

## CHAPTER 1

### 1.0 INTRODUCTION

Lignocellulosic biomass materials can be fractionated into biopolymer constituents by pulping or steam explosion technology. Treating various biomass resources by steam explosion has been studied by many researchers.<sup>5,7,18, 19,25</sup> The studies were carried either with batch or continuous reactor. In steam explosion, the biomass is, in principle, pressurized with high steam pressure for a certain period of time, followed by sudden decompression. This explosive discharge changes the starting material (solid) into a fibrous mulch by a combination of mechanical and chemical action. In case of wood chips, the explosion causes defibrillation of chips into fiber bundles, and partial hydrolysis of cellulose, other carbohydrates, lignin and volatile components.<sup>4</sup>

After the explosion step, there are many opportunities on how to use this product (steam exploded fibers, SEF). Chemical components such as cellulose, hemicellulose and lignin from SEF are studied by many investigators.<sup>3,13,16,20,26,32,37</sup> The industry manufacturing such products as pulp and paper, textiles and composites, should consider the SEF source as a raw material. In addition, SEF from biomass should also be considered as a source for the production of fuel and energy by enzymatic treatment.<sup>10,17,28,29,31,38</sup>

SEF can be treated with chemicals to isolate one or several interesting chemical components. For example, hemicelluloses (water soluble) and lignin (alkali soluble) are components that can be separated from SEF by water and alkali extractions. The residue of this operation, the insoluble components (water and alkali washed fibers), are almost free from hemicelluloses and lignin. The question is, how clean are the product-fractions after each treatment?

To answer this question, a quantitative “Clean Fractionation Concept” should be established. This concept, which describes the effectiveness of the separation of biomass into individual components or isolated fractions, is to provide quantitative information on how clean the isolated products (fractions) are after each treatment. Therefore, the objectives of this thesis are to create and test a tool for determining quantitatively the degree to which lignocellulosic biomass can be fractionated into constitutive polymer components. The important data needed for reaching this goal are:

- i. Mass fraction of selective biomass fractions.
- ii. Chemical composition of selective biomass fractions.

With that information, detailed mass fractionation will be established by summative analysis, to provide a rapid quantitative assessment of the fractionation behavior of biomass resources.

## CHAPTER 2

### 2.0 LITERATURE REVIEW

#### 2.1 Lignocellulosic Biomass Materials

Lignocellulosic biomass is the most abundant material in the world. Its sources range from trees to agricultural residues. Long ago, these materials were used as firewood, building materials and animal food. Nowadays, lignocellulosic materials are not just used in their old ways, their applications have expanded into the fiber level as in pulp and paper products. In some cases, the use of lignocellulosics is proceeding to the level of the chemical component itself. For example, cellulose, which is a major chemical constituent of lignocellulosic materials can be used for fibers in the textile industry; while lignin is used as an adhesive component in the composite industry.

#### 2.2 Lignocellulose Structure

##### 2.2.1 *Cell Wall Structure*

For general discussion purposes, the structure of wood is usually used as example for lignocellulose. The basic model of the wood cell wall structure is well described and understood.<sup>8,11,30</sup> In nature, the layers of the cell wall structure are illustrated using a wood model shown in Figures 1 and 2. The relative thickness of the layers is illustrated in Figure 3.

Between the cells, there is a component that acts as glue to join the cells together. It is known as middle lamella (ML). Toward inside, the cell wall called the primary wall (P). The primary wall can be divided into an outer and an inner surface. The arrangement of the microfibrils in the primary wall are increasingly disperse from inner to outer surface. Following the primary wall is the secondary wall, which consists of three layers. They are

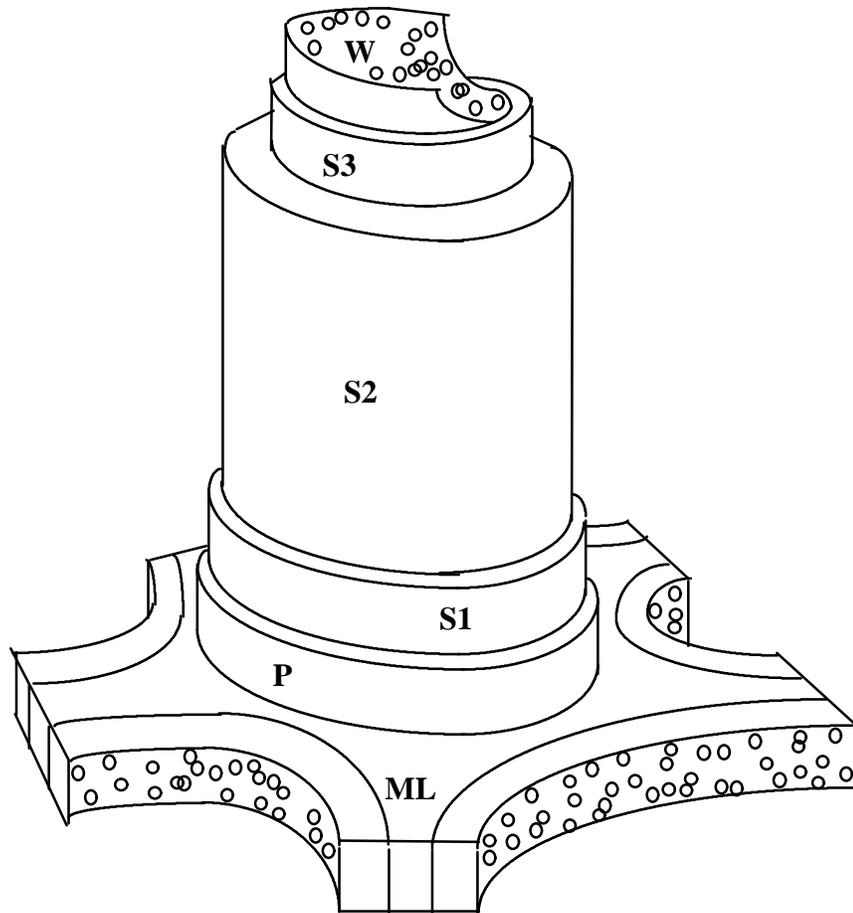


Figure 1. Schematic illustration of the cell wall of wood cells which generally applies to many cells in both softwoods and hardwoods.<sup>8</sup>

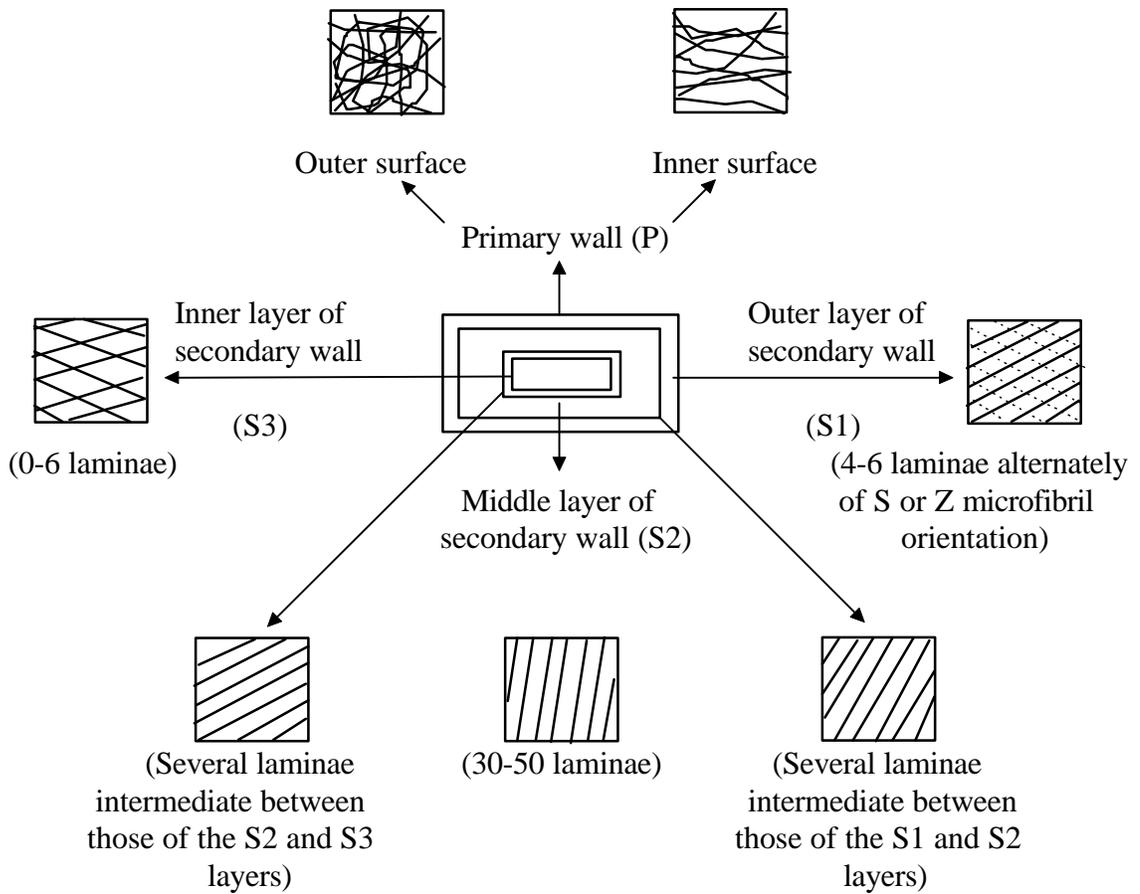


Figure 2. Schematic illustration of the layers of wood fibers.<sup>44</sup>

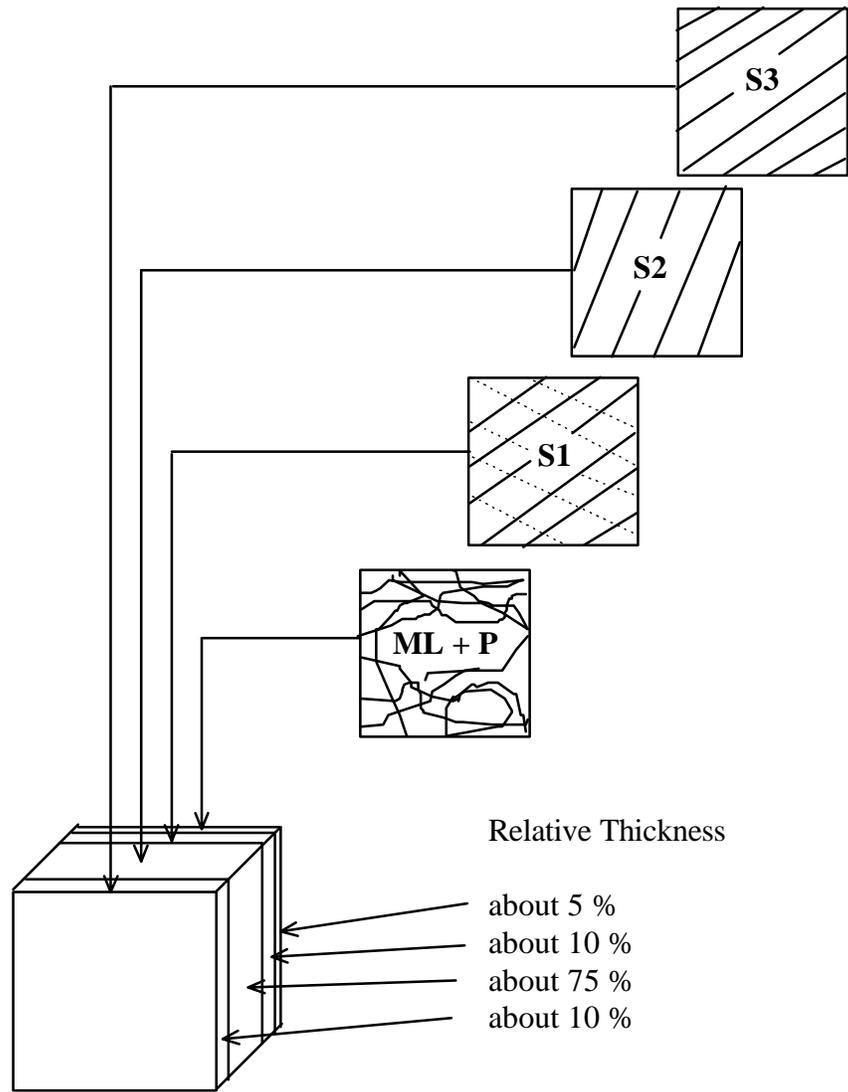


Figure 3. Schematic illustration of the relative thickness of cell wall layers for wood fibers.<sup>12</sup>

outer layer (S1), middle layer (S2) and inner layer (S3). In the outer layer of the secondary wall (S1), the microfibrils are oriented in a cross-helical structure (S helix and Z helix). The middle layer of the secondary wall (S2), which is the thickest layer, has relatively consistent orientation of microfibrils. In contrast, the microfibrils of in the inner layer of the secondary wall (S3) may arrange in two or more orientations. Lastly, in some cases, there is a warty layer (W) on the inner surface of the cell wall. In addition, some authors<sup>11</sup> mention that there is a tertiary wall (T) between S3 and W.

### **2.2.2 Chemical Structure**

The chemical components of lignocellulose can be divided into four major components. They are cellulose, hemicelluloses, lignin and extractives. Generally, the first three components have high molecular weights and contribute much mass, while the latter component is of small molecular size, and it is available in little quantity (Table 1). Based on weight percentage, cellulose and hemicelluloses are higher in hardwoods compared to softwoods and wheat straw, while softwoods have higher lignin content. Wheat straw has a high percentage of extractives. Chemical composition data of red oak wood (*Quercus rubra*) from some authors<sup>2,11,36</sup> are presented in Table 2.

#### **2.2.2.1 Cellulose**

The cellulose content of wood varies between species in the range of 40-50 %. Some lignocellulosic materials can have more cellulose than wood (Table 3). Cellulose is a linear polymer chain which is formed by joining the anhydroglucose units into glucan chains. These anhydroglucose units are bound together by  $\beta$ -(1,4)-glycosidic linkages. Due to this linkage, cellobiose is established as the repeat unit for cellulose chains (Figure 4). The degree of polymerization (DP) of native cellulose is in the range of 7,000-15,000.

$$DP = \frac{\text{Molecular weight of cellulose}}{\text{Molecular weight of one glucose unit}}$$

Table 1. Average chemical composition of softwoods, hardwoods and wheat straw.<sup>39</sup>

Chemical component	Weight, % of dry material		
	Softwoods	Hardwoods	Wheat straw
Cellulose	42 ± 2	45 ± 2	36 ± 5
Hemicellulose	27 ± 2	30 ± 5	27 ± 3
Lignin	28 ± 3	20 ± 4	11 ± 3
Extractives	3 ± 2	5 ± 3	26 ± 5

Table 2. Chemical composition of red oak. (*Quercus rubra*).

Reference	Chemical composition, % of oven dry wood									
	Holo-cellulose	Alpha cellulose	Cellulose	Hemi-cellulose	Pentosan	Lignin	Klason lignin	Extractives	Hot water	Ash
Fengel and Wegener <sup>11</sup>	49.2	-	-	-	24.1	21.8	-	-	5.2	0.1
Biermann <sup>2</sup>	69.1	46.0	-	-	21.5	23.9	-	-	-	-
Pettersen <sup>36</sup>	69	46	-	-	22	-	24	-	6	0.4
Pettersen <sup>36</sup>	-	-	42.2	33.1	-	20.2	-	4.4	-	0.2

Table 3. Cellulose content of various lignocellulosic materials.<sup>11</sup>

Lignocellulosic materials	Cellulose content, %
Cotton	95-99
Ramie	80-90
Bamboo	40-50
Wood	40-50
Bark	20-30

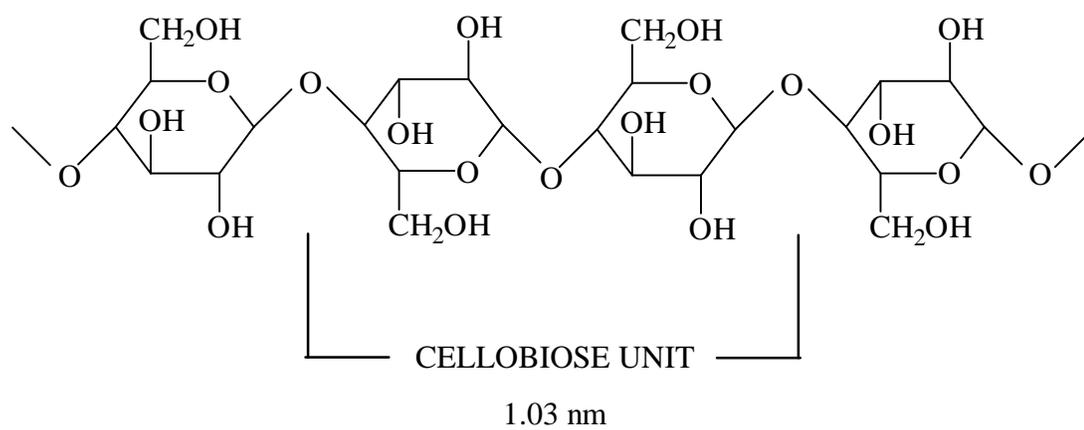


Figure 4. Schematic illustration of the cellulose chain.<sup>11</sup>

By forming intramolecular and intermolecular hydrogen bonds between OH-groups within the same cellulose chain and the surrounding cellulose chains, the chains tend to arrange in parallel and form a crystalline supermolecular structure. Then, bundles of linear cellulose chains (in the longitudinal direction) form a microfibril which is oriented in the cell wall structure.

#### **2.2.2.2 Hemicelluloses and Starch**

Unlike cellulose, hemicelluloses consist of different monosacharide units. In addition, the polymer chains of hemicelluloses have short branches and are amorphous. Because of the amorphous morphology, hemicelluloses are partially soluble or swellable in water. The backbone of the chains of hemicelluloses can be a homopolymer (generally consisting of single sugar repeat unit) or a heteropolymer (mixture of different sugars). Formulas of the sugar component of hemicelluloses are listed in Figure 5. Among the most important sugar of the hemicelluloses component is xylose.

In hardwood xylan, the backbone chain consists of xylose units which are linked by  $\beta$ -(1,4)-glycosidic bonds and branched by  $\alpha$ -(1,2)-glycosidic bonds with 4-O-methylglucuronic acid groups. In addition, O-acetyl groups sometime replace the OH-groups in position C2 and C3 (Figure 6 A). For softwood xylan, the acetyl groups are fewer in the backbone chain. However, softwood xylan has additional branches consisting of arabinofuranose units linked by  $\alpha$ -(1,3)-glycosidic bonds to the backbone (Figure 6 B).

Among the carbohydrates components, starch is the only structure that has linear and branched chains. The linear chain is known as amylose (Figure 7 A). Their anhydroglucose units are linked by  $\alpha$ -(1,4)-glycosidic bonds. In the case of branched chains, which is known as amylopectin (Figure 7 B), the backbone is like amylose but it also has  $\alpha$ -(1,6)-glycosidic bonds at the branch position.

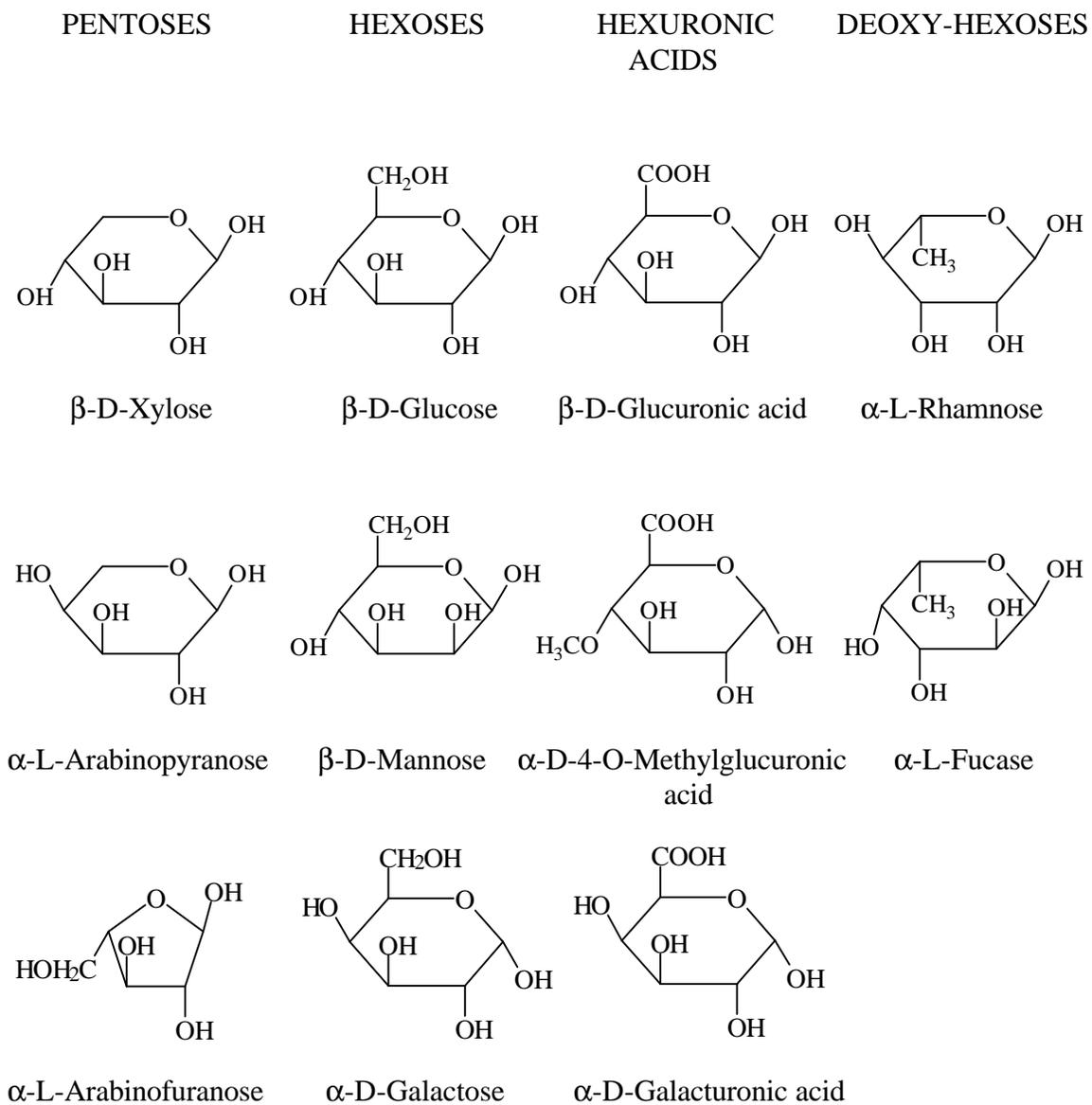
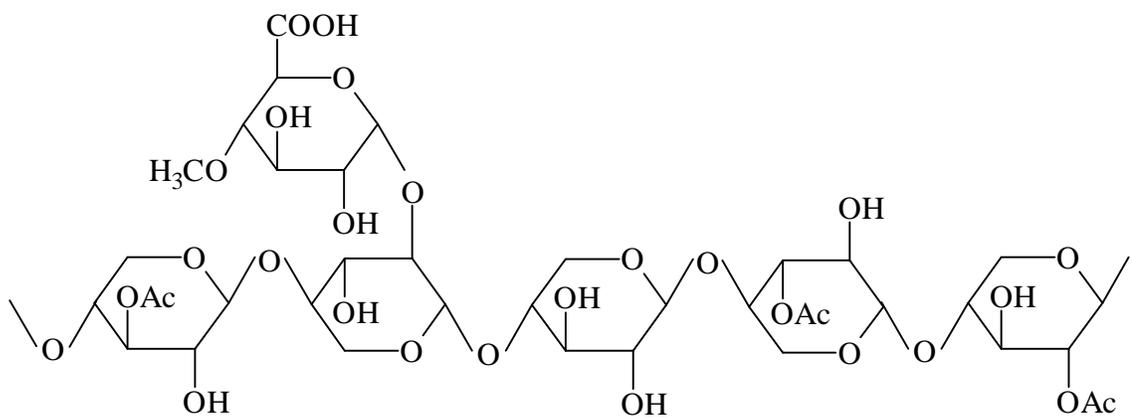
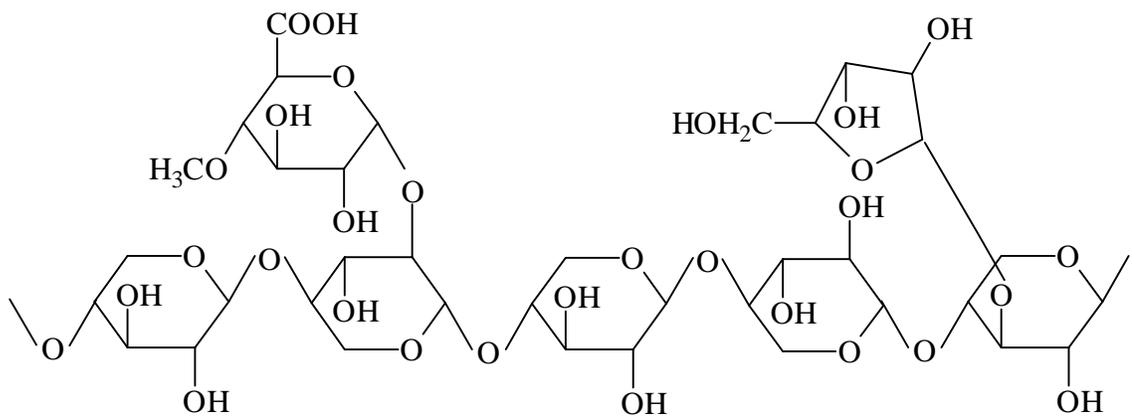


Figure 5. Schematic illustration of sugar units of hemicelluloses.<sup>11</sup>



**A**



**B**

Figure 6. Schematic illustration of xylans:<sup>11</sup>  
 A - Partial xylan structure from hardwood chain.  
 B - Partial xylan structure from softwood chain.

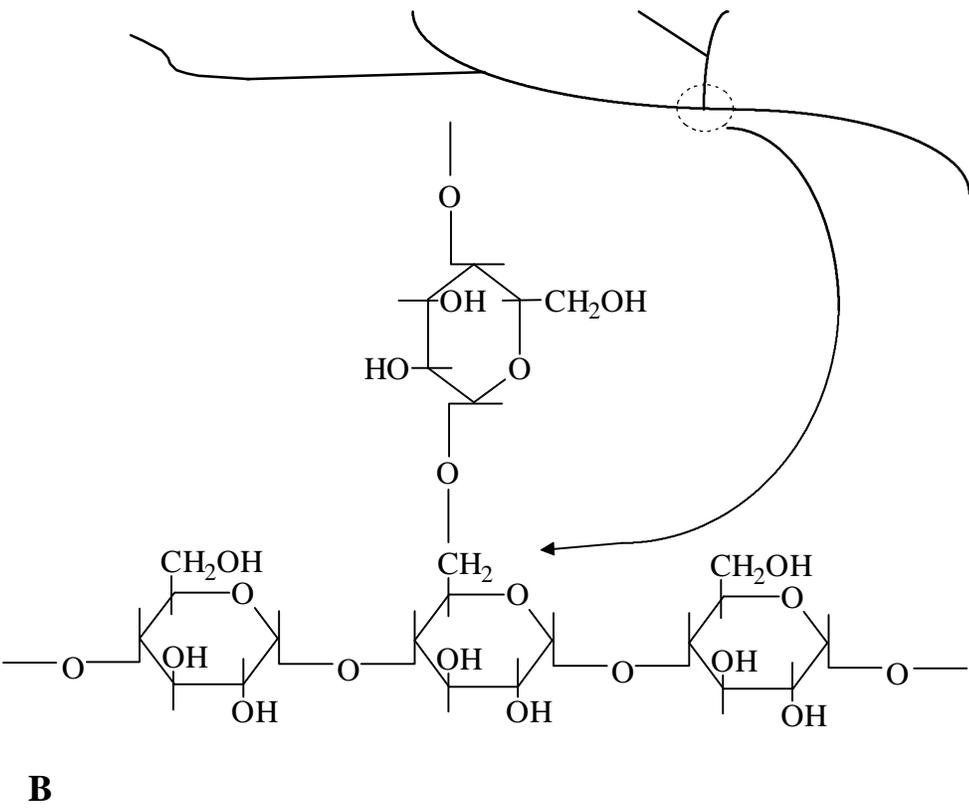
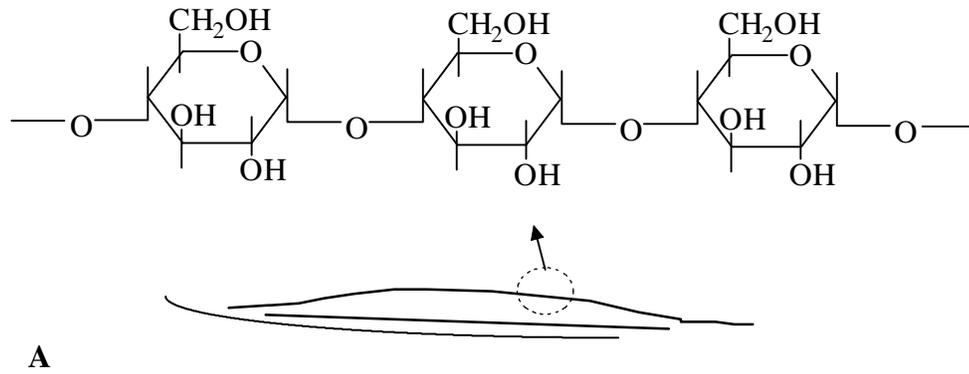


Figure 7. A schematic illustration of starch.<sup>45</sup>  
 A - Amylose chain.  
 B - Amylopectin chain.

### 2.2.2.3 Lignin

Lignin is a complex, crosslinked polymer that forms a large molecular structure. Lignin gives mechanical strength to wood by gluing the fibers together (reinforcing agent) between the cell walls. Lignin also serves as a disposal mechanism for metabolic waste.<sup>44</sup> The monomeric building units of lignin are shown in Figure 8. The guaiacyl unit is dominant in the softwoods.<sup>14</sup> In contrast, syringyl units are dominant in hardwoods.<sup>33</sup>

### 2.2.2.4 Extractives

Extractives are the organic substances which have low molecular weight and are soluble in neutral solvents. Resin (combination of the following components: terpenes, lignans and other aromatics), fats, waxes, fatty acids and alcohols, terpenes, tannins and flavonoids are categorized as extractives. They only represent between 4-10 % of the total weight of dry wood, and the contents of extractives vary among wood species, geographical site and season. The extractives can be found mostly in resin canal and ray parenchyma cells and small amount in middle lamella and cell walls of tracheids. Some extractives are toxic and this is an advantage for the wood to resist attack by fungi and termites.

Tannins are the main component of the red oak extractives. Natural tannins can be subdivided into hydrolyzable and condensed tannins.<sup>21</sup> The hydrolyzable tannins are classified as gallotannins (yielding gallic acid after hydrolysis) and ellagitannins (yielding ellagic acid after hydrolysis), Figure 9 A. The condensed tannins are widely used in leather industries as chemical treatments. The main structures in the condensed tannins are catechins type (Figure 9 B).

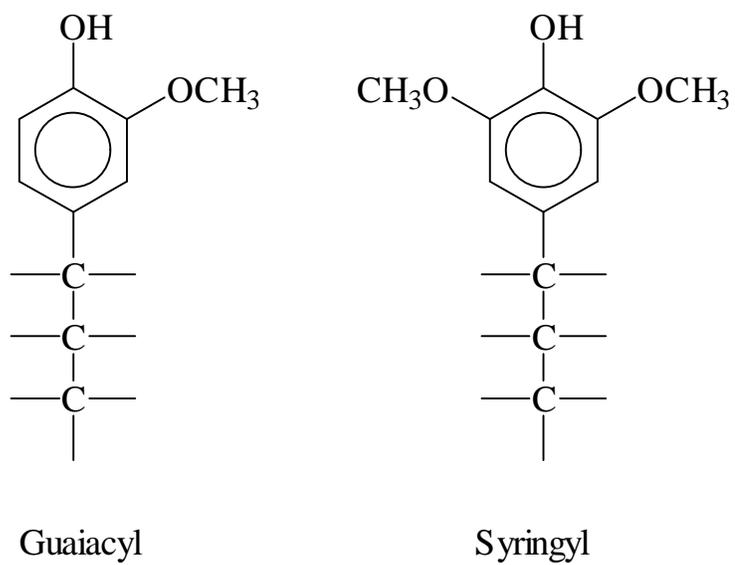


Figure 8. Schematic illustration of building units of lignin.<sup>33</sup>

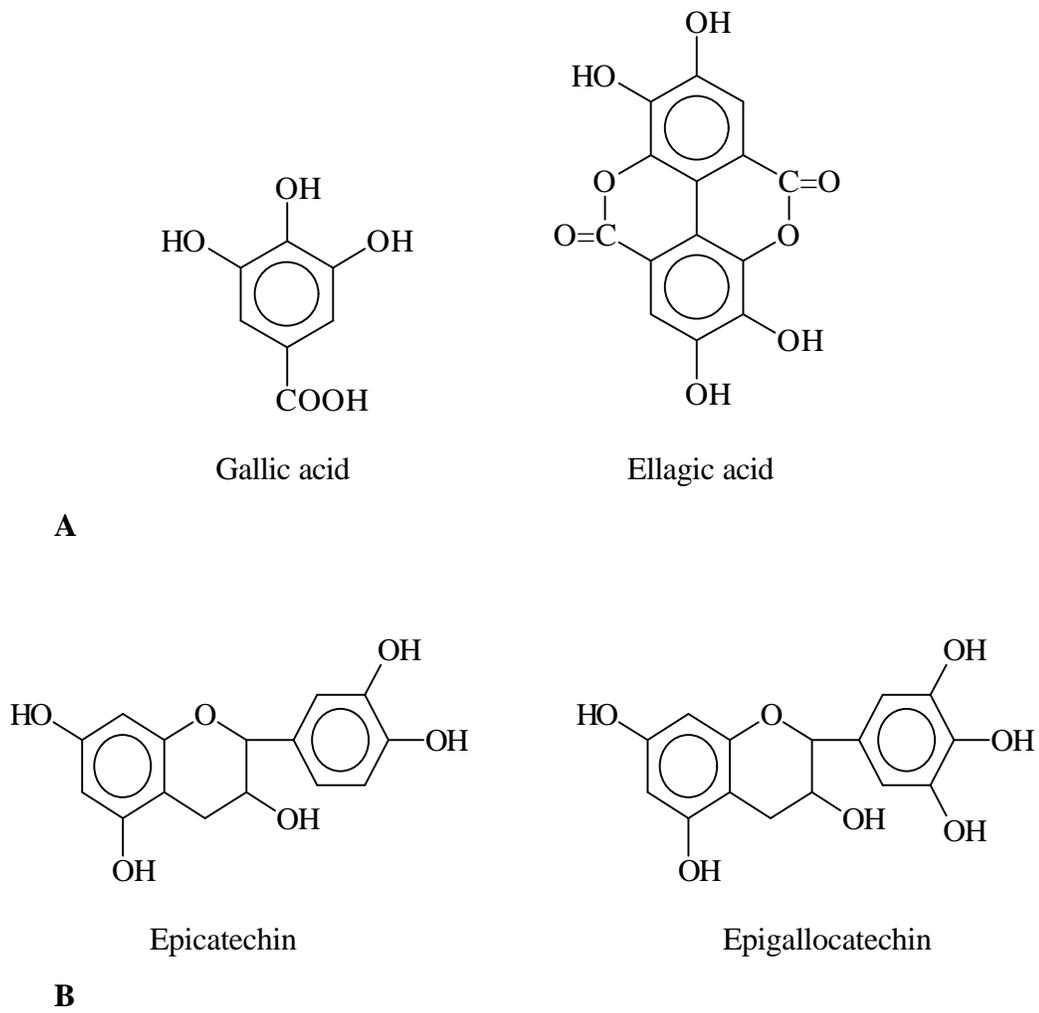


Figure 9. Schematic illustration of tannins in red oak.<sup>21</sup>

A - Hydrolysis products of hydrolyzable tannins.

B - Flavonoid molecules, members of the class of condensed tannins.

### 2.3 Steam Explosion

Steam explosion technology as a method to defibrillate lignocellulosic materials was studied about 60 years ago.<sup>24</sup> Then, the separation technique with steam explosion was improved by using batch<sup>1,9</sup> and continuous (stake)<sup>6</sup> reactors.

The treatment of lignocellulosic resources with high pressure steam, for short periods of time, followed by sudden decompression (explosion) represents a simple treatment for biomass that achieves fiberization or “mulching” by a combination of chemical and mechanical action. The parameters controlling the steam explosion technique are reaction temperature ( $T_r$ ) and retention time ( $t$ ). Then the relationship between  $T_r$  and  $t$  has been defined as severity ( $R_o$ )<sup>7,35</sup>

$$R_o = \int_0^t \exp [ ( T_r - T_b ) / 14.75 ] dt$$

$R_o$  - severity

$T_r$  - reaction temperature, °C

$T_b$  - base temperature (100 °C)

$t$  - retention time, minute

Marchessault<sup>25</sup> mentions that the steam explosion is an autohydrolysis process.

Effects of this process on biomass are:

- i. Cleavage of some accessible glycosidic links.
- ii. Cleavage of  $\beta$ -ether linkages of lignin
- iii. Cleavage of lignin-carbohydrate complex bonds.
- iv. Minor chemical modification of lignin and carbohydrates.

## 2.4 Fractionation

In order to obtain constitutive chemical products (cellulose, hemicelluloses and lignin) from exploded fibers, fractionation must be carried out. The most dramatic consequences of steam explosion on the structure and behavior of lignocellulosic materials is the extensive solubility of the biomass in neutral solvents and/or alkali. The fractionation of steam exploded biomass into water-soluble, alkali-soluble, and insoluble fractions by sequential treatment with hot water and hot alkali has been studied.<sup>13,15,22,32</sup>

As Myerly et al.<sup>33</sup> and Bozell et al.<sup>3</sup> have pointed out in the past, efficient separation of constitutive biomass components, similar to the separation of crude oil components by distillation, constitutes one of the major obstacles to the efficient utilization of renewable resources. However, such separation is mandatory if sustainably derived plant (renewable) resources are to advance as feedstocks for chemicals and materials that are presently obtained from fossil carbon resources. Fractionation technology by means other than distillation are available, and these include solvent extraction. The extraction of lignin and non-crystalline carbohydrates (hemicelluloses or polyoses) is commercially practiced by the pulp and paper industry. However, while the paper industry has managed to define conditions under which cellulose-rich pulp fibers can be isolated and purified efficiently, the process fails to achieve efficient fractionation performance by wasting (through under-utilizing the non-cellulosic biomass as process fuel) half of the plant resource. By contrast, Bozell et al.<sup>4</sup> have defined the following criteria for the development of efficient biomass fractionation processes:

- i. Selectivity - Separation of constitutive components with minimum cross contamination.
- ii. Accessibility - Each constitutive component must be easily accessible/recoverable after fractionation.
- iii. Recoverability - Each constitutive component must be recoverable in high yield.

- iv. Utility - Each constitutive component must become available in useful form without the need for extensive additional purification.
- v. Economics - The process must be economically viable.

To this end, Bozell et al.<sup>4</sup> have defined a “Clean Fractionation” process for lignified biomass that is based on biomass treatment with a ternary mixture of organic solvents, methyl isobutyl ketone (MIBK), ethanol and water, in the presence of acid at elevated temperature. The clean fractionation process advocated yields the three principal biomass components, cellulose, hemicelluloses, and lignin, in three different process fractions.

Clean biomass fractionation into constitutive components, however, is not limited to organosolv pulping techniques alone. Any hydrolytic pretreatment of biomass may render the resource fractionatable. Pretreatments with aqueous steam, hydrothermal or autohydrolytic pretreatments, etc., are all qualified to render biomass fractionatable. The availability of a diverse set of biomass fractionation technologies requires the definition of a “cleanness-parameter”: This parameter is to provide a quantitative (numerical) assessment for component recoverability with minimum cross contamination.

This is to introduce a quantitative clean fractionation concept that is based on a combination of mass fractions and summative analysis data. This evaluation is to produce rigorous and quantitative parameters for comparing different fractionation methodologies with respect to the principal parameters of clean fractionation: component separation and recovery in high yield with minimal cross contamination.

## CHAPTER 3

### 3.0 MATERIALS AND METHODS

#### 3.1 Materials

##### 3.1.1 *Starting Material for Steam Explosion*

Red Oak (*Quercus rubra*) chips were used as a starting material (SM). They were obtained freshly from a pilot scale wood chipper located at the Southern Forest Experiment Station, Pineville, Louisiana. The chips were screened with 5/8 inch sieve.

##### 3.1.2 *Materials for Extractions*

For water extraction, steam exploded fibers (SEF) from the steam explosion step were treated with tap water. Fibers from the water extraction step, called water extracted fibers (WEF), were treated with alkali, acetic acid and ethanol. Chemicals that were used in those extractions were:

- I. Sodium hydroxide, (Aldrich Chemical Company, Inc.).
- ii. Ethanol, (Aldrich Chemical Company, Inc.).
- iii. Acetic acid, (Aldrich Chemical Company, Inc.).

##### 3.1.3 *Materials for Chemical Analysis*

Materials that were used in chemical analysis included:

- I. D-Galactose, (Aldrich Chemical Company, Inc.).
- ii. D-Xylose, (Aldrich Chemical Company, Inc.).
- iii. D-Arabinose, (Aldrich Chemical Company, Inc.).
- iv.  $\alpha$ -D-Glucose, (Aldrich Chemical Company, Inc.).
- v. D-Mannose, (Aldrich Chemical Company, Inc.).

- vi. Erythritol, (Aldrich Chemical Company, Inc.)
- vii. Barium hydroxide, (Aldrich Chemical Company, Inc.)
- viii. 2-furaldehyde, (Aldrich Chemical Company, Inc.)
- ix. 5-(hydroxymethyl)-2-furaldehyde, (Aldrich Chemical Company, Inc.)
- x. Sulfuric acid, (Aldrich Chemical Company, Inc.)
- xi. H<sup>+</sup> resin, (Bio-Rad)
- xii. CO<sub>3</sub><sup>2-</sup> resin, (Bio-Rad)

## 3.2 Methods

### 3.2.1 *Steam Explosion*

A two cubic foot batch digester steam explosion unit located at the Thomas M. Brooks Forest Products Center of Virginia Tech under the supervision of Robert S. Wright was used in this study. A diagram of the batch steam explosion unit is shown in Figure 10. Five conditions of steam explosion were run on red oak samples. They were at severity R<sub>o</sub> 5,000, 10,000, 15,000, 20,000 and 35,000. The specific conditions are shown in Table 4. The solids content of the red oak chips was determined prior to processing using dryind balance (Ohaus model MB 200).

Before any sample was fed into the steam explosion chamber, all valves were closed except for valve ① (Figure 10). For each batch, about 3/4 bucket of chips (ca. 2 kg dry weight) were put into the chamber through valve ①. After this valve was closed, steam was released into the chamber via valve ②. The pressure and temperature of the steam were controlled from a boiler. Time was kept constant while pressure and temperature were varied in relation to severity chosen. Valve ③ was released when a desired “cooking” time had been reached. Simultaneously, an explosive expansion of the steam occurred and an exploded sample (called as steam explosion fibers, SEF) was collected in a container. The fibers (SEF) were weighed, packed into a plastic bag and stored in a cold room.

Table 4: Steam explosion conditions of red oak.

Severity,		Time, Minute	Pressure, Bar	Temperature, °C
R <sub>o</sub>	Log R <sub>o</sub>			
5,000	3.70	2.5	19	212
10,000	4.00	2.5	23	222
15,000	4.18	2.5	26	228
20,000	4.30	2.5	28	232
35,000	4.54	5.0	28	232

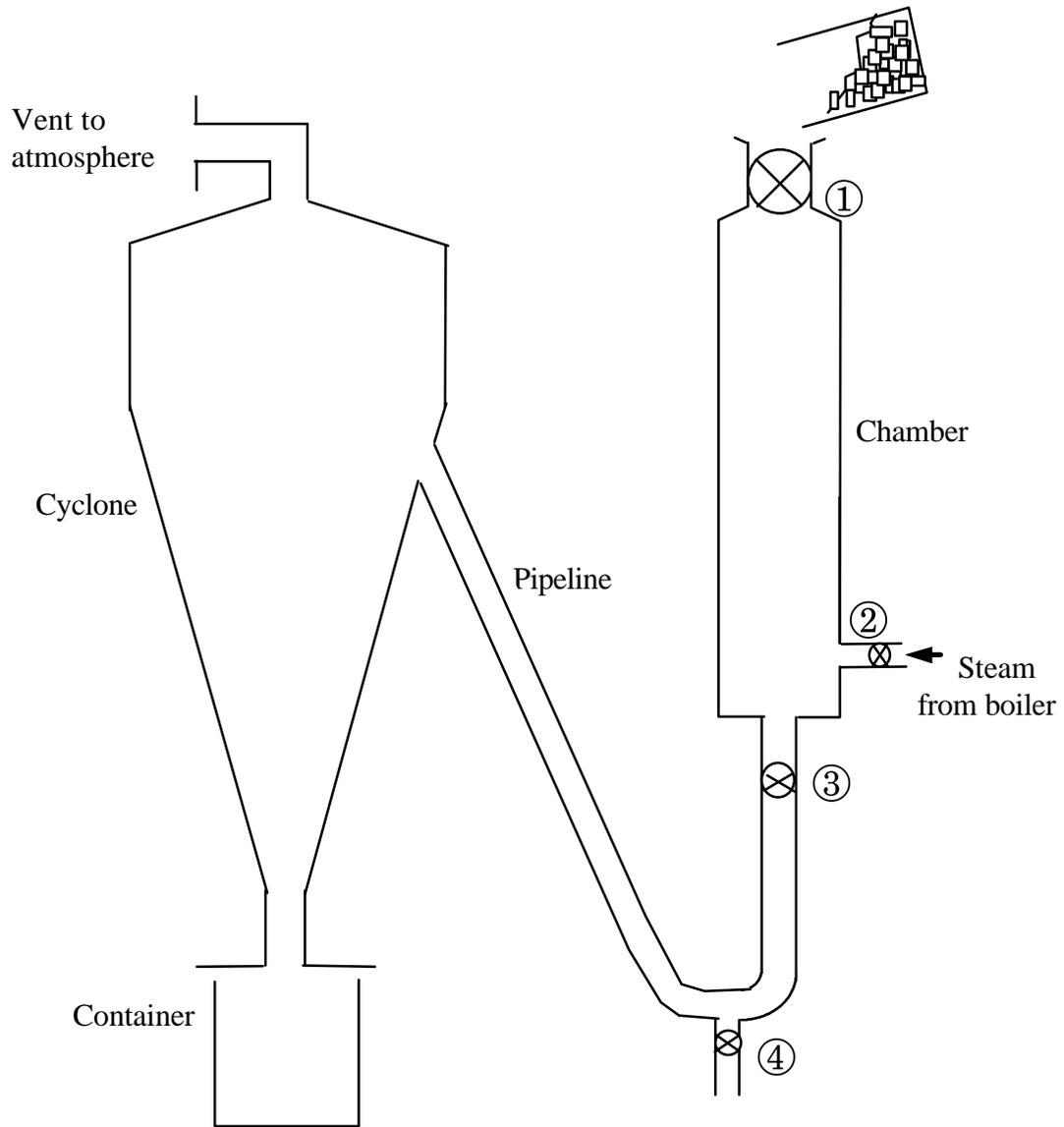


Figure 10. Schematic illustration of the batch steam explosion unit at T. M. Brooks Forest Products Center of Virginia Tech.

### **3.2.2 Extractions**

The SEF from the steam explosion at different severity factors were used in the extraction steps. Four kinds of extractions were performed in this experiment. They were water, aqueous alkali, acetic acid and ethanol extractions. The acetic acid and ethanol extractions were done to compare efficiency of delignification with alkali extraction. Only the SEF of severity  $R_0$  35,000 were used for the acetic acid and ethanol extractions. The solid to liquor ratio (S : L) of all extraction was 1 : 8. This ratio was based on the dry weight of solid fibers over the weight of liquor (w/w). The water, alkali and acetic acid extractions were carried out in the same apparatus as shown in Figure 11. On the other hand, the ethanol extraction was run using a Parr pressure reactor (Figure 12). The various samples collected from the steam explosion (step 1) and the extraction steps (step 2 and step 3) at different severities are summarized in Table 5.

#### **3.2.2.1 Water Extraction**

After the SEF were taken out from the cold room, the solids content of each fibers sample was determined. Based on the solids content, 200 g dry weight of SEF were charged into a 5 L beaker. Then, water was added to bring the total S : L ratio to 1 : 8 and the mixture was stirred with a stirring rod. The beaker was heated to 60 °C using a hot plate for 1/2 hour. The slurry was stirred for every 3 minutes. After 1/2 hour, the slurry was allowed to cool at room temperature. Then, it was centrifuged for 3 minutes using a Williams centrifuge. About 400 mL of the liquor was collected and poured into an ice cube mold. The liquor was frozen in a -70 °C freezer prior to being freeze dried. This liquor was designated as water extracted liquor (WEL). The remaining liquor was discarded. The fibers remaining on the centrifuge screen were washed 3 times with 400 mL of water before they were collected, weighed and placed into a plastic bag. These fibers were designated as water extracted fibers (WEF) and stored in a refrigerator.

Table 5. Sample designation of fibers processed by steam explosion and post treatment.

Severity, $R_o \times 10^3$	Steam exploded fibers	Water extracted fibers	Water extracted liquor	Alkali extracted fibers	Acetic acid extracted fibers	Ethanol extracted fibers
5	SEF-5	WEF-5	WEL-5	AEF-5	-	-
10	SEF-10	WEF-10	WEL-10	AEF-10	-	-
15	SEF-15	WEF-15	WEL-15	AEF-15	-	-
20	SEF-20	WEF-20	WEL-20	AEF-20	-	-
35	SEF-35	WEF-35	WEL-35	AEF-35	AcEF-35	EtEF-35

### 3.2.2.2 Alkali Extraction

The apparatus used for alkali extraction is the same as that used for water extraction (Figure 11). The WEF of different severity were taken out from the refrigerator and their solids content was determined. About 20 % (w/w of dry fibers) strength of sodium hydroxide and 50 g dry weight of WEF were used in this extraction. The solid to liquor ratio was 1 : 8. Therefore, extra water was added based on WEF solids content and the solid to liquor ratio. The WEF was placed into a 5 L beaker. Sodium hydroxide (10 g) based on dry WEF was dissolved in 50 mL of water in a 100 mL beaker. This solution was poured into the 5 L beaker and the mixture was stirred with a stirring rod. Then, the remaining water was poured into the beaker to bring the total S : L ratio to 1 : 8. Next, the beaker was heated to 60 °C for 1/2 hour. The slurry was stirred every 3 minutes. After 1/2 hour, the slurry was left at room temperature to cool. Then, it was centrifuged for 3 minutes. The liquor was collected into a plastic bottle. This liquor was called alkali extracted liquor (AEL). Unlike WEL, the AEL was not subjected to any further analysis. The fibers on the centrifuge screen were washed 3 times with 400 mL of water before it were collected and weighed. These fibers were called alkali extracted fibers (AEF). The AEF then were placed into a plastic bag and stored in the refrigerator.

### 3.2.2.3 Acetic Acid Extraction

The acetic acid extractions were performed on the WEF-35 sample only. 50 g dry weight of SEF were filled into a 5 L beaker (Figure 11). The solid to liquor ratio was 1 : 8 and the acetic acid strength in the extraction was 80 % (w/w). Therefore, 320 g of acetic acid was added into the beaker. Then, extra water was added to the mixture. The rest of the procedure was the same as the alkali extraction procedure. However, the liquor portion was called acetic acid extracted liquor (AcEL), and the fibers were designated as acetic acid extracted fibers (AcEF).

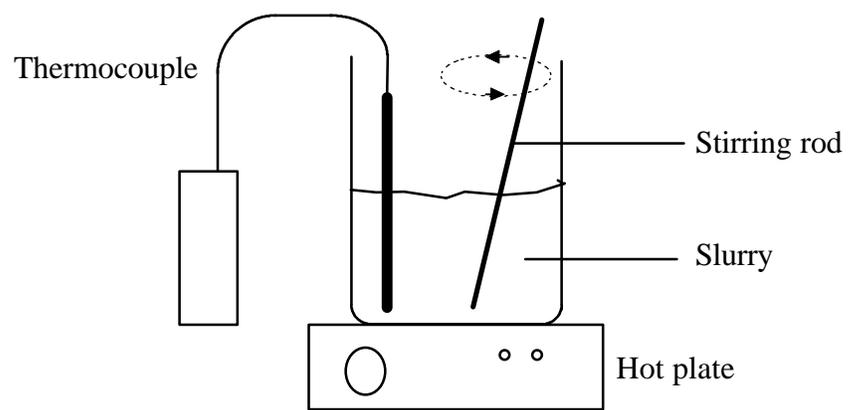


Figure 11. Schematic illustration of water, alkali and acetic acid extractions apparatus.

#### 3.2.2.4 Ethanol Extraction

Only WEF-35 sample was used in this extraction. 100 g of the dry WEF were placed into a Parr reactor. The Parr reactor is illustrated in Figure 12. The target of ethanol concentration in this extraction was 70 % (w/w). With the solid to liquor ratio of 1 : 8, 560 g of ethanol and extra water were poured into the reactor. Then the reactor was sealed with bolt nuts. The temperature was set at 85 °C and the pressure was measured at 10 psi. The reaction was run for 2 hours and stirred manually at 10 minute intervals. After 2 hours, the pressure was released through valve #1 (Figure 12). When the temperature had dropped to room temperature, the slurry was collected via valve #2.

Then, the fibers suspension were centrifuged like the other extractions. Like the alkali and acetic acid extractions, nothing else was being done to the ethanol extracted liquor (EtEL). The ethanol extracted fibers (EtEF) were weighed, packaged and stored in the refrigerator.

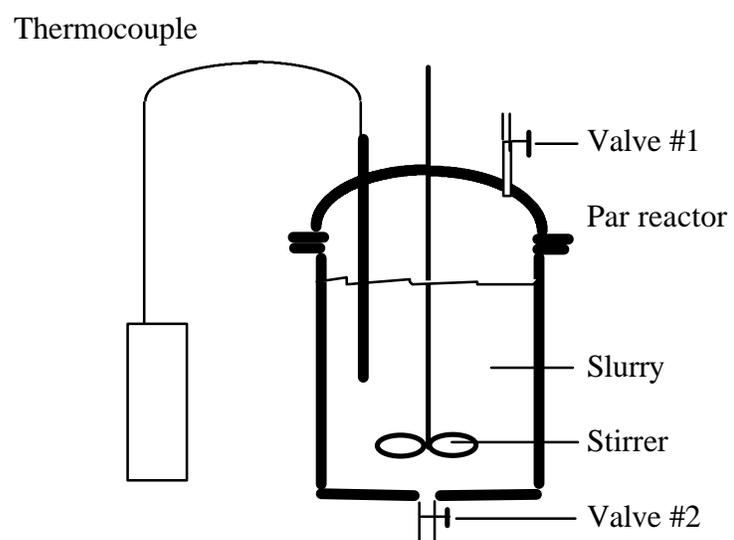


Figure 12. Schematic illustration of the Parr reactor used for ethanol extraction.

### **3.2.3 Chemical Analysis (Summative Analysis)**

Chemical analysis procedures<sup>23,24</sup> in this study were done at the Forest Products Laboratory of Virginia Tech. The procedures were modified to fit the equipments of this laboratory. The chemical analysis consists of acid hydrolysis, gravimetric determination of acid insoluble and carbohydrates analysis. Since the analysis was performed on non-extracted wood, and since the red oak contains tannins, the acid insoluble component consisted of lignin and tannins combined. This component is called “non-carbohydrates”.

#### **3.2.3.1 Acid Hydrolysis**

The red oak chips (SM) and the biomass fractions (WEF, AEF, AcEF and EtEF) of different severity were dried in a vacuum oven (Napco model 5831) overnight at 65 °C. The dry samples were ground in a Wiley Mill with 40 mesh screen. Then, they were placed into 4 mL vials at room temperature. For WEL, the frozen liquor was transferred into a freeze drying bottle. This was freeze dried for 3 days. Finally, the solid product of the WEL was crushed with a spatula and transferred into the 4 mL vial at room temperature.

Prior to the acid hydrolysis, all the powder samples in the vials were dried overnight in a vacuum oven at 65 °C. After the samples had been dried, they were quickly weighed (ca. 0.1000 ± 0.0010 g) before being stored in the 50 mL glass bombs. Then, 1 mL of 72 % of sulfuric acid was pipetted into each glass bomb. A stirring rod was used to stir the mixture homogeneously. Afterward, the glass bombs were placed in a 30 °C water bath. The mixture was stirred at 10 minutes interval during this hydrolysis. After 1 hour, the glass bombs were taken out from the water bath. The stirring rod from each glass bomb was transferred into a 125 mL cone flask which was filled with 30 mL of water. The stirring rod was rinsed thoroughly in order to remove the remaining fibers on it. Then, the water from that flask was poured into the glass bomb and capped firmly with its lid. Next, the glass bombs were autoclaved for 1 hour at 120 °C (103 kPa). The autoclave model was Vernitron Verniclave 2000. After 1 hour, the autoclave had been allowed to cool to below 100 °C (about 20 minutes) before the door was opened.

Then, each glass bomb content was filtered using a 250 mL suction flask. This filter was dried at 105 °C in the oven and weighed before it was used for filtration. The residue in the filter was used to determine the Klason lignin (including tannins) content. The acid hydrolyzate in the suction flask was transferred into a 100 mL volumetric flask. Then, the volumetric flask was filled to the mark with distilled water. This acid hydrolyzate was poured into a labeled brown bottle and stored in the refrigerator.

### **3.2.3.2 Non-Carbohydrates (Lignin and Tannins)**

The analysis for non-carbohydrates content of each sample involved the determination of acid insoluble (Klason) and acid soluble lignin. The Klason lignin (including tannins) was determined from the weight of the acid hydrolysis residue that was left on the filter after the acid hydrolysis process. The acid soluble lignin (including tannins) was determined using an UV/VIS instrument.

#### **3.2.3.2.1 Klason Lignin (Including Tannins)**

The fine sintered glass filter which was layered with nylon filter was dried in the oven at 105 °C overnight. Then, this dry filter was weighed to get its initial weight. After the filtration following acid hydrolysis, that filter was again dried and weighed. The difference between the initial and the second weight was the Klason lignin and tannins weight.

#### **3.2.3.2.2 Acid Soluble Lignin (Including Tannins)**

For acid soluble lignin (including tannins) analysis, a UV/VIS Spectrophotometer Perkin-Elmer was used. The scanning range of wavelength was set from 190 to 350 nm and 3 % sulfuric acid was used as a reference solution. By using a pipette, 1 mL of hydrolyzate was transferred into a 10 mL volumetric flask (dilution ratio was 1 : 9).

Then the volumetric flask was filled with 3 % sulfuric acid. This solution was used in the UV/VIS instrument to determine the absorbance of acid soluble lignin (including tannins) at the wavelength of 205 nm. The dilution ratio and absorbance values were recorded for calculation.

### **3.2.3.3 Carbohydrates**

Carbohydrate analysis consisted of two parts. The first part was the analysis of furaldehyde(s), (2-furaldehyde and 5-[hydroxymethyl]-2-furaldehyde). These volatile substances were retained in the capped glass bomb during acid hydrolysis. The second part was the sugar analysis. In this part, glucose, xylose, galactose, arabinose and mannose were determined. Both the volatile substances and the sugar analysis were measured using HPLC.

#### **3.2.3.3.1 Furaldehyde(s)**

The HPLC instrument used for this experiment was Waters 501 with a single Bio-Rad Carbo-H guard column (4.6 x 30 mm). Sulfuric acid (0.01 M) was used as the mobile phase. The flow rate was set at 0.8 mL/min; the operating temperature was room temperature; and the pressure was 400 psi.

A mixture of 2-furaldehyde (2-F) and 5-[hydroxymethyl]-2-furaldehyde (HMF) calibration solution was prepared. About 20  $\mu$ m of this calibration mixture was injected into the HPLC. Its chromatogram was used as a reference. Then, acid hydrolyzate of each sample was injected into the HPLC. The data from this chromatogram were used for calculation.

#### **3.2.3.3.2 Sugars**

A different HPLC instrument was used for sugar analysis. The HPLC model for this experiment was Waters 510 using a Bio-Rad Carbo-P guard column (4.6 x 30 mm) in line with Bio-Rad "Polypore" Aminex HPX-87P analytical column (7.6 x 300 mm). Deionized, degassed, distilled water was used as the mobile phase. The flow rate was set

at 1.0 mL/min; the operating pressure was 1,150-1,250 psi; and the column temperature was 85 °C. Two calibration solutions were prepared for reference. The calibration mixture #1 consisted of glucose, galactose, mannose and erythritol (internal standard). While the calibration mixture #2 was composed of xylose, arabinose and erythritol.

For each sample, the acid hydrolyzate was neutralized to pH 5.3 with barium hydroxide. Acid hydrolyzate (10 mL) was pipetted into a 50 mL beaker with stirring bar. Erythritol (1 mL) was added as an internal standard (4.0 g/L concentration). The neutralization was monitored using a pH meter (Fisher Scientific Model 50) while the saturated barium hydroxide solution was added. About 13 mL of the neutralized sample was pipetted into the centrifuge tube of a Sorval unit. The sample was centrifuged at 8,000 rpm for 5 minutes.

After that, the clear supernatant from the centrifuge tube was transferred into a 50 mL round bottom flask. This liquid was then evaporated by using a rotary evaporator. The water bath temperature of the rotary evaporator was set at 40 °C. The evaporation was stopped when the volume of the liquid was about 2 mL. Next, the concentrated liquid was eluted through an ion exchange resin bed. The set-up of the ion exchange resin bed is shown in Figure 13.

The ion exchange bed was prepared using a Bio-Rad disposable “Poly-prep” column. The column was first filled with about 0.2 mL of Bio-Rad AG 50W-X8, 100 - 200 mesh H<sup>+</sup> resin. Then, 0.4 mL of Dowex 1X-8, 200 - 400 mesh CO<sub>3</sub><sup>2-</sup> resin was added next. This column was attached to a stand and a vial was placed at the bottom of the column.

After the concentrated liquid was eluted through the ion exchange resin bed, about 0.5 mL of distilled water was applied to wash the resin bed. About 20 µm of this sample was injected into the HPLC and its chromatogram was analyzed for calculation.

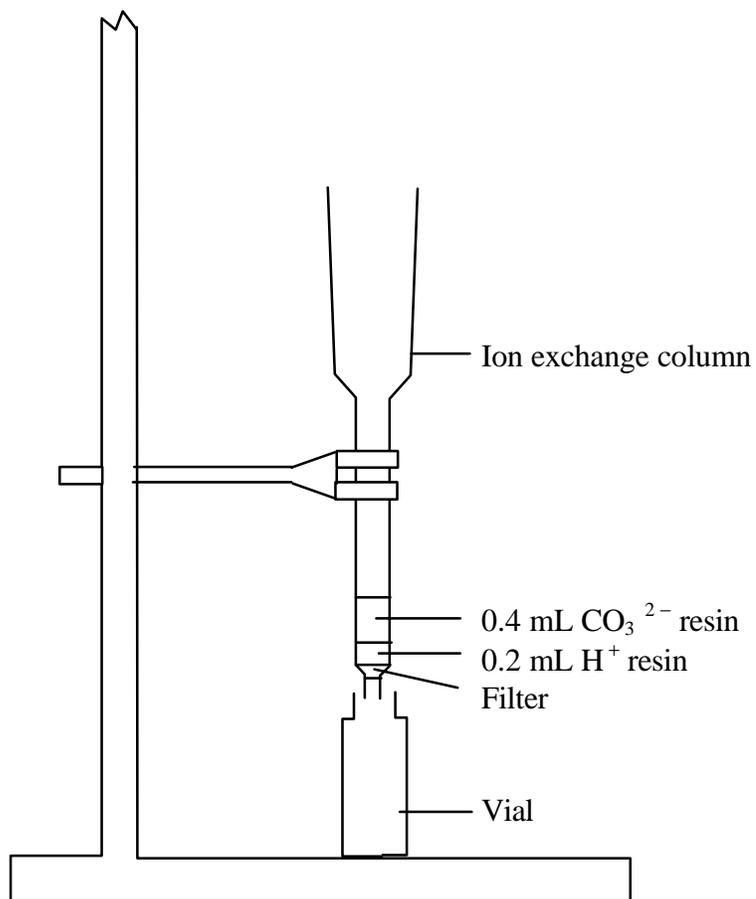


Figure 13. Schematic illustration of the ion exchange bed set-up used for sugar analysis.

## CHAPTER 4

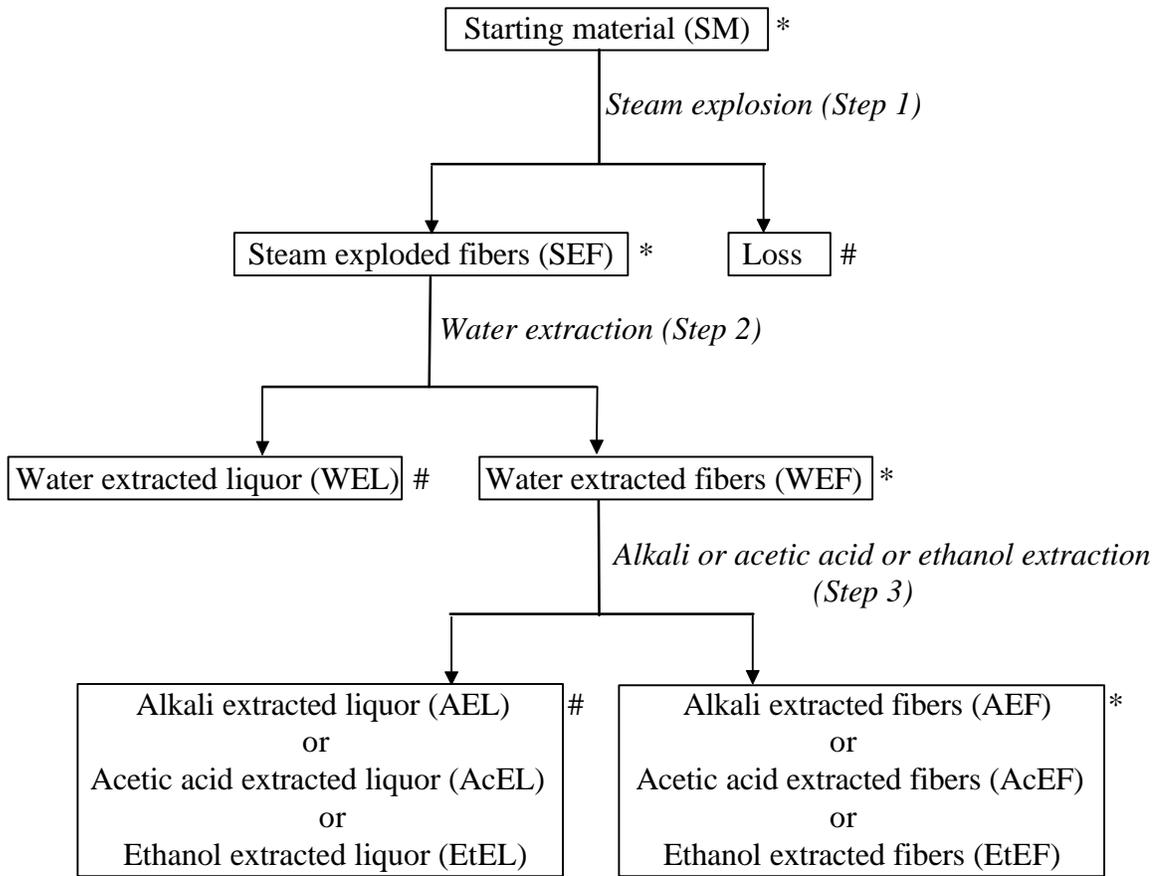
### 4.0 DATA INTERPRETATION

#### 4.1 Mass Fraction

Mass fraction calculation involves data from the steam explosion and the extraction steps. A biomass fractionation diagram is shown in Figure 14. The mass fractions of SM, SEF, WEF, AEF, AcEF and EtEF were determined directly from the biomass sample weights. The mass fractions of WEL, AEL, AcEL, EtEL and Loss, were determined by difference. In the extraction steps, only small scale experiments (50 g fiber solids) were run while explosions involved about 2 kg dry samples on average. Extracted data were converted to large scale and the weight of SM was always used as reference. The terms “solids content” and “weight” are very important in this chapter. For illustration purposes, the data of red oak samples at severity  $R_o$  15,000 or  $\text{Log } R_o$  4.18 were used.

##### 4.1.1 *Steam Explosion*

Three mass fractions can be obtained from the steam explosion step; they are the mass fraction of SM, SEF and Loss (Figure 14). The mass fractions of SM and SEF are determined experimentally and the mass fraction of Loss is calculated by difference, by subtraction of SEF from SM.



\* - experimental data  
 # - calculated data (by difference)

Figure 14. Schematic biomass fractionation diagram.

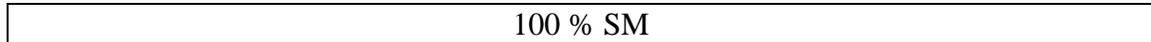
#### 4.1.1.1 Starting Material (SM)

The weight of the starting material is used as a reference; its mass fraction is considered 100 %.

Total weight of SM = 2,584 g; solids content of SM = 74.1 %

$$\begin{aligned} \therefore \text{Total dry weight of SM} &= [ \text{Total weight of SM} \times \text{Solids content of SM} ] \\ &= [ 2,584 \text{ g} \times 74.1 \% ] = 1,915 \text{ g} \end{aligned}$$

A graphical mass fraction distribution of SM is as follows:



#### 4.1.1.2 Steam Exploded Fibers (SEF)

After the steam explosion step, SEF was collected in the container and weighed.

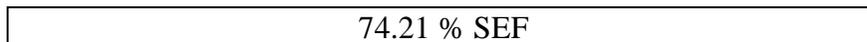
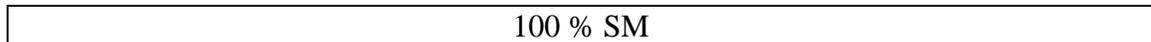
The total dry weight of this biomass fraction and its mass fraction are calculated as follow;

Total weight of SEF = 3,579 g; solids content of SEF = 39.7 %

$$\begin{aligned} \therefore \text{Total dry weight of SEF} &= [ \text{Total weight of SEF} \times \text{Solids content of SEF} ] \\ &= [ 3,579 \text{ g} \times 39.7 \% ] = 1,421 \text{ g} \end{aligned}$$

$$\begin{aligned} \text{Mass fraction of SEF} &= \left[ \frac{\text{Total dry weight of SEF}}{\text{Total dry weight of SM}} \right] \times 100 \\ &= \left[ \frac{1,421 \text{ g}}{1,915 \text{ g}} \right] \times 100 = 74.21 \% \end{aligned}$$

A graphical mass fraction distribution of SM and SEF is as follows:



#### 4.1.1.3 Loss

The unrecovered biomass fraction after steam explosion is called Loss. The mass fraction of Loss is calculated by difference as shown:

$$\begin{aligned}\text{Mass fraction of Loss} &= [\text{Mass fraction of SM} - \text{Mass fraction of SEF}] \\ &= [100 \% - 74.2 \%] = 25.8 \%\end{aligned}$$

A graphical mass fraction distribution of SM, SEF and Loss is as follows:

100 % SM	
74.2 % SEF	25.8 % Loss

#### 4.1.2 Water Extraction

The mass fractions of WEF and WEL can be determined from the water extraction step. All extractions were conducted on small scale and the results were extrapolated to the scale of a complete batch experiment; 200 g of dry matter equivalent SEF was used for each determination. The results from the small scale experiments were converted to full scale and the total dry weight of SM is used as a reference in order to get the actual mass fractions of WEF and WEL (Figure 14).

##### 4.1.2.1 Water Extracted Fibers (WEF)

Based on the small scale experiment with 200 g dry SEF, with 1 : 8 solid to liquor ratio, and with 39.7 % SEF solids content, the weight of SEF, the amount of liquor, and the amount of water needed for this extraction can be calculated as follows:

Dry weight of SEF = 200 g ; solids content of SEF = 39.7 %

$$\therefore \text{Weight of SEF} = \left[ \frac{\text{Dry weight of SEF}}{\text{Solids content of SEF}} \right] \times 100$$

$$= \left[ \frac{200 \text{ g}}{39.7 \%} \right] \times 100 = 504 \text{ g}$$

For 200 g dry weight of SEF, 1,600 g of liquor was needed for the extraction (1 : 8 ratio).

Moisture from SEF = [ Weight of SEF - Dry weight of SEF ]

$$= [ 504 \text{ g} - 200 \text{ g} ] = 304 \text{ g}$$

∴ Water needed = [ Weight of liquor - Moisture from SEF ]

$$= [ 1,600 \text{ g} - 304 \text{ g} ] = 1,296 \text{ g}$$

After the extraction, the weight of WEF and its solids content were measured.

Therefore, dry weight of WEF can be calculated and converted to a full scale to get its mass fraction.

Weight of WEF = 348 g ; solids content of WEF = 41.8 %

$$\therefore \text{Dry weight of WEF} = \left[ \frac{\text{Weight of WEF} \times \text{Solids content of WEF}}{100} \right]$$

$$= \left[ \frac{348 \text{ g} \times 41.8 \%}{100} \right] = 145 \text{ g}$$

$$\therefore \text{Total dry weight of WEF} = \left[ \frac{\text{Dry weight of WEF}}{\text{Dry weight of SEF}} \right] \times \text{Total dry weight of SEF}$$

$$= \left[ \frac{145 \text{ g}}{200 \text{ g}} \right] \times 1,421 \text{ g} = 1,032 \text{ g}$$

$$\text{Mass fraction of WEF} = \left[ \frac{\text{Total dry weight of WEF}}{\text{Total dry weight of SM}} \right] \times 100$$

$$= \left[ \frac{1,032 \text{ g}}{1,915 \text{ g}} \right] \times 100 = 53.9 \%$$

A graphical mass fraction distribution of SM, SEF, Loss and WEF is as follows:

100 % SM	
74.2 % SEF	25.8 % Loss
53.9 % WEF	

#### 4.1.2.2 Water Extracted Liquor (WEL)

The water soluble biomass fraction from the water extraction step is called WEL.

This was determined as shown:

$$\begin{aligned} \text{Mass fraction of WEL} &= [ \text{Mass fraction of SEF} - \text{Mass fraction of WEF} ] \\ &= [ 74.2 \% - 53.9 \% ] = 20.3 \% \end{aligned}$$

A graphical mass fraction distribution of SM, SEF, Loss, WEF and WEL is as follows:

100 % SM	
74.2 % SEF	25.8 % Loss
53.9 % WEF	20.3 % WEL

#### 4.1.3 Alkali Extraction

Similar to the water extraction, the fractionation by alkali extraction was carried out on a small scale.

##### 4.1.3.1 Alkali Extracted Fibers (AEF)

By using 50 g dry WEF and 1 : 8 of solid to liquor ratio, 400 g of liquor was used in this extraction. The calculation on the alkali extraction was different from the water extraction due to the addition of sodium hydroxide. The weight of sodium hydroxide was included in the weight of the liquor.

Dry weight of WEF = 50 g ; solids content of WEF = 41.9 %

$$\begin{aligned} \therefore \text{Weight of WEF} &= \left[ \frac{\text{WEF dry weight}}{\text{WEF solids content}} \right] \times 100 \\ &= \left[ \frac{50 \text{ g}}{41.9 \%} \right] \times 100 = 119 \text{ g} \end{aligned}$$

With the addition of 20 % sodium hydroxide based on WEF dry weight, the moisture from WEF and the weight of sodium hydroxide should be considered in the determination of the amount of water required.

$$\begin{aligned} \text{Weight of sodium hydroxide} &= \left[ \frac{20 \% \times \text{Dry weight of WEF}}{100} \right] \\ &= \left[ \frac{20 \% \times 50 \text{ g}}{100} \right] = 10 \text{ g} \end{aligned}$$

$$\begin{aligned} \text{Moisture from WEF} &= [ \text{Weight of WEF} - \text{Dry weight of WEF} ] \\ &= [ 119 \text{ g} - 50 \text{ g} ] = 69 \text{ g} \end{aligned}$$

$$\begin{aligned} \therefore \text{Water needed} &= [ \text{Weight of liquor} - (\text{Moisture of WEF} + \text{Weight of sodium hydroxide}) ] \\ &= [ 400 \text{ g} - (69 \text{ g} + 10 \text{ g}) ] = 321 \text{ g} \end{aligned}$$

After the extraction, the mass fraction of WEF can be converted to the full scale equivalent:

Weight of AEF = 108 g ; solids content of AEF = 30.3 %

$$\begin{aligned} \therefore \text{Dry weight of AEF} &= \left[ \frac{\text{Weight of AEF} \times \text{Solids content of AEF}}{100} \right] \\ &= \left[ \frac{108 \text{ g} \times 30.3 \%}{100} \right] = 33 \text{ g} \end{aligned}$$

$$\begin{aligned} \therefore \text{Total dry weight of AEF} &= \left[ \frac{\text{Dry weight of AEF}}{\text{Dry weight of WEF}} \right] \times \text{Total dry weight of WEF} \\ &= \left[ \frac{33 \text{ g}}{50 \text{ g}} \right] \times 1,032 \text{ g} = 681 \text{ g} \end{aligned}$$

$$\begin{aligned} \text{Mass fraction of AEF} &= \left[ \frac{\text{Total dry weight of AEF}}{\text{Total dry weight of SM}} \right] \times 100 \\ &= \left[ \frac{681 \text{ g}}{1,915 \text{ g}} \right] \times 100 = 35.5 \% \end{aligned}$$

A graphical mass fraction distribution of SM, SEF, Loss, WEF, WEL and AEF is as follows:

100 % SM	
74.2 % SEF	25.8 % Loss
53.9 % WEF	20.3 % WEL
35.5 % AEF	

#### 4.1.3.2 Alkali Extracted Liquor (AEL)

The mass fraction of AEL is obtained by difference.

$$\begin{aligned} \text{Mass fraction of AEL} &= [ \text{Mass fraction of WEF} - \text{Mass fraction of AEF} ] \\ &= [ 53.9 \% - 35.5 \% ] = 18.4 \% \end{aligned}$$

A graphical mass fraction distribution of SM, SEF, Loss, WEF, WEL, AEF and AEL is as follows:

100 % SM	
74.2 % SEF	25.8 % Loss
53.9 % WEF	20.3 % WEL
35.5 % AEF	18.4 % AEL

#### 4.1.4 *Acetic Acid and Ethanol Extractions*

The acetic acid and ethanol extractions were performed using the red oak samples at the severity of  $R_o$  35,000 or  $\text{Log } R_o$  4.54 only. However, the calculation procedure for both extractions is identical to that of the alkali extraction. The mass fractions of AcEF and AcEL or EtEF and EtEL are calculated in an analogous manner.

#### 4.2 **Chemical Composition (i.e., Summative Analysis)**

The biomass fractions of SM, WEF, WEL, AEF, AcEF and EtEF were analyzed to determine cellulose (taken as glucose polymer), other carbohydrates (taken as polymer of all non-glucose sugars) and non-carbohydrates content (Klason and acid soluble lignin and tannins). Figures 15 and 16 show how those samples were transformed to end up with 3 major chemical components. Although no experiment was run on biomass fractions of SEF, Loss, AEL, AcEL and EtEL, their chemical composition can be calculated (see chapter 4.3 for detail). For example, AEF sample from red oak at severity  $R_o$  15,000 ( $\text{Log } R_o$  4.18) is presented in the chemical composition calculation. A dry weight of AEF sample used in this analysis was 0.1005 g.

#### 4.2.1 Non-Carbohydrates (i.e., Lignin and Tannins)

Non-carbohydrates are a combination of Klason and acid soluble lignin and tannins. Klason lignin and tannins data are obtained gravimetrically and acid soluble lignin and tannins data are obtained from the analysis of the acid hydrolyzate by UV/VIS spectrometry at 205 nm wavelength.

$$\begin{aligned} \text{Weight of Klason lignin and tannins} &= \left[ \begin{array}{l} \text{Weight of the filter with} \\ \text{acid hydrolysis residue} \end{array} - \begin{array}{l} \text{Initial weight} \\ \text{of the filter} \end{array} \right] \\ &= [ 23.5070 \text{ g} - 23.4959 \text{ g} ] = 0.0111 \text{ g} \\ \text{Klason lignin and tannins (\%)} &= \left[ \frac{\text{Weight of Klason lignin and tannins}}{\text{Weight of dry AEF sample}} \right] \times 100 \\ &= \left[ \frac{0.0111 \text{ g}}{0.1005 \text{ g}} \right] \times 100 = 11.04 \% \end{aligned}$$

Absorbance measurements and dilution factors are used to calculate the percentage of acid soluble lignin in the samples. In addition, the amounts of 2-F and HMF from the HPLC Carbo-H chromatogram are applied in the calculation in accordance with the procedure of Kaar et al.<sup>23</sup>

$$\begin{aligned} \text{Acid soluble lignin and tannins (\%)} &= \left[ \frac{\left[ \frac{\text{Abs}}{b} \times [ \text{DF} \times \text{V} ] \right] - [ A_{2\text{-F}/\text{HMF}} \times \text{V} \times [ M_{2\text{-F}} + M_{\text{HMF}} ] ]}{[ A_{\text{ASL}} \times \text{W} ]} \right] \times 100 \\ &= \left[ \frac{\left[ \frac{0.1042}{1 \text{ cm}} \times [ 10 \times 100 \text{ mL} ] \right] - \left[ 19.14 \text{ mL mg}^{-1} \text{ cm}^{-1} \times 100 \text{ mL} \times \left[ \frac{1.996 \mu\text{g mL}^{-1} + 6.569 \mu\text{g mL}^{-1}}{1 \times 10^3 \mu\text{g mg}^{-1}} \right] \right]}{[ 110 \text{ mL mg}^{-1} \text{ cm}^{-1} \times 1.005 \text{ g} \times 1 \times 10^3 \text{ mg g}^{-1} ]} \right] \times 100 \\ &= 0.79 \% \end{aligned}$$

Where;

Abs = absorbance of acid soluble lignin and tannins at 205 nm

b = length of the UV/VIS cell, cm

DF = dilution factor

V = hydrolyzate volume, mL

$A_{2-F/HMF} = 19.14 \text{ mL mg}^{-1} \text{ cm}^{-1}$  = absorptivity of an equal mixture of 2-F and HMF at 205 nm

$M_{2-F}$  = concentration of 2-F in hydrolyzate  
(amount from HPLC Carbo-H),  $\mu\text{g mL}^{-1}$

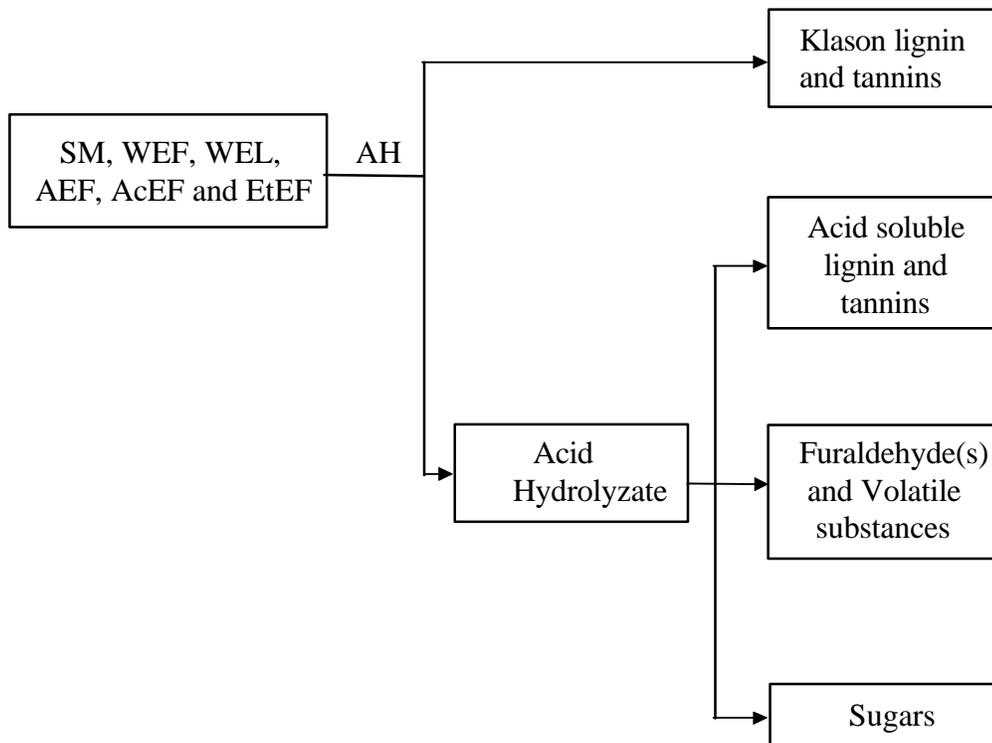
$M_{HMF}$  = concentration of 2-HMF in hydrolyzate  
(amount from HPLC Carbo-H),  $\mu\text{g mL}^{-1}$

$A_{ASL} = 110 \text{ mL mg}^{-1} \text{ cm}^{-1}$  = absorptivity of acid soluble lignin and tannins at 205 nm

W = weight of dry sample, g

Then, the actual amount of non-carbohydrates in the sample is the sum of Klason and acid soluble lignin and tannins.

$$\begin{aligned} \therefore \text{Non-carbohydrates (\%)} &= [ \text{Klason lignin and tannins} + \text{Acid soluble lignin and tannins} ] \\ &= [ 11.04 \% + 0.79 \% ] = 11.83 \% \end{aligned}$$



AH - acid hydrolysis process

Figure 15. Schematic flow chart of the acid hydrolysis (AH) and the constituent analysis of the acid hydrolyzate used for determining the chemical composition of biomass fractions.

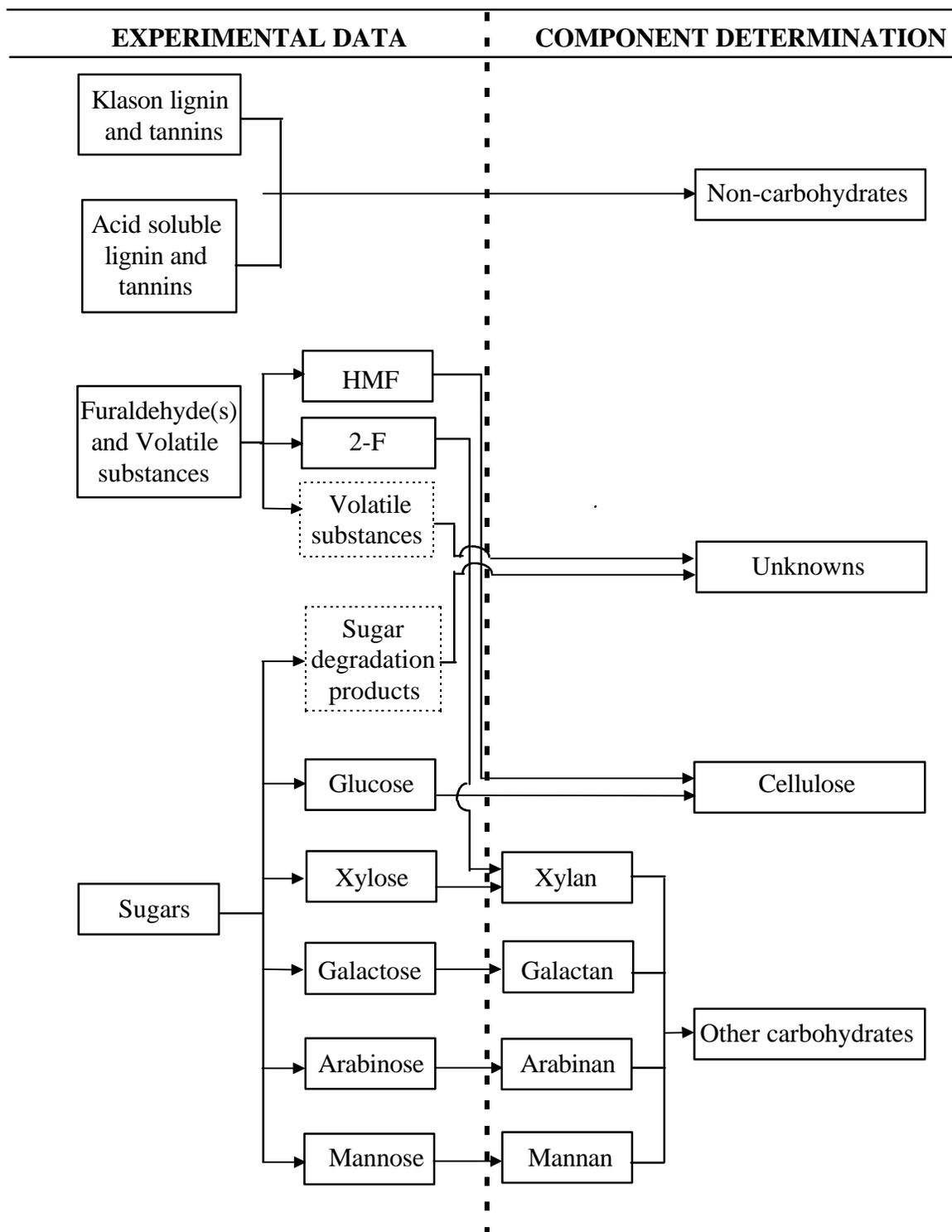


Figure 16. Schematic illustration of the data interpretation protocol: non-carbohydrates, cellulose and other carbohydrates are the components determined directly from experimental data.

#### 4.2.2 Cellulose

The percentages of HMF (from the furaldehyde(s) determination) and glucose (from sugar analysis) are combined to calculate the amount of glucan in the sample.

Glucan is taken to be synonymous with cellulose.

Amount of HMF (from HPLC Carbo-H) =  $6.569 \mu\text{g mL}^{-1}$ .

$$\begin{aligned} \text{HMF (\%)} &= \left[ \frac{\text{Amount of HMF}}{\left[ \frac{\text{Weight of dry sample}}{\text{Volume of hydrolyzate}} \right]} \right] \times 100 \\ &= \left[ \frac{6.569 \mu\text{g mL}^{-1} \times 1 \times 10^6 \text{ g } \mu\text{g}^{-1}}{\left[ \frac{0.1005 \text{ g}}{100 \text{ mL}} \right]} \right] \times 100 = 0.65 \% \end{aligned}$$

From the HPLC Carbo-P chromatogram, area of glucose = 11 236 152, Response factor (RF) of glucose = 0.850, area of erythritol = 952 189 and amount of erythritol (internal standard) =  $4.00 \text{ g L}^{-1}$ .

$$\begin{aligned} \text{Glucose (\%)} &= \left[ \frac{\left[ \frac{\text{Area of glucose} \times \text{RF of glucose}}{\text{Area of erythritol}} \right] \times \text{Amount of erythritol}}{\left[ \frac{\text{Weight of dry sample}}{\text{Volume of hydrolyzate}} \right] \times \text{Dilution factor}} \right] \times 100 \\ &= \left[ \frac{\left[ \frac{11\,236\,152 \times 0.850}{4\,952\,189} \right] \times 4.00 \text{ g L}^{-1} \times 1 \times 10^{-3} \text{ L mL}^{-1}}{\left[ \frac{0.1005 \text{ g}}{100 \text{ mL}} \right] \times 10} \right] \times 100 \\ &= 76.76 \% \end{aligned}$$

Then, further calculation is done to get the percentage of cellulose in the sample.

$$\begin{aligned} \therefore \text{Cellulose (\%)} &= [ (\text{Glucose} \times 0.9) + (\text{HMF} \times 1.473) ] \\ &= [ (76.76 \% \times 0.9) + (0.65 \% \times 1.473) ] = 70.04 \% \end{aligned}$$

#### 4.2.3 Other Carbohydrates

All sugars that are determined in this analysis, excluding glucose, are classified as monosaccharides from other carbohydrates. 2-F is also assumed to be derived entirely from other carbohydrates.

$$\text{Amount of 2-F (from HPLC Carbo-H)} = 1.996 \mu\text{g mL}^{-1}$$

$$\begin{aligned} \text{2-F (\%)} &= \left[ \frac{\text{Amount of 2-F}}{\left[ \frac{\text{Weight of dry sample}}{\text{Volume of hydrolyzate}} \right]} \right] \times 100 \\ &= \left[ \frac{1.996 \mu\text{g mL}^{-1} \times 1 \times 10^{-6} \text{ g } \mu\text{g}^{-1}}{\left[ \frac{0.1005 \text{ g}}{100 \text{ mL}} \right]} \right] \times 100 = 0.20 \% \end{aligned}$$

From HPLC Carbo-P , area of xylose = 11 236 152 and RF of xylose = 0.964.

$$\text{Xylose (\%)} = \left[ \frac{\left[ \frac{\text{Area of xylose} \times \text{RF of xylose}}{\text{Area of erythritol}} \right] \times \text{Amount of erythritol}}{\left[ \frac{\text{Weight of dry sample}}{\text{Volume of hydrolyzate}} \right] \times \text{Dilution factor}} \right] \times 100$$

$$\begin{aligned}
&= \left[ \frac{405\,382 \times 0.964}{4\,952\,189} \right] \times 4.00 \text{ g L}^{-1} \times 1 \times 10^{-3} \text{ L mL}^{-1} \\
&= \left[ \frac{\left[ \frac{0.1005 \text{ g}}{100 \text{ mL}} \right] \times 10}{\left[ \frac{405\,382 \times 0.964}{4\,952\,189} \right] \times 4.00 \text{ g L}^{-1} \times 1 \times 10^{-3} \text{ L mL}^{-1}} \right] \times 100 \\
&= 3.14 \%
\end{aligned}$$

Area of galactose (from HPLC Carbo-P) = 0

Area of arabinose (from HPLC Carbo-P) = 0

Area of mannose (from HPLC Carbo-P) = 0

Percentages of galactose, arabinose and mannose are also determined using the same formula as xylose. However for the area of sugar and its RF, their values should be used respectively. Finally, other carbohydrates are calculated as follow:

$$\begin{aligned}
\therefore \text{Other carbohydrates} &= [ \text{Xylan} + \text{Galactan} + \text{Arabinan} + \text{Mannan} ] \\
(\%) &= [ [(\text{xylose} \times 0.88) + (2\text{-F} \times 1.375)] + (\text{galactose} \times 0.9) \\
&\quad + (\text{arabinose} \times 0.88) + (\text{mannose} \times 0.9) ] \\
&= [ (3.14 \% \times 0.88) + (0.20 \% \times 1.375) + (0 \% \times 0.9) \\
&\quad + (0 \% \times 0.88) + (0 \% \times 0.9) ] \\
&= [ 3.04 \% + 0 \% + 0 \% + 0 \% ] = 3.04 \%
\end{aligned}$$

#### 4.2.4 Unknowns

The sum of all chemical components (cellulose, other carbohydrates and non-carbohydrates) must not be > 100 %. Undetermined chemical components (i.e., unknowns) are classified as any chemical component that has escaped detection during chemical analysis.

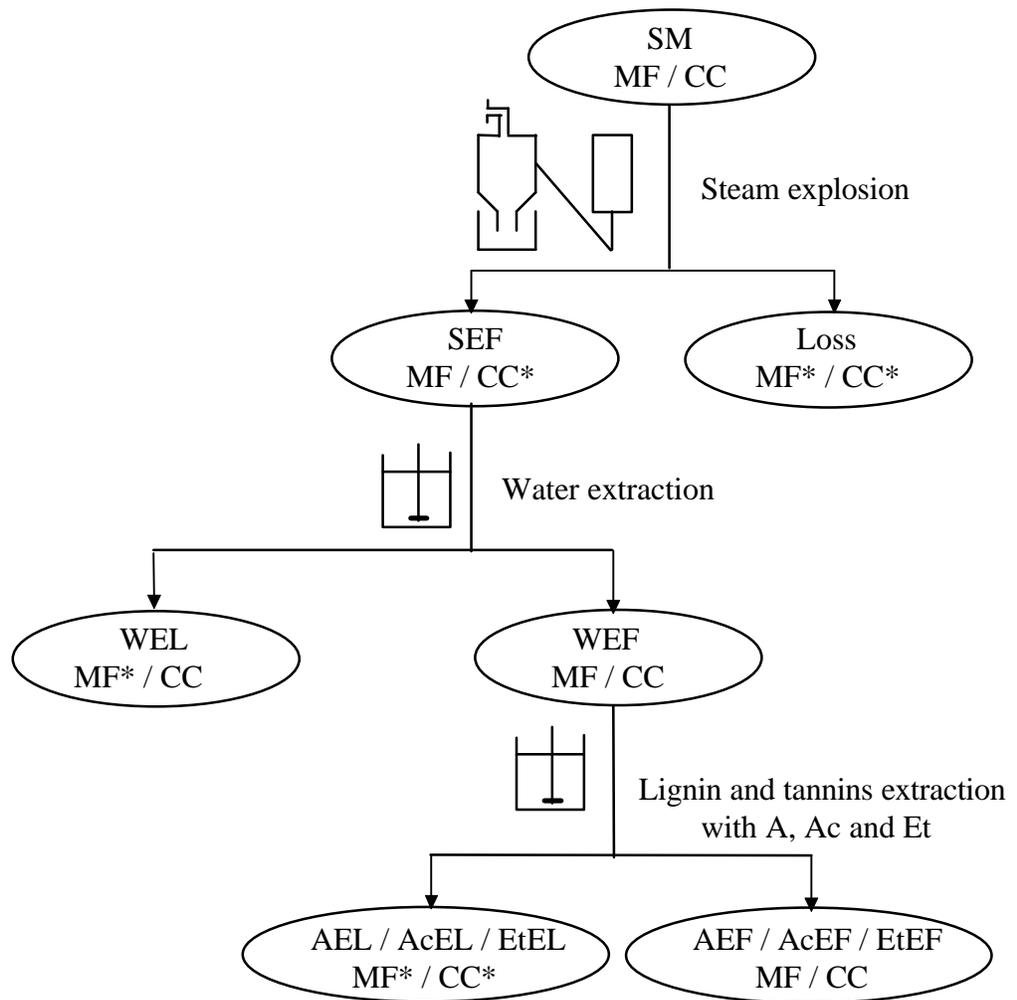
$$\begin{aligned}
\therefore \text{Unknowns} (\%) &= [ 100 \% - (\text{Cellulose} + \text{Other carbohydrates} + \text{Non-carbohydrates}) ] \\
&= [ 100 \% - (70.04 \% + 3.04 \% + 11.83 \%) ] = 15.09 \%
\end{aligned}$$

Table 6. Mass fraction data of red oak at  $R_o$  15,000.

Biomass fraction	Mass fraction, % of SM
SM	100.0
SEF	74.2
Loss	25.8
WEF	53.9
WEL	20.3
AEF	35.5
AEL	18.4

Table 7. Chemical composition of red oak at R<sub>o</sub> 15,000.

Biomass fraction	Chemical composition of each chemical component, % of biomass fraction			
	Cellulose	Other carbohydrates	Non-carbohydrates	Unknowns
SM	35.46	18.76	28.95	16.83
WEF	45.73	2.84	38.08	13.35
WEL	4.35	47.82	16.49	31.34
AEF	70.04	3.04	11.83	15.09



- MF - experimental mass fraction
- MF\* - calculated mass fraction
- CC - experimental chemical composition
- CC\* - calculated chemical composition
- A - alkali
- Ac - acetic acid
- Et - ethanol

Figure 17. Schematic mass fraction and chemical composition flow chart including experimental and calculation data.

### 4.3 Combination of Mass Fractionation and Summative Analysis Data

Steam explosion and extraction steps provide information about mass fraction (Table 5). The chemical composition of each individual mass fraction is determined from the chemical analysis (Table 6). However, not all biomass fractions can be accounted for as shown in Figure 17. For example, a Loss fraction cannot be analyzed at all. Therefore, mass fractionation and summative analysis (chemical composition) data can be combined to obtain additional information.

#### 4.3.1 Starting Material (SM)

The mass fraction (MF) and chemical composition (CC) of SM is determined in accordance with Figure 17. The data in Table 8 will be used when normalization calculation is done for other biomass fractions.

#### 4.3.2 Water Extracted Liquor (WEL)

In WEL, 4.35 % of total biomass fraction was found to be cellulose (from chemical composition data). However, since WEL comprises only 20.3 % of total SM, a normalization calculation should be applied to convert the chemical composition of WEL and its contribution to the mass balance into its relative content. In the example, the product of cellulose content of WEL and the mass fraction of WEL is equal to how much of the cellulose was present in the WEL fraction.

$$\begin{aligned} \text{Normalized cellulose of WEL} &= \left[ \frac{\text{Cellulose of WEL}}{\text{Total CC of WEL}} \right] \times \text{Mass fraction of WEL} \\ &= \left[ \frac{4.35 \%}{100.00 \%} \right] \times 20.3 \% = 0.88 \% \end{aligned}$$

Now we know that 0.88 % of total cellulose in SM was present in WEL. All other chemical components (other carbohydrates, non-carbohydrates and unknowns) of SM present in WEL are calculated based on the above formula and summarized in Table 9.

An example of how the normalization of the WEL chemical composition data are to be viewed is illustrated in Figure 18. The chemical composition gives the percentage of each individual chemical component based on summative analysis. Normalization of the chemical composition provides information on the specific component in specific mass fraction.

#### 4.3.3 *Alkali Extracted Fibers (AEF)*

In the AEF fraction, the other carbohydrates component is chosen for the example calculation. The other carbohydrates content of AEF was 3.04 % by chemical analysis; its mass fraction was 35.5 %.

$$\begin{aligned} \text{Normalized other carbohydrates of AEF} &= \left[ \frac{\text{Other carbohydrates of AEF}}{\text{Total CC of AEF}} \right] \times \text{Mass fraction of AEF} \\ &= \left[ \frac{3.04 \%}{100.00 \%} \right] \times 35.5 \% = 1.08 \% \end{aligned}$$

#### 4.3.4 *Water Extracted Fibers (WEF)*

The example calculation for the normalization of the WEF involves non-carbohydrates. The mass fraction of WEF is 53.9 % and its non-carbohydrates was found to be 38.08 %.

$$\begin{aligned} \text{Normalized non-carbohydrates of WEF} &= \left[ \frac{\text{Non-carbohydrates of WEF}}{\text{Total CC of WEF}} \right] \times \text{Mass fraction of WEF} \\ &= \left[ \frac{38.08 \%}{100.00 \%} \right] \times 53.9 \% = 20.53 \% \end{aligned}$$

Table 8. Chemical composition of starting material (SM).

Chemical component of SM	Chemical composition, % of SM
Cellulose	35.46
Other carbohydrates	18.76
Non-carbohydrates <sup>1)</sup>	28.95
Unknowns	16.83
Total chemical composition of SM	100.00

1) Includes all acid insoluble, primarily lignin and tannins

Table 9. Normalized chemical composition of WEL

Chemical component of WEL	Chemical composition <sup>1)</sup> , % of biomass fraction	Normalized chemical composition <sup>2)</sup> , % of SM
Cellulose	4.35	0.88
Other carbohydrates	47.82	9.71
Non-carbohydrates	16.49	3.35
Unknowns	31.34	6.36
	100.00	20.30

1) Chemical composition of WEL based on chemical analysis

2) Chemical composition of WEL (normalized to SM)

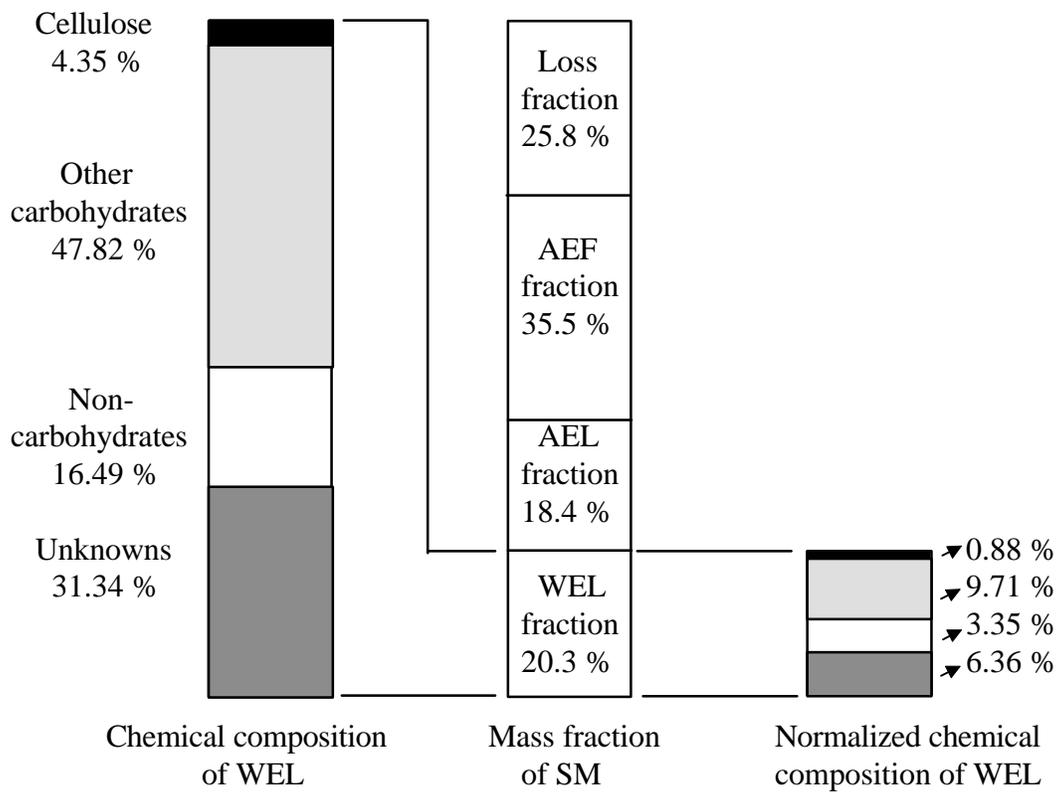


Figure 18. Schematic presentation of chemical composition, mass fraction and normalized chemical composition data for the case of the WEL fraction.

Table 10. Normalized chemical composition of AEF

Chemical component of AEF	Chemical composition <sup>1)</sup> , % of biomass fraction	Normalized chemical composition <sup>2)</sup> , % of SM
Cellulose	70.05	24.84
Other carbohydrates	3.04	1.08
Non-carbohydrates	11.84	4.24
Unknowns	15.07	5.34
	100.00	35.50

1) Chemical composition of AEF based on chemical analysis

2) Chemical composition of AEF (normalized to SM)

Table 11. Normalized chemical composition of WEF

Chemical component of WEF	Chemical composition <sup>1)</sup> , % of biomass fraction	Normalized chemical composition <sup>2)</sup> , % of SM
Cellulose	45.73	24.65
Other carbohydrates	2.84	1.52
Non-carbohydrates	38.08	20.53
Unknowns	13.35	7.20
	100.00	53.90

1) Chemical composition of WEF based on chemical analysis

2) Chemical composition of WEF (normalized to SM)

#### 4.3.5 *Alkali Extracted Liquor (AEL)*

Unlike the previous calculations, no chemical composition data are available for AEL. However, one can calculate the chemical composition of AEL based on AEF and WEF data. The unknowns component is used in the example. Either normalized or non-normalized, both chemical compositions can be calculated by difference.

$$\begin{aligned} \text{Normalized unknowns of AEL} &= \left[ \begin{array}{c} \text{Normalized unknowns} \\ \text{of WEF} \end{array} - \begin{array}{c} \text{Normalized unknowns} \\ \text{of AEF} \end{array} \right] \\ &= [ 7.20 \% - 5.34 \% ] = 1.86 \% \end{aligned}$$

#### 4.3.6 *Loss*

The last calculation for summative analysis is concerned with the Loss fraction. Normalized chemical composition of the Loss fraction is illustrated with cellulose in the example. Similar to AEL, both chemical compositions can be calculated by difference.

$$\begin{aligned} \text{Normalized cellulose of Loss} &= \left[ \begin{array}{c} \text{Normalized} \\ \text{cellulose of SM} \end{array} - \left( \begin{array}{c} \text{Normalized} \\ \text{cellulose of WEL} \end{array} + \begin{array}{c} \text{Normalized} \\ \text{cellulose of WEF} \end{array} \right) \right] \\ &= [ 35.46 \% - ( 0.88 \% + 24.65 \% ) ] = 9.93 \% \end{aligned}$$

#### 4.3.7 *Outcomes Assessment of Mass Fractionation and Summative Analysis*

By combining mass fractionation and summative analysis data, the distribution of cellulose, other carbohydrates, non-carbohydrates and unknowns across individual mass fractions is derived in normalized form. This combination represents an outcomes assessment that provides quantitative information on biomass component-flow through the steam explosion process.

Table 12. Normalized chemical composition of AEL

Chemical component of AEL	Chemical composition <sup>1)</sup> , % of biomass fraction	Normalized chemical composition <sup>2)</sup> , % of SM
Cellulose	-0.95	-0.18
Other carbohydrates	2.46	0.45
Non-carbohydrates	88.44	16.27
Unknowns	10.05	1.86
	100.00	18.40

1) Chemical composition of AEL based on difference calculation of WEF - AEF

2) Chemical composition of AEL (normalized to SM)

Table 13. Normalized chemical composition of Loss

Chemical component of Loss	Chemical composition <sup>1)</sup> , % of biomass fraction	Normalized chemical composition <sup>2)</sup> , % of SM
Cellulose	38.46	9.93
Other carbohydrates	29.18	7.53
Non-carbohydrates	19.66	5.07
Unknowns	12.69	3.27
	100.00	25.80

1) Chemical composition of Loss based on chemical analysis

2) Chemical composition of (normalized to SM)

Table 14. Outcomes assessment of mass fractionation and summative analysis of red oak at R<sub>o</sub> 15,000.

Chemical composition	Mass fractions, % of SM				Total, % of SM
	WEL	AEL	AEF	Loss	
Cellulose	0.88	-0.18	24.84	9.93	35.47
Other carbohydrates	9.71	0.45	1.08	7.53	18.77
Non-carbohydrates	3.35	16.27	4.24	5.07	28.93
Unknowns	6.36	1.86	5.34	3.27	16.83
<b>Total, % of SM</b>	<b>20.30</b>	<b>18.40</b>	<b>35.50</b>	<b>25.80</b>	<b>100.00</b>

#### 4.4 Clean Fractionation

A “Clean Fractionation” table and diagram can be created from the outcomes assessment data (Table 14). The mass retention at each process step and in each mass fraction stage is calculated based on the normalized chemical composition data. This is illustrated for the example of cellulose in the following calculation. The result from this calculation is listed in Table 15 and presented in graphical form in Figure 19.

$$\begin{aligned} \text{Mass retention of cellulose in stage 1} &= \left[ \frac{\text{Normalized cellulose of SM}}{\text{Cellulose of SM}} \right] \times 100 \\ \text{(SM)} &= \left[ \frac{35.47 \%}{35.47 \%} \right] \times 100 = 100 \% \end{aligned}$$

$$\begin{aligned} \text{Mass retention of cellulose in stage 2} &= \left[ \frac{\text{Normalized cellulose of SEF}}{\text{Cellulose of SM}} \right] \times 100 \\ \text{(SEF)} &= \left[ \frac{25.54 \%}{35.47 \%} \right] \times 100 = 72.00 \% \end{aligned}$$

$$\begin{aligned} \text{Mass retention of cellulose in stage 3} &= \left[ \frac{\text{Normalized cellulose of WEF}}{\text{Cellulose of SM}} \right] \times 100 \\ \text{(WEF)} &= \left[ \frac{24.66 \%}{35.47 \%} \right] \times 100 = 69.52 \% \end{aligned}$$

$$\begin{aligned} \text{Mass retention of cellulose in stage 4} &= \left[ \frac{\text{Normalized cellulose of AEF}}{\text{Cellulose of SM}} \right] \times 100 \\ \text{(AEF)} &= \left[ \frac{24.84 \%}{35.47 \%} \right] \times 100 = 70.02 \% \end{aligned}$$

Table 15. Clean fractionation of red oak at R<sub>0</sub> 15,000.

Chemical component	Mass retention, % of SM			
	Stage 1 (SM)	Stage 2 (SEF)	Stage 3 (WEF)	Stage 4 (AEF)
Cellulose	100.00	72.00	69.52	70.02
Other carbohydrates	100.00	59.88	8.15	5.75
Non-carbohydrates	100.00	82.47	70.90	14.65
Unknowns	100.00	80.57	42.78	31.73

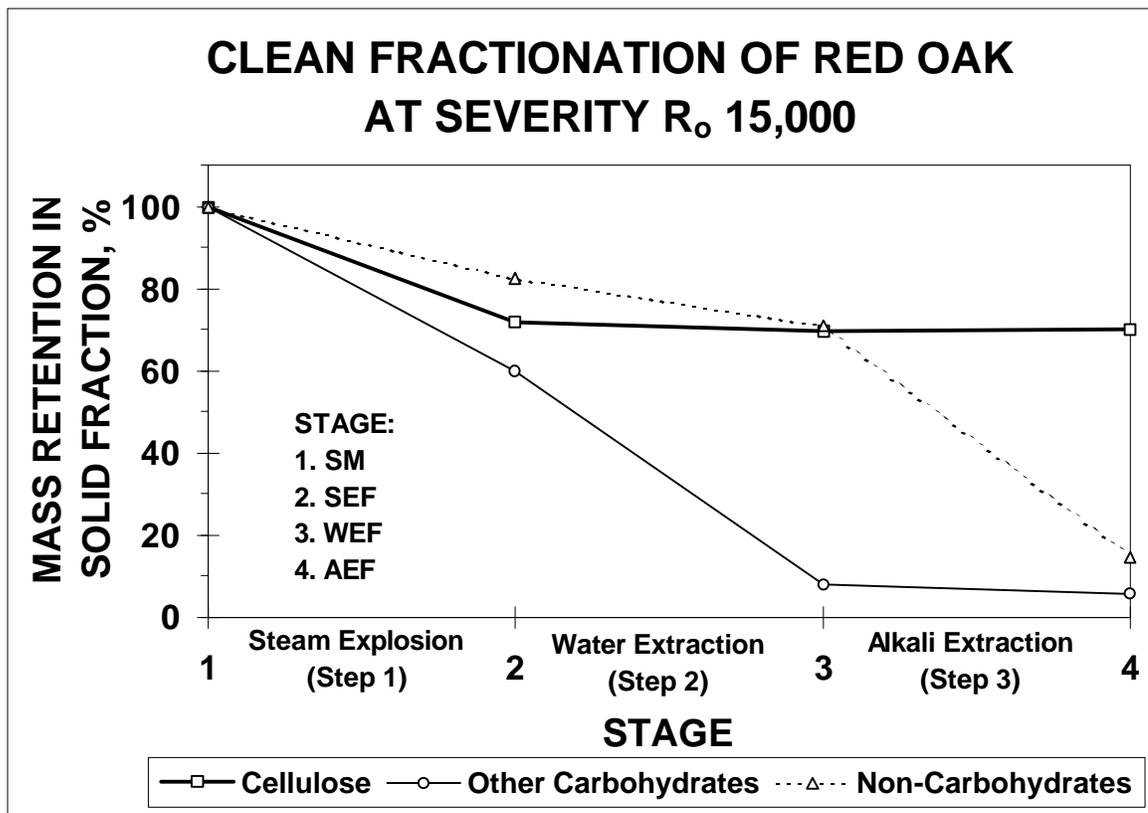


Figure 19. Plot of the clean fractionation for red oak at  $R_0$  15,000.

From Table 15, the final calculation is to get a normalized clean fractionation slope of each step. It could be obtained by subtracting between the 2 stages. Using the other carbohydrates fraction as an example ;

$$\begin{aligned} \text{Slope of other carbohydrates at step 1 per 1 step} &= \left[ \begin{array}{l} \text{Mass retention of other} \\ \text{carbohydrates at stage 1} \end{array} - \begin{array}{l} \text{Mass retention of other} \\ \text{carbohydrates at stage 2} \end{array} \right] \\ &= [ 100.00 \% - 59.88 \% ] = 40.12 \% \end{aligned}$$

$$\begin{aligned} \text{Slope of other carbohydrates at step 2 per 1 step} &= \left[ \begin{array}{l} \text{Mass retention of other} \\ \text{carbohydrates at stage 2} \end{array} - \begin{array}{l} \text{Mass retention of other} \\ \text{carbohydrates at stage 3} \end{array} \right] \\ &= [ 59.88 \% - 8.15 \% ] = 51.73 \% \end{aligned}$$

$$\begin{aligned} \text{Slope of other carbohydrates at step 3 per 1 step} &= \left[ \begin{array}{l} \text{Mass retention of other} \\ \text{carbohydrates at stage 3} \end{array} - \begin{array}{l} \text{Mass retention of other} \\ \text{carbohydrates at stage 4} \end{array} \right] \\ &= [ 8.15 \% - 5.75 \% ] = 2.40 \% \end{aligned}$$

A table of clean fractionation slope parameters is created as shown in Table 16.

Table 16. Clean fractionation slope parameters of red oak at R<sub>0</sub> 15,000.

Chemical component	Slope parameter, % of mass retention per step		
	Step 1 (steam explosion)	Step 2 (water extraction)	Step 3 (alkali extraction)
Cellulose	28.00	2.48	-0.50
Other carbohydrates	40.12	51.73	2.40
Non-carbohydrates	17.53	11.57	56.25
Unknowns	19.43	37.79	11.05