chemicals must be significantly greater than the cost incurred to the manufacturer because of increased wood usage. A more detailed economic analysis would have to be completed after more detailed study to determine true economic return.

Wood usage and mill production figures are based on values for a new OSB manufacturing facility built by Louisiana Pacific (LP) in Clarke County Alabama which is scheduled for a late 2007 start-up. According to LP press releases, the plant will consume an estimated 1.1 million tons of wood and produce 700 million square feet (¾ inch thickness basis) (MMSF¾) annually [Louisiana Pacific Corporation (2004)]. These values are similar

to many OSB plants in the Southeastern region of the United States [Harris (2007)]. The cost of raw wood is estimated from Timber-Mart researched values for the Southeast in 2007. Timber-Mart quotes a delivered market price of $26-$33 per ton on pine pulpwood [Harris (2007)]. For economic analysis, the higher of these values will be used for raw material costs. It is assumed that the delivered wood averages 30% moisture content by mass and contains 5% extractives by mass (similar to that observed in this study). Wood usage will be corrected based on these values.

A review of the literature indicates that the economic value of the chemicals produced directly through the extraction process is too low to justify direct sale [Paster (2003), Werpy (2004)]. As such, exploration of products that can be derived from the chemicals produced from the extraction process must be explored.

For a variety of reasons, the conversion of lignocellulosic materials into bio-based ethanol for fuel sources is an attractive option. For ethanol production, the carbohydrates produced from hydrolysis are isolated via separation. These carbohydrates are fermented by bacteria, enzymes, or yeasts into ethanol [Duff (1996)]. Ethanol is an attractive value added product because there is a large, pre-established market. Ethanol is being blended into fuels as an eco-friendly option to gasoline itself and is currently used as a gasoline additive to replace methyl tertiary butyl ether [Sun (2002)]. Previous research has indicated that the hydrolysis byproducts HMF and furfural, even in dilute quantities, act as inhibitors to the conversion of carbohydrates to ethanol by microorganisms [Palmqvist (2000)]. Extraction under the most severe conditions of this study produce only small amounts of HMF (and no detectable levels of furfural) in the hydrolysate. Ethanol yields will approach a maximum without the presence of these inhibitors [Palmqvist (2000)]. Literature indicates that for

softwood derived carbohydrates, ethanol conversions from both cellulose and hemicellulose derived carbohydrates average near 40% by mass [Olssen (1996)]. This conversion value will be used for economic analysis. Literature also indicates that a reasonable market price for corn-derived ethanol is $1.25 per US gallon, or $0.67 per pound of ethanol [Kaylen (2000)]. Because lignocellulosic ethanol will compete economically with corn based ethanol, it is assumed that market price for lignocellulosic ethanol will have to be near that of the corn-derived ethanol. Equations for economic calculations can be found in Appendix B.

The revenue generated from the volume of ethanol that could be produced through low and mid temperature extraction is less than the incremental cost increase incurred due to breakage and mass loss (Table 11). At 120ºC and 20 mins only trace amounts of carbohydrates are produced by the extraction processes, and therefore little to no ethanol could be produced from hydrolysates produced at this extraction condition. The resulting damage and mass loss would require an additional input of 5.9 million dollars worth of raw wood annually to ensure product densities remain unchanged when OSB is manufactured

Table 11: Revenue Generation From Ethanol Production

Extraction Conditions					
Temp, ºC	Hold Time, Mins	Incremental Wood Cost Increase $/year	Ethanol Conversion Ton ethanol/ton raw wood	Ethanol Revenue $/year	Net Revenue $/year
120	20	$5,900,000	0.00%	$0	-$5,900,000
120	40	$6,300,000	0.15%	$550,000	-$5,800,000
120	60	$7,400,000	0.21%	$790,000	-$6,600,000
140	20	$7,600,000	0.29%	$1,100,000	-$6,500,000
140	40	$9,400,000	1.02%	$3,900,000	-$5,500,000
140	60	$9,900,000	1.45%	$5,600,000	-$4,300,000
160	20	$12,000,000	2.95%	$12,000,000	$0
160	40	$13,000,000	3.72%	$15,000,000	$2,000,000
160	60	$14,000,000	4.09%	$17,000,000	$3,000,000

with extracted flakes. At 120ºC and 40 mins and 120ºC and 60 mins, ethanol can be produced, however ethanol is produced in too small of quantities to counteract the increase in incremental wood costs incurred through damage and mass loss. Extractions at 120ºC and 40 mins and 120ºC and 60 minutes result in annual revenue losses of 5.8 and 6.6 million dollars respectively. Ethanol yields would increase with increasing severity. Extractions at 140ºC produce higher ethanol yields than 120ºC extractions, but ethanol production at 140 ºC cannot counteract the incremental increase in wood cost.

This analysis indicates that extracting at 160ºC may yield enough raw material to make ethanol production economically viable. Annual revenue ranges from 2 million dollars (160ºC, 40 mins) to 3 million dollars (160ºC, 60 mins). Operation here is not practical relative to the goal of the project, and returns are most likely too low to counteract capital, utility, and operating expenses [Seider (1998)]. The levels of cellobiose present in the hydrolysate indicate cellulose has been hydrolyzed and that the mechanical strength of the cell wall may be negatively impacted as a result. Hydrolysates produced at an extraction temperature of 160°C contain far more chemical species than hydrolysates produced at milder conditions. Product separation would be more complex and costly as a result [Kochergin (2006)], and the presence of HMF at this extraction temperature would inhibit the conversion of carbohydrates to ethanol [Palmqvist (2002)]. From these data, ethanol does not seem to be a viable candidate as a value added product.

A review of the literature indicates that levulinic acid is a high return chemical that can be derived from biomass. Levulinic acid is generally used as a building block chemical for fuel additives, polymers, resins, and herbicides [Paster (2003), Werpy (2004)]. This

compound is currently derived from petroleum feedstocks and has a market value of $4-6 per pound [Werpy (2004), Paster (2003)]. Levulinic acid is produced from an acid catalyzed degradation of HMF, which itself is derived from the dehydration of lignocellulosic hexoses [Sjostrom (1993)]. Literature indicates that reasonable conversions from hexoses to levulinic acid are 65% [Fitzpatrick (1996)].

Levulinic acid provides a much higher economic return than ethanol (Table 12). The only condition that does not generate net revenue is 120°C and 20 mins, with a net loss of 5.9 million dollars annually. Economic returns are even higher for hydrolysates produced at an extraction temperature of 140°C. Net revenue ranges from 29 million dollars annually (140°C, 20 mins) to 160 million dollars annually (140°C, 60 mins). These higher returns could help offset the additional capital, utility and operating expenses that would be required for the separation and conversion of hexoses to high purity levulinic acid.

Economic returns on levulinic acid reach a maximum value at 160°C and 60 mins (430 million dollars annually). Again, the levels of cellobiose present in the hydrolysate

Table 12: Revenue Generated From Levulinic Acid Production

Extraction Conditions					
Temp, °C	Hold Time, Mins	Incremental Wood Cost Increase $/year	Levulinic Acid Conversion ton LA/ton raw wood	Levulinic Acid Revenue $/year	Net Revenue $/year
120	20	$5,900,000	0.00%	$0	-$5,900,000
120	40	$6,300,000	0.15%	$19,000,000	$13,000,000
120	60	$7,400,000	0.21%	$27,000,000	$20,000,000
140	20	$7,600,000	0.29%	$37,000,000	$29,000,000
140	40	$9,400,000	1.02%	$120,000,000	$110,000,000
140	60	$9,900,000	1.45%	$170,000,000	$160,000,000
160	20	$12,000,000	2.95%	$340,000,000	$330,000,000
160	40	$13,000,000	3.72%	$410,000,000	$400,000,000
160	60	$14,000,000	4.09%	$440,000,000	$430,000,000

produce concerns that the strength of the cell wall has been compromised at this extraction temperature. Although the yield is high, so too is the number of components present in the hydrolysate. This would likely result in the required product separations being both costly and complex [Kochergin (2006)].

E. Process Scale-Up

The basic economic analysis indicates that further, more detailed research should be carried out on this process to determine if large scale implementation is possible. However, some changes in experimentation and instrumentation should occur to ensure future research is more practical for industrial scale processes.

This research investigated extractions at a 40:1 mass ratio of solvent (water) to substrate (dry, extractives free wood flakes). This ratio was necessitated by the geometry of the Parr reactor in which extractions occurred. Wood flakes could only align in one direction in the reaction chamber, as the length of the wood flake was near the diameter of the reaction chamber (length of average wood flake = 7.87 cm, diameter of reaction chamber = 9 cm). Flakes had to be stacked one on top of the other inside of the reaction chamber. The clearance between the temperature probe and mixing apparatus and the bottom of the reactor chamber allowed a maximum of 9 flakes inside the reactor during each extraction. For proper temperature control of the extraction, the temperature probe had to be sufficiently submerged in liquid phase inside of the reactor. This necessitated roughly 200 mL of water. These geometric constraints set the solvent to substrate ratio for the research, however, a 40:1 ratio is hardly practical in an industrial environment [Kubikova (2000)]. Capital investment for large volume reactors, piping and transfer pumps for high volumes of water would be

prohibitively expensive [Seider (1998)]. As such, future research should focus on extractions using less water. A 10:1 ratio appears to provide total wetting of the wood flake surface area and would be an appropriate ratio for further studies.

The amount of dry mass charged for each extraction should be increased. Research in this study was limited by the amount of wood that could physically fit in the reactor (9 flakes, approximately 5 grams). It is hypothesized that some of the variation in the chemical component concentration data is high because of this. In this study, inter-batch flakes were chosen at random, and likely came from different trees and different areas within the same trees. These flakes could contain different chemistries and may have had profound impact on the chemistry of the hydrolysates [McMillin (1968), Jones (2006)]. The recommendation for future research is to use 1 kg batches of wood to minimize the effect flake to flake variation has on the hydrolysate compositions. A 10:1 ratio of solvent to substrate would require 10 Liters of reactor volume.

Wood losses due to breakage have a considerable impact on the economic returns of the process. In this study, mechanical agitation was achieved with a blade impeller. Mixing via agitation was provided to aid both mass and heat transfer during extraction and should be considered a requirement for batch processes [Mosier (2005)]. However, mixing can be achieved in methods that would most likely lead to less damaging of the flake. A circulation of the liquid phase during the extraction via pumping should provide adequate mixing without the possibility of physical interactions between wood flakes and the mixing apparatus [Hu, (2008)].

As noted previously noted, deficiencies exist in the method utilized for HPLC hydrolysate characterization. Current HPLC methodologies result in substantial peak

widening. This peak widening prevents baseline separation of carbohydrate peaks and required the researcher to use peak intensity, rather than the traditional area under the peak, for carbohydrate quantification. Literature reports other HPLC characterizations methods have been used with greater success in achieving near baseline separation of carbohydrates [Agblevor (2004)]. Methods in literature also utilize different detectors for characterization in order to overcome the shortcomings of refractive index detectors (i.e. weak sensitivity and incompatibility with gradient elution) [Nogueira (2005)]. Pulsed amperometric, evaporative light scattering and electrochemical detectors have been cited in literature as viable alternatives to refractive index detection [Torimura (1997), Cheng (2001), Li (2007)]. These methods were not chosen for this study because they are not as widely used as the NREL characterization method. Future research may benefit from utilizing a different hydrolysate HPLC method.

The results of these proposed experiments and characterizations could be used for more detailed economic analysis. If the economics of levulinic acid prove marginal, further hydrolysate processing may provide a chemical species with a higher economic return.

Investigation of the mechanical property changes undergone by the substrate would also be beneficial. Prior research has indicated that the polymeric properties of wood are heavily influenced by chemical processing [Kelley (1987), Dave (1992), Hon (1992), Olsson (1992), Wolcott (1994), Sugiyama (1998), Bjorkman (2000), Olsson (2004)]. Changes in the viscoeleastic properties of the wood flake may have profound influence on the behavior of the wood under heat and pressure, and undesirable consequences may arise from industrial pressing of extracted flakes [Wolcott (1994)]. Tensile testing could also be performed to

determine with certainty whether the removal of small amounts of cellulose is detrimental to the strength of the flake.

5. Conclusions

Various levels of extraction of lignocellulosic material can be accomplished over the range of reactor temperatures and isothermal hold times investigated, although there is significant overlap at 120 and 140°C. This level of extraction can have a profound impact on the chemical identity of the woody biomass, as extraction hydrolyzes cellulose and hemicellulose polymers while solubilizing small quantities of lignin. The changes in the chemical composition of the wood flake can be used to classify the flake by the condition through which it was extracted. Reaction conditions also have a profound effect on the chemical composition of the liquid phase hydrolysates, as hydrolysis of biomass samples yield carbohydrates which can react further to produce co-products. The changes in the chemical composition of the hydrolysates can be used to classify the hydrolysate by the condition through which it was extracted. These chemical changes are also manifested through changes in the physical characteristics of the substrate. Extraction can produce significant levels of breakage when reactor agitation is accomplished through mechanical means. The fiber saturation point of extracted flakes is also reduced as extraction severity increases.

Results seem promising that rapid NIR techniques can be used to predict the physical changes the extraction process would have on wood flakes (percent change in dry mass). Prediction of hydrolysate chemical yields seems a bit less accurate, but some components can be modeled (hydrolysate concentrations of xlose and mannose, and the percent lignin solubilized).

HPLC data indicate that only small amounts of hemicellulose are extracted at a temperature of 120ºC, as only trace amounts of hemicellulose derived monosaccharides are

present in the hydrolysates. The concentrations of chemicals produced at this temperature are not high enough to be economically viable as value added products. At an extraction condition of 140ºC and 40 mins, cellulose is extracted and may indicate the strength of the cell wall has been compromised. Extraction of cellulose increases with reaction severity causing greater and greater concern about cell wall strength of wood flakes subjected to extraction at 160ºC. Also, the number of chemical species present in the hydrolysate at 160ºC indicates a higher degree of energy and capital expenditure would be required for product separation. In regards to the goal of this product, further studies should focus around extraction that occurs at a temperature of 140ºC with a residence time of 20 minutes. Hydrolysates produced at these extraction conditions have the highest potential as value added processes to the manufacture of OSB because of a better balance between the number of components, component quantities, and relatively small levels of physical changes in the substrate wood flake.

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Appendices

Appendix A

Summary of HPLC Calibrations

The R^2 value for the linear fits to the calibration data ranged from 0.9999 (mannose, Run #2) to 0.9887 (acetic acid, Run #2) (Table 12). The error of prediction of the concentration of the standards solution with the calibration curve ranged from 0.53% (xyltiol, Run #2) to 9.75% (cellobiose, Run #2). The highest prediction errors occur at the lowest product concentrations as the detector limit is approached.

Table 13: Summary of HPLC Calibration Runs

	Calibration Concentrations, g/L					
	Cal Pt. 1	Cal Pt. 2	Cal Pt. 3	Cal Pt. 4	R^2	Max Prediction Error
	Run #1 **Run #2**	Run #1 **Run #2**	Run #1 **Run #2**	Run #1 **Run #2**	Run #1 **Run #2**	Run #1 **Run #2**
Cellobiose	20.04 **15.00**	13.73 **12.22**	7.31 **4.34**	0.97 **0.52**	0.9993 **0.9940**	6.96% **9.75%**
Glucose	20.44 **14.96**	14.00 **12.18**	7.46 **4.32**	0.99 **0.05**	0.9931 **0.9953**	5.71% **6.00%**
Xylose	20.12 **14.88**	13.78 **12.12**	7.34 **4.30**	0.98 **0.51**	0.9993 **0.9958**	3.82% **5.05%**
Galactose	20.60 **15.12**	14.11 **12.31**	7.52 **4.37**	1.00 **0.05**	0.9992 **0.9954**	7.07% **5.90%**
Mannose	19.98 **15.23**	13.68 **11.58**	7.29 **4.57**	0.97 **1.07**	0.9996 **0.9999**	6.74% **1.88%**
Xyltiol	5.92 **6.00**	5.65 **4.50**	2.00 **1.56**	0.45 **0.06**	0.9998 **0.9994**	7.72% **0.53%**
Lactic Acid	11.68 **12.00**	9.93 **9.00**	3.50 **3.12**	0.98 **1.20**	0.9941 **0.9950**	4.40% **1.39%**
Glycerol	9.06 **8.00**	7.70 **6.00**	2.75 **2.08**	0.84 **0.80**	0.9954 **0.9991**	5.31% **0.77%**
Acetic Acid	12.92 **12.00**	10.98 **9.00**	4.00 **3.12**	1.24 **1.20**	0.9990 **0.9887**	5.77% **2.00%**
HMF	5.50 **5.00**	4.68 **3.75**	1.38 **1.30**	0.50 **0.50**	0.9977 **0.9951**	9.38% **1.34%**
Furfural	4.92 **5.00**	4.45 **3.75**	1.60 **1.30**	0.43 **0.50**	0.9992 **0.9953**	5.09% **1.65%**

Appendix B

Calculations

Determination of reaction severity [Overend (1987)]

$$S_0 = \log\left\{\exp\left[\frac{T-100}{14.75}\right]\times t\right\}$$

Where
S_0 = Severity Factor
T = Temperature (ºC)
t = Residence Time (mins)

Determination of percent change in dry mass

$$\%CDM = \frac{DM_{After} - DM_{Before}}{DM_{Before}} \times 100\%$$

Where
%CDM = Percent Change in Dry Mass (%)
DM_{After} = Mass of Dry Wood Flakes After Extraction (g)
DM_{Before} = Mass of Dry Wood Flakes After Extraction (g)

Determination of percent change in mass at saturation

$$\%CMS = \frac{SM_{Equil} - DM_{After}}{DM_{After}} \times 100\%$$

Where
%CMS = Percent Change in Mass at Saturation (%)
SM_{Equil} = Mass of Saturated Wood Flakes After Equilibration (g)
DM_{After} = Mass of Dry Wood Flakes After Extraction (g)

Determination of percent lignin solubilized

$$\%LS = \frac{\frac{UV_{Abs} \times Vol_{Hydrol}}{1000 \times \varepsilon} \times 100\%}{\%Lignin_{Comp\ Anal} \times DM_{Before}}$$

Where
%LS = Percent Lignin Solubilized (%)
UV_{Abs} = Ultra Violet Absorption at 240 nm
Vol_{Hydrol} = Recovered Hydrolysate Volume After Extraction (mL)
ε = Absorptivity constant, a Value of 12 for Pine Species
$\%Lignin_{Comp\ Anal}$ = % of Dry Mass of Lignin in Compositional Analysis (%)
DM_{Before} = Dry Mass of Wood Flakes Before Extraction (g)

Determination of Hydrolysate Component Compositions

$$Comp_{Conc} = \frac{Intens_{Comp\ Pk} \times CC_{Slope}}{10}$$

Where
$Comp_{Conc}$ = Component Composition (g/L)
$Intens_{Comp\ Pk}$ = Intensity of Component Peak on Chromatogram (RIU)
CC_{Slope} = Slope of Component Calibration Curve (g/L per RIU)

Economic Calculations

Incremental Wood Cost Increase

$$IWCI = WC \times WU_{ANN}\left[(1 - \%CSA)(1 - \%CDM) - 1\right]$$

Where
IWCI = Incremental Wood Cost Increase (USD/year)
WC = Wood Cost (USD/ton)
WU_{ANN} = Annual Wood Usage (tons/year)
%CSA = Percent Change in Average Surface Area (%)
%CDM= Percent Change in Dry Mass (%)

Component Conversion

$$Comp_{Conv} = \frac{Comp_{Conc} \times Vol_{Hydrol}}{DM_{Before} \times 1000} \times \frac{1}{MC_{DE} \times EC} \times Y_R$$

Where
$Comp_{Conv}$ = Component Conversion (%)
$Comp_{Conc}$ = Component Composition (g/L)
Vol_{Hydrol} = Recovered Hydrolysate Volume After Extraction (mL)
DM_{Before} = Dry Mass of Wood Flakes Before Extraction (g)
MC_{DE} = Estimated Delivered Wood Moisture Content (30%)
E_C = Average Extractives Content (5%)
Y_R = Final Reaction Yields (%)

Component Revenue

$$Comp_{Rev} = Comp_{Val} \times Comp_{Conv} \times WU_{ANN}\left[(1 - \%CSA)(1 - \%CDM) - 1\right]$$

Where
$Comp_{Rev}$ = Component Revenue (USD/year)
$Comp_{Val}$ = Component Market Value (USD/ton)
WU_{ANN} = Annual Wood Usage (tons/year)
%CSA = Percent Change in Average Surface Area (%)
%CDM= Percent Change in Dry Mass (%)

Net Revenue

$$NR = Comp_{Rev} - IWCI$$

Where
NR = Net Revenue (USD/year)
$Comp_{Rev}$ = Component Revenue (USD/year)
IWCI = Incremental Wood Cost Increase (USD/year)

Vita

Clinton Sattler obtained his Bachelors Degree in Chemical Engineering at the Purdue University in West Lafayette Indiana in 2002. Following graduation, he was employed by the Fluor Corporation in Aliso Viejo California and designed, troubleshot, and simulated chemical processes common in the oil and gas industries. In 2005, Clinton was employed as the Technical Director of the Plum Creek Timber Company's MDF manufacturing facility in Columbia Falls Montana. He enrolled in the Chemical Engineering Masters of Science program at the University of Tennessee in 2006, and graduated from the program in 2008. His research interests focus on energy applications.