1-1-2012

Optimization of Hydrothermal Pretreatment and Membrane Filtration Processes of Various Feedstocks to Isolate Hemicelluloses for Biopolymer Applications

Badamkhand Sukhbaatar

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by

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A Dissertation
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
in Forest Resources
in the Department of Forest Products

Mississippi State, Mississippi

December 2012

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Title of Study: Optimization of hydrothermal pretreatment and membrane filtration processes of various feedstocks to isolate hemicelluloses for biopolymer applications

Pages in Study: 108

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Hemicelluloses (HC) are the second most abundant plant polysaccharides after cellulose, constituting 25-30% of plant materials. In spite of their abundance, HC are not effectively utilized. Recently, considerable interest has been directed to HC-based biomaterials because of their high oxygen barrier properties, which has potential in food packaging applications. In this study, HC were extracted from sugarcane bagasse and southern yellow pine using a hydrothermal technique which utilizes hot compressed water without catalyst. The parameters affecting the yield of extracted HC such as temperature, time and pressure, were tested and optimized. Eighty four percent of xylose was extracted from sugarcane bagasse at the optimum condition, 180 ^{o}C 30 min and 1 MPa pressure. In the case of southern yellow pine, 79% of the mannose was extracted at 190 ^{o}C for 10 min and 2 MPa pressure. Concentration and isolation of HC from bagasse and southern yellow pine HC extract were performed by membrane filtration and freeze drying systems. Isolated HC were characterized by FT-IR and ^{13}C NMR techniques and used as a starting material for film preparation. Films were prepared in

0/100, 50/50, 60/40, 70/30 and 80/20% ratios of HC and sodium carboxymethylcellulose (CMC). Thirty five percent of sorbitol (w/w of HC and CMC weight) was also added as a plasticizer. Films were evaluated by measuring water absorption, water vapor permeability (WVP), tensile property and oxygen barrier capability. At 55% relative humidity (RH) and 25 ^{o}C the water absorption of both sugarcane bagasse and southern yellow pine HC-based films tended to increase as HC content increased. The lowest WVP of sugarcane bagasse ($3.84e^{-12}$ g/Pa h m) and southern yellow pine HC films ($2.18e^{-12}$ g/Pa h m) were determined in 60/40 HC/CMC films. Tensile test results showed that as HC content increases the Young's modulus decreases, deflection at maximum load and percentage of strain at break increase. It implies that the film properties are changing from stiff to elastic. The oxygen permeability for 60/40 bagasse HC/CMC film was 0.005265 cc μm / (m^2 day kPa) and for 70/30 pine HC/CMC film was 0.007570 cc μm / (m^2 day kPa).

Keywords: Hemicelluloses, Biopolymers, Membrane Filtration, Food Packaging materials

DEDICATION

I would like to dedicate this work to my loving parents Dambadarjaa Sukhbaatar and Nyam Sanjaachultem, and my son Badral Zundui.

ACKNOWLEDGEMENTS

I would like to express my appreciation to my advisor Dr. El Barbary Hassan for his sincere help and guidance during this program. I would also like to state my gratitude to my committee members Dr. Moon G. Kim, Dr. Philip H. Steele, and Leonard L. Ingram Jr. for their support and guidance as committee members. I also would like to express my appreciation for assistance given by Qi Li, Dr. Hussein Abouyousef, colleagues and friends in Forest Products Department.

Finally, I would like to extend special appreciation to the most important people in my life: my father Dambadarjaa Sukhbaatar, my loving mother Nyam Sanjaachultem, my son Badral Zundui, and my brothers Sukhbaatar Bayarmanlai and Sukhbaatar Shirbazar and his family for their endless love and invaluable support and enthusiasm.

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CHAPTER I

INTRODUCTION

1.1 Background

Non-degradable plastics and polymers has become ubiquitous the product in our everyday life. Currently, 250 million tons of plastics from petroleum naphtha fraction are being produced (Rao, 2010), and this number is expected to increase. Plastics can be used in food packaging, textile, electronics, vehicle parts, coating, construction, bottles, containers and many other products. They are mostly non-degradable and can pollute waterways, endanger marine life and cause litter problems (Davis and Song, 2006). Plastic waste last hundreds of years in landfills and are a potential source of harmful chemicals when they break down (Curlee, 1991; Kale et al., 2007). Burning plastic waste is not a good material for disposal due to emission of toxic compounds (Singleton, 2001; Stein, 2002). Eighteen countries, for example, France, Italy, India, Bangladesh and others have banned or introduced a fee for the use of plastic bags. The United States has experienced a similar inclination in recent years. According to *USA Today*, the state of Hawaii has banned the use of non-biodegradable plastic bags in retail stores as well as paper bags that are not composed of at least 40 percent recycled paper. This ban becomes effective in July of 2015 (Bly, 2012). Several cities such as Seattle, Washington and Washington, DC have joined this movement. Eventually, this movement is expected to span the entire world. Thus, an urgent need of alternative- biodegradable polymers for packaging to replace plastics is essential.

Biopolymers are a type of polymers derived mostly from renewable sources. They are generally biodegradable and environmentally friendly. Biopolymers can be produced by chemical modification of biomaterials or by chemical synthesis. However, degradability of these biopolymers can be negatively affected. Mainly biopolymers are being used for plastic cups, straws, plastic silverware, packaging and various medical applications such tablet capsules and surgical implants.

Lignocellulosic biomass is a potential source of biopolymers and chemicals because it is renewable, abundant, and potentially available at lower costs than other agricultural crops (Sánchez and Cardona, 2008). Lignocellulosic biomass consists of complex polymer mixtures of cellulose, HC, lignin, and small amounts of a blend of extractives. Cellulose and HC are carbohydrates that constitute the majority of lignocellulosic biomass. Cellulose and HC can be used for diverse biopolymer production. For example, cellulose is the main biopolymer of lignocellulosic biomass and the Applications of cellulose-derived polymers have been known for many years. Cellulose is used in various cellophane packaging materials. Some of these include the packaging material for cigarettes and for various food products such as confectionary products, cereal, and crackers (Moon et al., 2011; Wibowo et al., 2006). Cellulose is also used in preparing the polymers such as rayon, lyocell, and bamboo textiles which are used for making clothes (Teli and Sheikh, 2012). Moreover, it is the main polymer used for the production of second generation bio-fuel (ethanol).

HC are another main component of lignocellulosic biomass. HC comprise of a group of non-crystalline hexoses including mannose, glucose and galactose, and pentoses including xylose and arabinose. Numbers of studies and developments on new chemicals and materials from cellulose and lignin have been conducted. However, HC have

remained relatively unexplored until quite recently. HC have numerous potential applications those can be used in the food industries, pharmaceuticals and in packaging industries.

Lignocellulosic biomass is promising alternative feedstock for bioethanol production. Due to the extensive interaction of cellulose, HC and lignin, and protective feature of lignin, the fermentable sugars are not readily accessible to the hydrolysates. Alternatively, removal of HC will create the pores that allow enzymes access easier to the cellulose. This will enhance the hydrolysis process of bioethanol production. Although bioethanol production is outside the scope of this study, byproducts from this study, HC removed residue can be a excellent source for bioethanol production.

For a range of practical application, HC need to be separated from the lignocellulosic components. Due to the complex structure of lignocellulosic biomass, the separation of HC is complicated. Therefore, the objectives of this project are to develop (1) efficient pretreatment and (2) separation methods for isolation of hemicellulosic components in an inexpensive manner from agricultural residue and forestry biomass prior to ethanol or chemical production, and use them as starting material for (3) biopolymer applications.

1.2 Composition of lignocellulosic biomass

Lignocellulosic biomass is an abundant and renewable material that offers numerous products such as energy, chemicals and polymeric materials. It has complex structure consisting of three main components including cellulose, HC and lignin (Figure 1.1) and small amounts of extractives. The structure can be illustrated as a cellulose chain embedded in a cross linked matrix of HC and surrounded by lignin. Due to the interaction

between cellulose, HC and lignin, and protective feature of lignin the hydrolysis process is limited.

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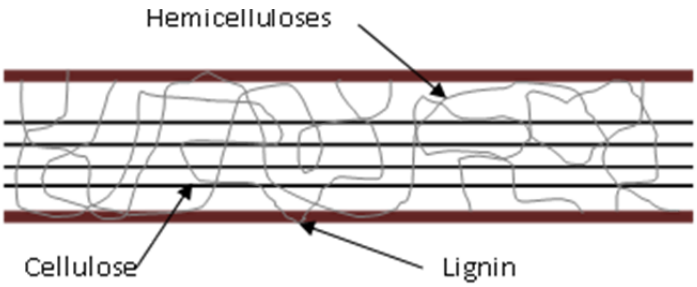


Figure 1.1 Structure of lignocellulosic biomass

1.2.1 Cellulose

Cellulose is the most abundant organic material on earth (Sjöström, 1993). Approximately 40-45% of the dry substance in most wood species is cellulose, located predominantly in the secondary cell wall (Ebringerová and Heinze, 2000). Cellulose is found in plants as microfibrils with dimension of 2-20 nm in diameter and 100- 40000 nm in length (www.btinternet.com). It is a linear homopolysaccharide composed of highly uniform β-(1→4) linked glucose units (Figure 1.2). Cellulose molecules form inter- and intra-molecular hydrogen bonds between each other that allow them to bundle

and aggregate together in the form of microfibrils. The regions with highly ordered microfibrils are termed crystalline region that alternates with less ordered region which is called the amorphous region. As a result, microfibrils build up to fibrils and further increase size to cellulose fibers with high tensile strength and stable chemical properties. The degree of polymerization (DP) of cellulose can reach as high as 10000 units depending on the feature of biomass (Sjöström, 1993). Cellulose is insoluble in water, dilute acids or alkalis at ambient temperature, however at moderate or high temperatures it hydrolyzes to D-glucose and its oligomers of various DP.

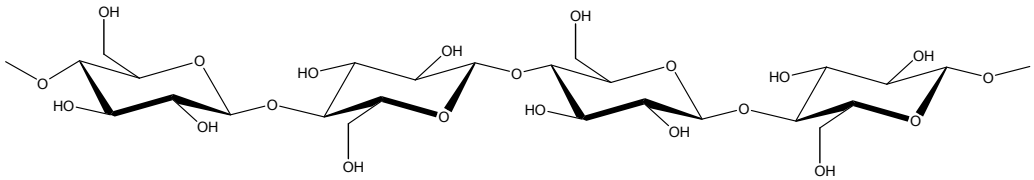


Figure 1.2 Structure of cellulose chain.

1.2.2 Hemicelluloses

HC are the second most abundant, heteropolysaccharides representing in general 15-35% of plant cell wall (Gírio et al., 2010). They are easily hydrolysable by acids into the monomeric sugars such as acetic, D-glucose (D-Glc), D-mannose (D-Man), D-galactose (D-Gal), D-xylose (D-Xyl), L-arabinose (L-Ara) and some organic acids such as D-glucuronic (D-GlcA), 4-O-methyl-D-glucuronic (4-O-Me-D-GlcA) and D-galacturonic acids (D-GalA) (Figure 1.3). HC have a relatively lower degree of polymerization (~200) and more branched structure than does cellulose.

The composition and the structure of HC in softwood and hardwood are considerably different from each other. Also, HC in various hardwood or softwood

species differ from each other qualitatively and quantitatively. Generally, HC can be divided in several types such as galactoglucomannan, arabinoglucuruonoxylan, arabinogalactan, glucuronoxylan and glucomannan.

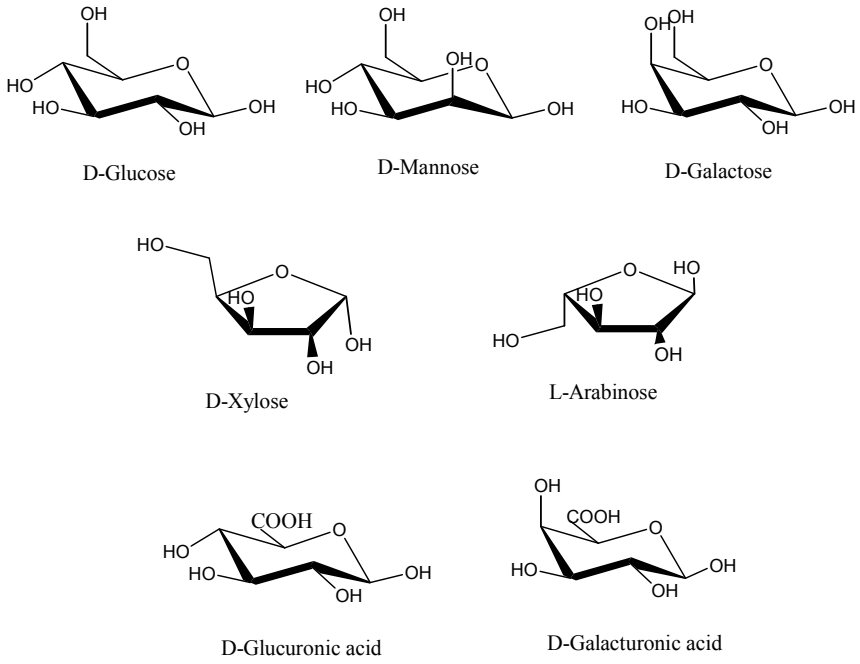


Figure 1.3 HC comprising monomeric sugars.

1.2.2.1 Softwood HC

Softwood HC generally consist of galactoglucomannan, arabinoglucuruonoxylan, arabinogalactan and other polysaccharides in minute quantities.

1.2.2.1.1 Galactoglucomannans

(GGM, O-acetyl-galactoglucomannans) are the major HC in softwoods (Figure 1.4) comprising 20-25% of the dry mass. GGM have a backbone consists of mannopyranose (Man*p*) and glucopyranose (Glc*p*) units connected together by β-(1→4) linkages and branched by galactopyranose (Gal*p*) residue at the carbon-6 on the Man*p*

units. The amount of attached galactose units varies. Based on the galactose content, GGM can be divided into two fractions. The fraction that has low galactose content in which the ratio of galactose: glucose: mannose is about 0.1:1:3 (less than 15%) (Ebringerová, 2005) is referred to as glucomannan (GM). The fraction that has high galactose content in which the ratio of related sugars is 1:1:3 is considered as GGM.

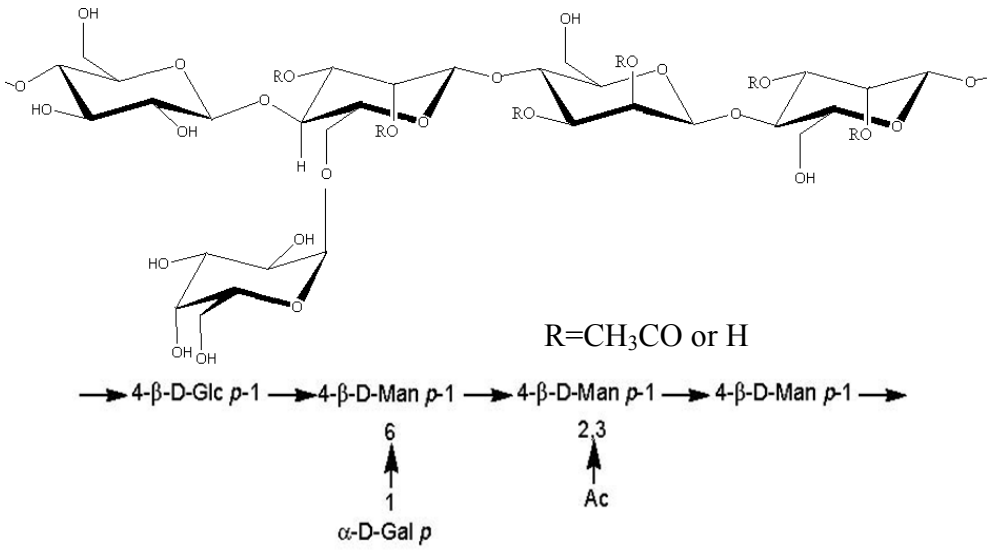


Figure 1.4 Structure and abbreviated formula of galactoglucomannan.

Another important aspect of the structure of GGM is that the hydroxyl groups at the carbons 2 and 3 of the backbone units are partially substituted by O-acetyl ($COCH_3$) groups. The content of acetyl groups is around 6%, commonly, one acetyl group per three to four backbone units (Ebringerová, 2005; Peng et al., 2012; Sjöström, 1993).

1.2.2.1.2 Arabinoglucuronoxylans

AGX, ((L-arabino)-D-glucurono-D-xylans) are the minor HC that occur in softwood. AGX have xylapyranan backbone connected by β-(1→4) glycosidic linkage. In

addition, it has single 4-O-methyl-D-glucuronic acid (MeGlcA) and α-L-arabinofuranosyl (α-L-Ara*f*) units partially substituted at carbon- 2 and carbon- 3 positions, respectively (Figure 1.5). The amount of 4-O-MeGlcA is two units per ten xylose units on average. In case of α-L-Ara*f*, the ratio is 1.3 α-L-Ara*f* units per ten xylose units. (Ebringerová, 2005; Sjöström, 1993).

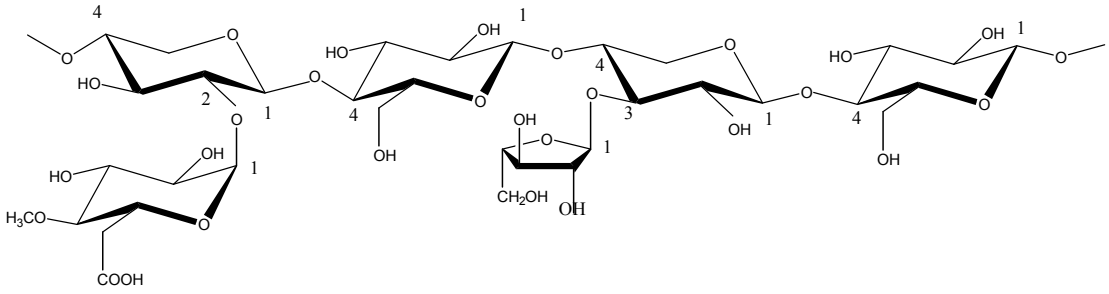


Figure 1.5 Structure of arabinoglucuronoxylan.

1.2.2.1.3 Arabinogalactans

AGs are the minor constituents in wood species except in heartwood of larches. AG has a highly branched structure that makes it readily water soluble. It has a backbone that consists of β-D-galactopyronose units connected with each other by (1→ 3) linkage and branched with β-D-galactopyronose residue almost by (1→ 6) linkage at every carbon- 6 position. Sometimes, L-arabinose can be attached instead of β-D-galactopyronose residue (Figure 1.6). There are also a few glucuronic acid residue occur in the molecule (Sjöström, 1993).

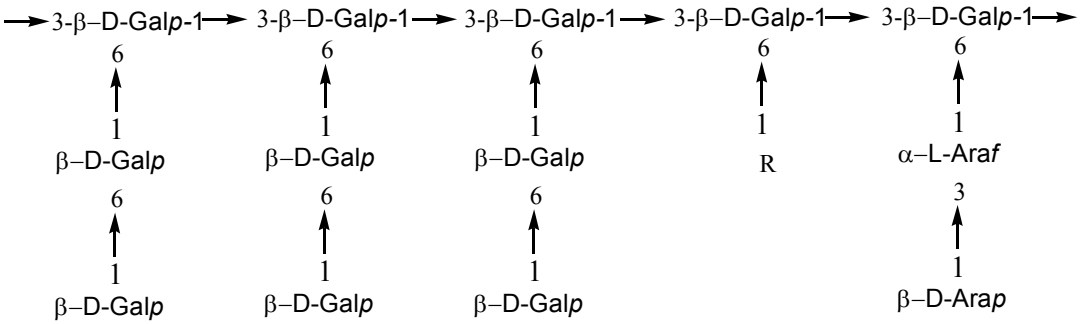


Figure 1.6 Abbreviated formula of arabinogalactan.

1.2.2.2 Hardwood and grass HC

Hardwood HC generally consists from glucuronoxylan, glucomannan and other minor amount of miscellaneous HC.

1.2.2.2.1 Glucuronoxylans

GXs (O-acetyl-4-O-methylglucurono-β-D-xylan) are the main components that occur in hardwood HC. GX has the backbone consists of β-D- xylopyranose units linked by (1→4) bonds. Commonly, xylose units in xylan chain contain an O-acetyl group at carbon 2 and carbon 3 positions on the average about seven O-acetyl groups per ten xylose units. In addition, it contains (1→2) linked 4-O-methyl glucuronic acid residues on the average one uronic acid per ten xylose units (Figure 1.7).

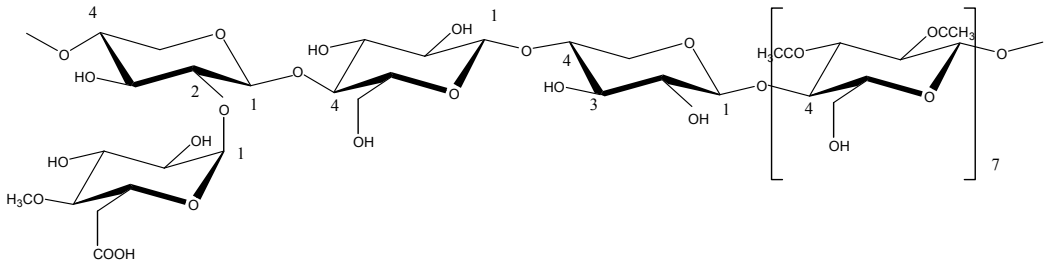


Figure 1.7 Structure of glucuronoxylan.

1.2.2.2.2 Glucomannans

GMs (D-gluco-D-Mannans) are the minor component (2-5%) of HC that occur in hardwood and grasses. The GM backbone consists of β-D- glucopyranose (Glc*p*) and β-D-mannopyranose (Man*p*) units linked by (1→4) bond. The ratio of Glc*p* and Man*p* residues is varies between 1:2 and 1:1, depending on wood species (Figure 1.8) (Sjöström, 1993).

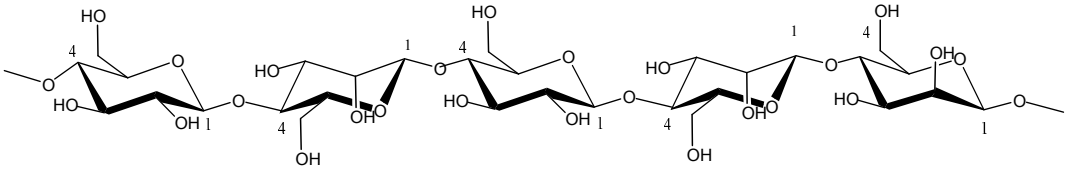


Figure 1.8 Structure of glucomannan.

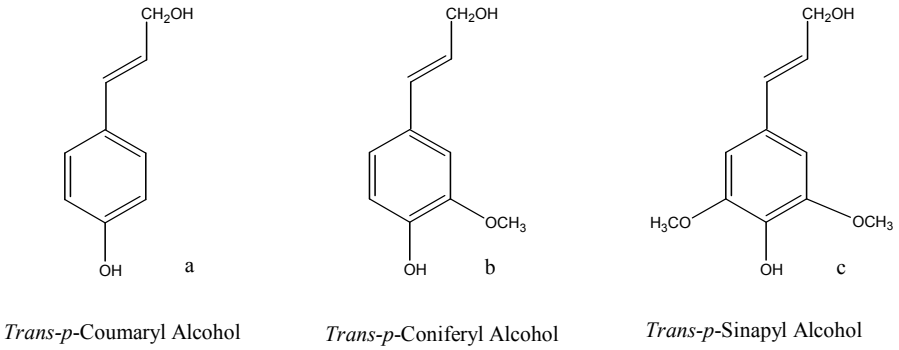
Other HC those exist in softwood HC are also exist in minute amount in hardwood HC as well.

1.2.3 Lignin

Lignin is a third major component in the cell wall that occupies 20-30%. The main function of the lignin is to cement wood fibers with each other, serve as stiffening agent within fibers and protect the cell wall from enzymatic degradation.

Lignins are three dimensional polymers consisting of phenylpropane units which are linked with each other in various ways forming a complicated structure. The structure of lignin is formed by enzymatically initiated free radical polymerization of lignin precursors (*trans*-*p*-coumaryl, *trans*-coniferyl and *trans*-sinapyl alcohols) (Figure 1.9). Softwood and hardwood lignins differ by their methoxyl group content and degree of their crosslinking. Softwood, guaiacyl lignin is formed from coniferyl alcohol (3-

methoxy-4-hydroxy-cinnamyl alcohol) (Figure 1.9 b). In hardwoods and grasses, guaiacyl-syringyl lignins are formed from coniferyl and sinapyl alcohols (3-methoxy-4-hydroxy-cinnamyl alcohol and 3, 5-dimethoxy-4-hydroxy-cinnamyl alcohol) (Lewin and Goldstein, 1991) (Figure 1.9 b and c). Due to their complexity, many features of lignin chemistry remain unclear (Sjöström, 1993). Lignin is deposited between cell wall, linked and closely associated with polysaccharides. The chemical bonds between lignin and carbohydrate is called lignin-carbohydrate complex (Figure 1.10). Isolation of pure lignin has not been achieved yet. However, lignin can be isolated fractionally with a certain degree of degradation. Chemical properties of the lignins vary from the methods used for isolation (Lewin and Goldstein, 1991).



Trans-p-Coumaryl Alcohol *Trans-p*-Coniferyl Alcohol *Trans-p*-Sinapyl Alcohol

Figure 1.9 Structure of lignin precursor alcohols.

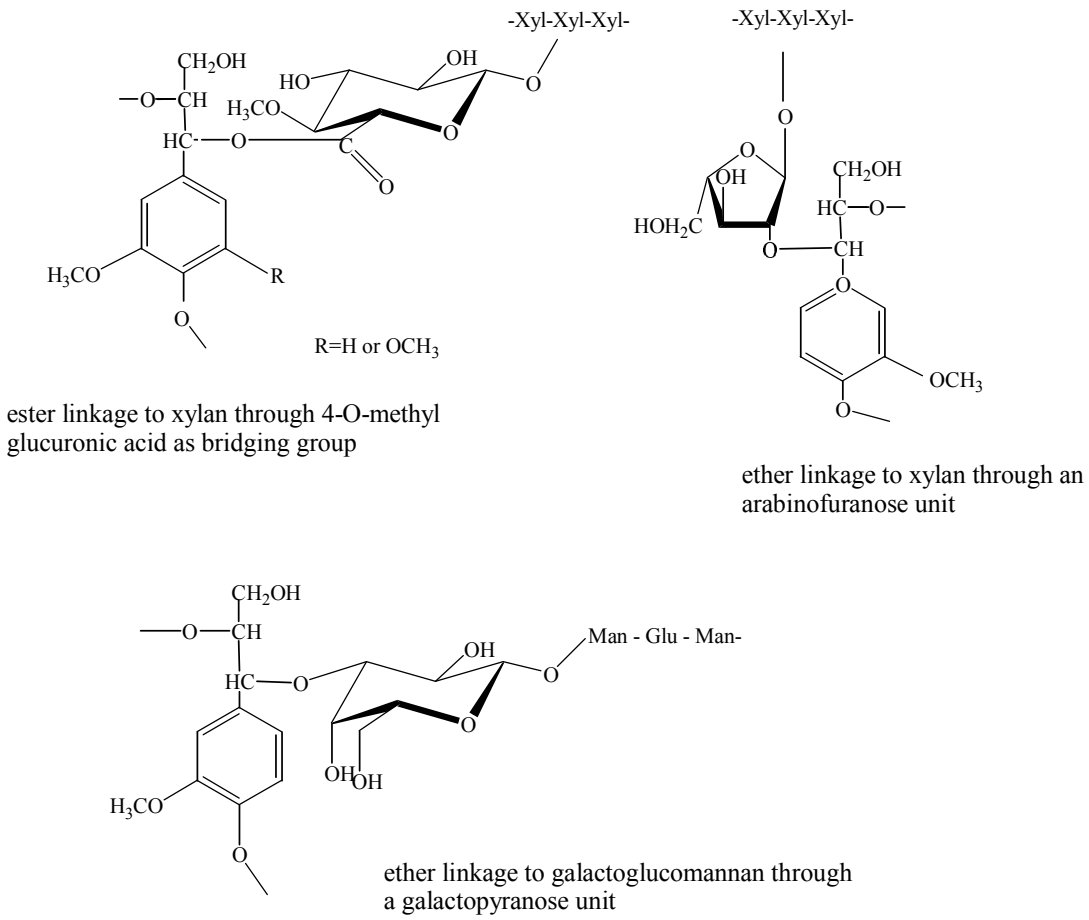


Figure 1.10 Most frequently suggested types of lignin-polysaccharide linkages (Fengel and Wegener, 1984; Sjöström, 1993).

1.2.4 Extractives

The content of extractives in lignocellulosic materials is approximately 5%. They represent small fraction but they comprise an extremely large variety of different compounds of both lipophilic and hydrophilic types. Extractives are soluble in neutral organic solvents or in water. Different types of extractives are necessary to maintain diverse biological functions. For instance, fats are energy source of the wood, whereas

low terpenoids, resin acids and phenolic substances protect the wood against microbiological damage or insect attack (Lewin and Goldstein, 1991; Sjöström, 1993).

1.3 Extraction and isolation of HC

Proper extraction of HC is difficult to achieve. Due to the lignin network as well as ester and ether lignin-carbohydrate linkages the extraction of HC from the cell wall of lignocellulosic material is limited. Also, due to the hydrogen bonds between polysaccharides, extractability of polysaccharides is impeded. Different biomass types with different properties require different techniques as well (Sjöström, 1993). Therefore, determining corresponding extraction and isolation techniques is important.

1.3.1 Pretreatment of lignocellulosic biomass.

Studies performed over the last few decades show that the pretreatment process extensively increases the extraction of hemicellulosic components. On the other hand, removal of HC increases the pore size in the biomass which enhances the accessibility of enzymes to the cellulose increasing the probability to be hydrolyzed for bio-ethanol production (Chandra, 2007). At the same time, extracted HC can be utilized for value-added product such as biopolymers. Pretreatment of lignocellulosic biomass has been investigated by using a variety of pretreatment techniques such as alkaline hydrolysis, dilute or strong acid hydrolysis, steaming with or without explosion, organosolv, and hydrothermal pretreatments for the extraction of HC.

1.3.1.1 Alkaline pretreatment

The alkaline pretreatment process typically utilizes sodium, potassium, calcium, ammonium hydroxides or alkaline solution of hydrogen peroxide. The mechanism of this process is based on saponification reaction of intermolecular ester bonds cross-linking

HC and other components (Singh, 2011). During this process most of the lignin is removed and small amounts of cellulose and slightly higher amounts of HC are dissolved as well (Figure 1.11) (Gírio et al., 2010). Operation temperature can start from ambient and the time can vary from seconds to days depending on the biomass and alkali to be used (Alvira et al., 2010).

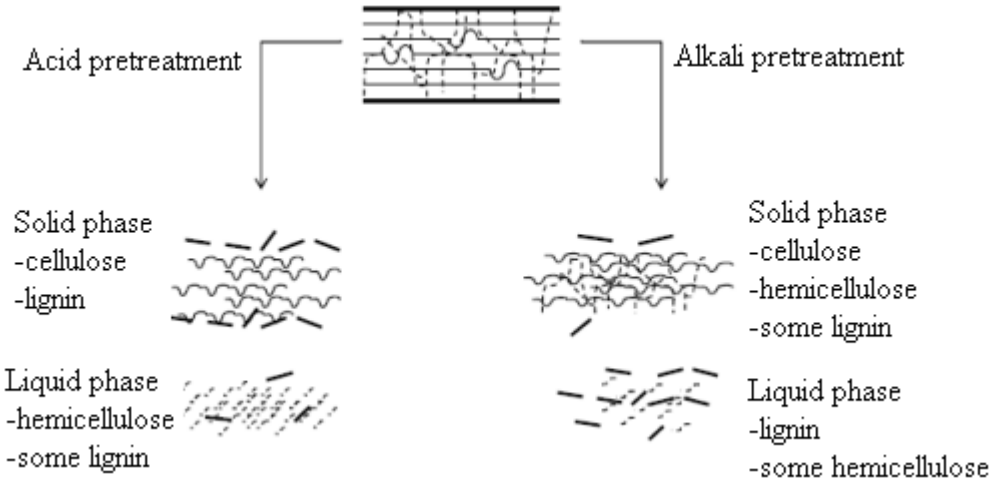


Figure 1.11 Fractionation of lignocellulosic biomass due to acid and alkali pretreatments (Keshwani, 2009).

Sodium hydroxide causes swelling of cellulose fiber. It partially disrupts the interaction among crystalline cellulose causing the reduction in the degree of polymerization and crystallinity and interruption of the lignin structure (Taherzadeh and Karimi, 2008). Calcium hydroxide pretreatment removes mainly lignin and acetyl groups from HC. It is reported that the ultrasonication assisted extraction slightly increases the extraction of HC (Hromádková and Ebringerová, 2003; Sun et al., 2004a). Alkaline solution of hydrogen peroxide removes even more lignin rather than HC (Carvalheiro et

al., 2008). Generally, alkaline pretreatment is suitable for removal of lignin content from the biomass.

1.3.1.2 Acid pretreatment

Acid hydrolysis pretreatment can be divided into dilute and strong acid pretreatments. These pretreatments mainly remove HC fractions. Depending on the concentration and nature of acids used, a small amount of lignin and cellulose are removed as well (Figure 1.11). Sulfuric acid is the most commonly used acid. Hydrochloric, phosphoric and nitric acids have been reported as being tested. Organic acids such as formic, fumaric and maleic acids are alternatives for HC removal from biomass.

In a recent study, Zhao and Liu (2012) reported that the hydrolysis with dilute formic acid removed more than 80% of lignin and HC from sugarcane bagasse. According toRocha et al. (2011), mixture of 1% (w/v) sulfuric acid and 1% (w/v) acetic acid pretreatment was able to extract more than 90% of HC from sugarcane bagasse. However, DP of HC was small and the concentration of HC degradation compounds was high.

Drawbacks of acid pretreatments are the production of furfural, hydroxymethylfurfural (HMF) and other toxic compounds which are formed due to the degradation of sugars. Equipment corrosion, high operation and maintenance costs are the big challenges of this process (Mosier et al., 2005). Furthermore, HC obtained as a result of the acid hydrolysis pretreatment have a considerably low degree of polymerization that hinders the probability of utilization in biopolymer application.

1.3.1.3 Organosolv pretreatment

The organosolvation method utilizes numerous organic and aqueous solvent mixtures, including methanol, ethanol, acetone, ethylene glycol and tetrahydrofurfuryl alcohol. As a result, mainly the lignin fraction of lignocellulosic material is solubilized. Combination of these organic solvents with acid catalysts such as hydrochloric acid, sulfuric acid, oxalic acid or salicylic acid allows extract increased amounts of HC than organosolv pretreatment alone.

Area et al. (2009) studied the effect of sulfuric and acetic acid catalysts on ethanol-water fractionation of sugarcane bagasse. The result showed that 0.5 g/l sulfuric acid catalyzed ethanol-water mixture was able to extract a maximum of 60% of total xylan and 87.5% of lignin at 160 ^{o}C for 120 min. The degradation of cellulose and arabinose was high during this pretreatment. Sannigrahi et al. (2010) treated loblolly pine sawdust with 65% ethanol/ water mixture catalyzed with 1.1% sulfuric acid at 170 ^{o}C for 60 min. As a result, 62% of total HC were extracted.

Compared to other chemical pretreatments, the organosolv pretreatment method provides relatively pure lignin that can be used for production of value-added chemicals. However, extraction of hemicellulosic components with this technique is inadequate. Moreover, HC are extracted with excessive amounts of lignin that requires an additional step for separation. Besides, this pretreatment requires expensive organic solvents, special extraction and separation techniques and facilities for removal and recovery of organic solvents from the system. It is because organic solvents may reduce the enzymatic and microbial action of further hydrolysis (Sun and Cheng, 2002). The organic solvents also were expensive to apply. Although the hydrolysis process is outside the

scope of this project, preparation of fermentable raw materials for further hydrolysis as a byproduct from this study of interest.

1.3.1.4 Ionic liquid (IL) pretreatment

IL pretreatment has recently received much attention for pretreating lignocellulosic biomass because this method is able to dissolve carbohydrates and lignin fractions and does not produce any toxins or explosive gases. ILs are salts, typically composed of large organic cations and small inorganic anions, which exist as liquids at relatively low temperatures; often at room temperature. Their solvent properties can be varied by adjusting the anion and the alkyl constituents of the cation. These properties include chemical and thermal stability, non-flammability, low vapor pressures and a tendency to remain liquid in a wide range of temperatures (Hayes, 2009). The main principle of the IL is that the anion of the salt forms a hydrogen bond with the sugar hydroxyl protons at a 1:1 ratio. As a result, the complex network of non-covalent interactions among cellulose, HC, and lignin is effectively disrupted and causes effective solubilization. Moreover, the formation of degradation products is minimal. This technique is relatively new and still expensive in terms of IL recovery.

1.3.1.5 Steam explosion pretreatments

Steam explosion is one of the most widely used pretreatments. It applies pressurized steam, at temperatures ranging from 140 to 240 ^{o}C, to the biomass for few seconds to several min following by a sudden release of the applied pressure. Due to the explosive pressure decrease, the fibers are separated. In addition, an autohydrolysis takes place due to the disruption of acetic acid from HC. The steam explosion technique has several advantages compared to chemical pretreatment techniques. It includes low

environmental impact, low capital investment, low hazardous chemicals utilization and complete sugar recovery (Avellar and Glasser, 1998). However, depending on severity factor, the steam explosion pretreatment causes degradation of HC and production of toxins such as HMF and furfural. The higher the severity factor, the higher the degradation of HC (Alvira et al., 2010).

1.3.1.6 Hydrothermal pretreatment

Hydrothermal treatment mainly solubilizes HC from lignocellulosic biomass. This pretreatment utilizes hot water at elevated temperature between 160 ^{o}C and 240 ^{o}C. Pressure is applied to maintain the water in the liquid state to provoke the alteration of lignocellulosic material structure. Also, the hydrothermal pretreatment does not require any catalyst or chemicals (Alvira et al., 2010).

Yoon et al. (2010b) studied the effect of the pre-extraction of HC of southern pine on the property and recovery capability of kraft pulp. Loblolly pine was extracted by hydrothermal pretreatment until 10% of total weight of the biomass was removed. The strength of paper prepared from HC pre-extracted pulp was not significantly different than the one prepared from the regular kraft pulp. However, minor reduction in tear strength was observed (Yoon et al., 2010a; Yoon et al., 2011; Yoon et al., 2010b). The result suggests that the pre-extraction of HC before pulping is economically beneficial because HC can be used for value-added products and chemicals instead of burning. In fact, HC have low heating value (Lyytikainen et al., 2011) and less cost-efficient to use through burning .

Laser et al. (2002) have compared steam explosion and hydrothermal pretreatments for HC removal from lignocellulosic biomass. The result revealed that the

hydrothermal had better performance than steam explosion and removed up to 80% of HC at 220 ^{o}C in 2 min.

Sasaki et al. (2003) found out that the hydrothermal extraction pretreatment is promising technique for isolating mainly HC and lignin as water soluble fraction at 220-230 ^{o}C in semi-batch reactor. Two-step pretreatment has been conducted to isolate increased amounts of HC and to obtain biomass with enhanced enzymatic digestibility. However, the amount of partially depolymerized lignin was increased as well (Walch et al., 1992).

Boussarsar et al. (2009) investigated the valorization of sugarcane bagasse by extracting xylose for xylitol production. At optimum extraction condition 170 ^{o}C for 2 hr 48.8% of xylose was extracted in the form of xylan oligomers and polymers with large distribution of degree of polymerization. The production of toxins produced due to the degradation of HC was minimal and the recovery and purity (78%) of xylose were high.

In general, hydrothermal pretreatment is an attractive technique for HC removal because it has a cost saving potential. It does not require any catalyst or chemicals addition. During this pretreatment, relatively low concentration of hemicellulosic degradation products are produced and higher pentosan recovery is achieved compared to other pretreatment techniques. The high energy required for water processing is the only disadvantage of this pretreatment (Alvira et al., 2010).

1.3.1.7 Evaluation of the pretreatment techniques

Table 1.1 summarizes the above discussion of pretreatment techniques. All these methods have advantages and disadvantages in terms of isolation of HC, cost efficiency, environmental impact and production of sugar degradation products. Since one of the

objectives of this project is to extract the maximum possible amount of HC, acid hydrolysis (dilute), hydrothermal and steam explosion techniques were considered.

Table 1.1 Comparison of different pretreatment technique performances.

	Pretreatments	Dissolve			Toxins	Require chemicals	Operation cost	Environmental harm
		Cellul	HC	Lignin				
1	Alkaline		***	*****		yes	*****	*****
2	Acid	**	*****	****	****	yes	*****	****
3	Organosolv		**	*****		yes	*****	*****
4	Ionic Liquid		***	****		yes	*****	
5	Hydrothermal	*	*****	**	*	no	**	
6	Steam explosion	*	*****	****	****	no/yes	***	

***** Maximum value

Dilute acid hydrolysis and steam explosion techniques are able to extract 60-80% of HC (Alvira et al., 2010; Gírio et al., 2010). However, the production of sugar degradation products overrides hydrothermal extraction technique. On other hand, the acid hydrolysis technique requires high operation cost than other two methods. Hydrothermal and steam explosion techniques have quite close performance; however, the steam explosion technique produces higher toxins and extracts relatively higher content of lignin than the hydrothermal extraction technique. Thus, the hydrothermal extraction technique was chosen for extraction of HC from lignocellulosic biomass. Hydrothermal extraction used in previous studies, reported in literature, utilized lengthy time periods (Boussarsar et al., 2009) and relatively high temperatures (Laser et al., 2002; Sasaki et al., 2003). Thus, one objective is to reduce the time and temperature of the hydrothermal extraction process to render hydrothermal extraction of HC economics.

1.3.2 Isolation of HC

Isolation of hemicellulosic components is complicated and a variety of methods have been reported in the literature. Depending on the desired degree of purity, different techniques may be employed. Ion-exchange resins are useful for desalination and removal of other undesired compounds (Jacobs et al., 2003; Vázquez et al., 2005). Solvent extraction has been employed to separate non-saccharide fractions. Membrane filtration technique is a relatively new technique that has been employed to separate oligosaccharides based on their molecular mass.

1.3.2.1 Ethanol precipitation

Isolation of hemicellulosic components from the wood was accomplished by O'Dwyer for the first time in 1926 by extracting with hot water and precipitating with alcohol (O'Dwyer, 1926). In 1947, Wise and Ratliff (1947) isolated hemicellulosic components from the chlorite holocellulose (cellulose and HC mixture) by fractionating with aqueous potassium hydroxide followed by acidification with acetic acid, and precipitating the hemicellulosic components with excess ethyl alcohol. Precipitation with a excess amount (four to six times the volume of the aliquot) of ethyl alcohol is currently widely applied (Mellinger-Silva et al., 2011; Peng et al., 2011; Peng et al., 2009; Peng et al., 2010; Seo et al., 2011). However, this technique is relatively expensive.

1.3.2.2 Ion exchange resin

The ion exchange resin technique is useful for desalination and removal of undesired compounds. In 1970, Blake et al. (1971) developed a method for separation of hemicellulosic component impurities by using dialysis and ion exchange resin from alkaline extracted fraction. Combination of ion exchange resin technique with

ultrafiltration allowed removal of colored compounds (Zeitoun et al., 2010). One of the big disadvantages of this technique is quite expensive.

1.3.2.3 Membrane filtration

Membrane filtration is one of the promising techniques for concentration and isolation of hemicellulosic materials from other materials. The membrane filtration method has been widely investigated in the food and pharmaceutical area. Some effective membranes are reported known for the separation of hemicellulosic components from aqueous fraction obtained by various pretreatment methods. Manasrah noted that the separation of HC from the extraction liquor of hydrothermal depends on membrane cut-off and surface material. The membrane UC030 with cut-off 30000 g/mol followed by membrane GE-5 or ETNA01PP with cut-off 1000 g/mol separated relatively pure HC (Manasrah 2008). Moreover, the separation of HC was conducted with hydrophilic and hydrophobic membranes. It appeared that hydrophilic membranes such as ETNA01PP, ETNA10PP (composite fluoro polymer, hydrophilic) and UFX5 (polysulphone, hydrophilic) have much more potential than hydrophobic membranes for separation of HC from the extracted liquor (Persson et al., 2009; Persson and Jönsson, 2010).

The main principle of the membrane filtration technique is that the separation of components in any solvent media can be achieved based on the difference of their molecular sizes. The separation process is usually pressure or vacuum driven. Solvent media introduced for separation is called the feed solution. Particle matter in the feed solution that has larger size than the pore size of an engineered barrier is rejected by it, primarily, through a size-exclusion mechanism. Particles rejected by the membrane go to

the retentate fraction, but particles that passed through the membrane go to the permeate fraction (Figure 1.12) (Cheryan, 1998).

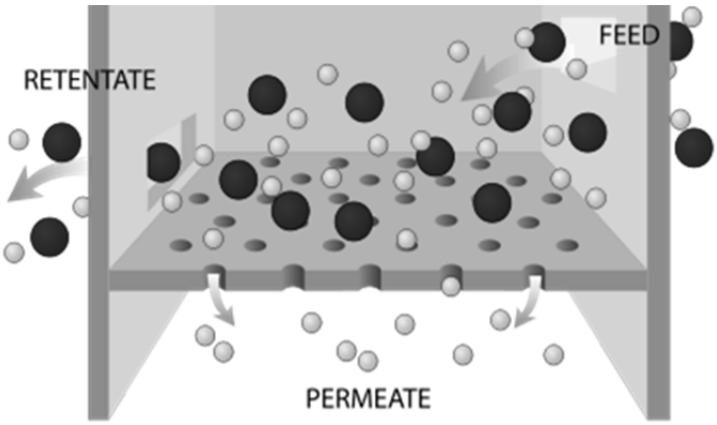


Figure 1.12 Schematic representation of membrane filtration technique. (http://en.wikipedia.org/wiki/Passive_transport

One of the classification methods of membranes filtration is based on membrane pore sizes. It can be classified as microfiltration, ultrafiltration, and nanofiltration. Microfiltration membranes have 4-0.02 µm pore sizes, ultrafiltration membranes have 0.2-0.02 µm pore sizes, and nanofiltration membranes have <0.002 µm pore sizes. Membranes can also be classified as hydrophilic and hydrophobic based on the material of which the surface material is made. The membrane filtration technique has numerous advantages such as ease and efficiency to use; low energy consumption, adjustable separation capability, and the process can be performed in various conditions. However, fouling, flux decline and relatively short life time of the polymer membranes are drawbacks of this method (Cheryan, 1998).

A major limiting step in membrane technology is “fouling” of the membrane. Fouling represents a decline in a flux with time of operation. The basic evaluation of the

degree of fouling is measured with the pure water flux (PWF) of the membrane. Flux decline should be measured when operating parameters such as pressure, temperature, flow rate, and feed concentration are kept constant (Cheryan, 1998). PWF can be expressed by following equation 1.1:

$$A_w = \frac{J_w}{P_t} = \frac{L}{bar\ h\ m^2} \quad (1.1)$$

Where,

A_w is pure water permeability,

J_w is the water flux or flow rate in unit volume per unit area per unit time,

P_t is applied transmembrane pressure.

The main advantages of this technique are the low operating cost, lacking need of additives and modifiers, continuous production, reliability and ease of combination with other separation methods.

1.4 Application of the HC

Hemicellulosic polysaccharides are gaining importance in the food industry, pharmaceutical industry and food packaging application (Figure 1.13).

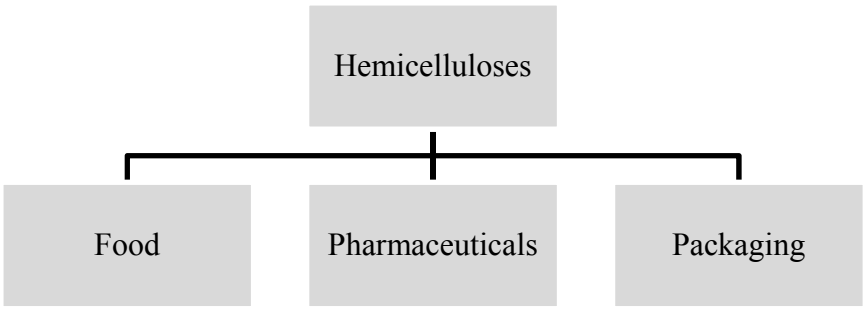


Figure 1.13 Major application of HC.

1.4.1 In the food industry

During the 1980's polysaccharides started to gain importance as food ingredients in Japan and Europe. They are considered to provide health benefits such as non-carcinogenicity, promotion of the growth of good bacteria in the colon and low caloric value. Food grade oligosaccharides consist of a mixture of 2 to10 sugar unit polysaccharides based on galactose, fructose, palatinose, maltose, isomaltose, gentiose, and many others. Compared to the sucrose sugars, the sweetness of the polysaccharides is three to six times lower and has lower caloric value which makes them suitable for low caloric value food preparation. In fact, they are used in food production for enhancing food flavors; to alter the freezing temperature of foods, or to prevent the excessive drying and maintain a low water activity in food, which is convenient in controlling microbial contamination (Crittenden and Playne, 1996).

Some polysaccharides have prebiotic functions such as promoting the growth of colon friendly bacteria which helps to maintain a healthy digestive system, stimulate immunoresponse of human organisms, enhances the possibility of increasing mineral absorption. Also, it helps keep cholesterol and triglycerides in the blood at moderate levels (Geier et al., 2007; Manning and Gibson, 2004; Marteu, 2001; Tuohy et al., 2003; Vitali et al.).

O-acetylgalactoglucomannans (AcGGM or mannans), the main HC of soft wood, are mostly used as a guar gum in industry. Guar gums are usually applied in textile, pharmaceutical, cosmetics, paper, explosive and mining industries. Mannans have higher thickening properties than any other natural sugars. Thus, mannans are also used in industry mainly as emulsifying, thickening, stabilizing, and gelling agents. They are also considered to have nutritional, medical and health effects (Willför et al., 2008). AcGGM

postulated have an ability to reduce serum cholesterol and attenuate blood glucose level (Shimizu et al., 1991).

Xylooligosaccharides, main HC that can be obtained from hardwood or plants, are predominantly used in production of antioxidant compounds and low calorie sweeteners as xylitol. Also, xylooligosaccharides are used for both prebiotic and symbiotic health drinks and detoxifier of fermentation media (Crittenden and Playne, 1996; Cruz et al., 1999; Moure et al., 2001; Vázquez et al., 2005)

1.4.2 In the pharmaceutical industry

Polysaccharides of different origin are being investigated to find application in the pharmaceutical field such as the precursor of antiviral and antitumoral drugs. For instance, sulfated oligoxylans (oligoxylans obtained from beechwood) mimic most biological actions of natural heparins (anticoagulant) *in vitro*, including inhibition of the human immunodeficiency virus (Stone et al., 1998). Sulfated xylogalactan from red seaweed *Northogenia fastigiata* (Damonte et al., 1996) and xylomannan from algae (Pujol et al., 1998) showed antiviral activity against herpes simplex virus. Fractions of xylose based polysaccharides and water soluble lignin have cytotoxic effect that can reduce the viability of leukemia cell lines derived from acute lymphoblastic leukemia (Ando et al., 2004).

Different applications were proposed based on indigestible properties of polysaccharides. For instance, xylooligosaccharides which are indigestible polysaccharides, promote the production of *Bifidobacterium bifidum* in the colon (Matsushima and Takagi, 2012). In case of fructo-oligosaccharides, they are used to

prevent gastrointestinal infections and to reduce the duration of diarrhea episodes in human (Mellinger-Silva et al., 2011).

Another area for utilization of polysaccharides is the controlled drug delivery in human body. Nano- and micro- size xylans deliver the drugs into the body at controlled rates (Garcia et al., 2001). Chemically modified polysaccharides from spruce are used for preparation of hydrogels (Gabrielii et al., 2000; Söderqvist et al., 2001).

1.4.3 In the packaging material production industry

Historically, the research on HC was more concentrated in production of sugars, chemicals, fuels, etc. Currently, developing environmentally benign and biodegradable packaging materials while reducing packaging waste are receiving greater attention. The first HC biopolymer, HC acetate, was produced early in 1949 by Smart and Whistler (Hansen and Plackett 2008). Since then a number of studies have been reported. Films produced from HC tend to have low oxygen and aroma permeability. These are one of the desirable features in food packaging. In fact, a large part of commercial production of food products deals with packaging that extends the shelf life of the food by controlling the oxygen and aroma transport (Miller and Krochta, 1997).

Höije et al. (2005) studied the effect of arabinoxylan isolation methods on the film properties. Dilute acid, HCl/NH_4OH and ethanol delignification, dilute acid and chlorite delignification and enzymatic pretreatment methods followed by sodium hydroxide hydrolysis were evaluated in terms of the yield and the structure of the isolated arabinoxylan. Also, the film properties prepared out of isolated arabinoxylan were compared as well. The highest yield, approximately 57% of arabinoxylan was obtained by dilute acid and chlorite delignification pretreatment. The molecular weights of the

arabinoxylan were between 35000 and 45000 g/mol. Films prepared from the arabinoxylan were strong, stiff and brittle and highly hydroscopic.

Gröndahl et al. (2004) studied the oxygen barrier properties of glucuronoxylan films isolated from aspen wood. Unmodified xylan films were brittle, thus, plasticizers such as sorbitol and xylitol were used in concentrations of 20, 35 and 59 wt %. Tensile testing revealed that increasing the plasticizer content resulted in a reduction of strength and increase in elongation. Specially, addition of sorbitol had a remarkable effect on elongation. Oxygen permeability of the film plasticized with 35 wt% sorbitol was 0.21 cm^3 μm m^{-2} d^{-1} kPa^{-1} at 50% RH. This value was identical to the value for a polyvinyl alcohol which is considered an excellent barrier material.

Gröndahl and Gatenholm (2007) research results showed that the films prepared from barley husk arabinoxylan and aspen glucoronoxylan have different tensile properties at corresponding plasticizer content. Barley husk arabinoxylan films had higher stress at break and strain at break than the aspen glucoronoxylan films. The glucuronoxylan films were semicrystalline, whereas the arabinoxylan films were mainly amorphous with small crystalline peaks detected by Wide Angle X-ray Scattering. Both the glucuronoxylan and arabinoxylan films had low oxygen permeability and it was reported that those films can be used in packaging for oxygen-sensitive products.

Hartman et al. (2006) studied the oxygen barrier potential of AcGGM films. The HC with approximate molecular weight of 10000 g/mol were isolated by ultrafiltration from process water of thermomechanical pulping. Films were prepared with 21-25% of plasticizers such as sorbitol, glycerol and xylitol. In addition, alginate and carboxymethylcellulose (CMC) were utilized for film formation with the ratio of 2:1 AcGGM: alginate/CMC. The storage modulus of the films decreased with increasing

humidity. The result was more pronounced with the film plasticized with glycerol. Tertiary mixture of AcGGM, plasticizer and alginate or CMC exhibited better mechanical properties at elevated humidity than binary mixture of AcGGM and plasticizer. Elongation of the films was high for the films plasticized with glycerol; however, the samples containing alginate or CMC the elongation was small. Oxygen permeabilities were lowest for the AcGGM-alginate and AcGGM-CMC films. Among the plasticized films the film containing sorbitol had the lowest oxygen permeability.

Goksu et al. (2007) studied the effect of lignin on cotton waste and birchwood xylan based films. Different films from commercial purified birch wood xylan with or without lignin content was prepared and compared. The birch wood xylan without lignin formed small separate patches, although when the lignin content increased the number of patches decreased. Approximately 1% of lignin was required to produce self-supporting films.

Zhu et al. (2011) studied the effect of not highly refined polysaccharide rich wood hydrolysate fraction for the preparation of the films. In the polysaccharide rich fraction a fair amount of inter-molecular and intra-molecular HC-lignin interactions were still preserved. Researchers noted that due to the preserved native interactions the barrier feature of the films was higher than the barrier feature of the pure AcGGM based films.

Mixing of xylan with other biopolymers such as alginate and carboxymethylcellulose improved the oxygen barrier property of xylan films. Also, some plasticisers like glycerol, sorbitol and xylitol significantly change the properties of hemicellulosic films creating more flexible polymers.

Based on literature search the objectives of the study can be accomplished by (1) extracting HC by hydrothermal extraction technique. Compared to other techniques, the

hydrothermal extraction produces a high yield of HC with minimum content of HC degradation products. Since the objective of the study is to develop an economical technique, the reduction of applied temperature and time will lead to an optimum approach. (2) Isolation and concentration of HC can be approached by a membrane filtration technique. This technique is relatively easy and inexpensive to operate. (3) Application of HC in the packaging industries is in its early stage compared to the application of HC in food and pharmaceutical industries. Thus, the third objective can be approached by preparing HC based films with low oxygen permeability by fine-tuning the ratio of HC, plasticizer and film forming agents.

CHAPTER II

MATERIALS AND METHODS

2.1 Materials

2.1.1 Sugarcane bagasse

As a representative of an annual grass and native plant in the south, sugarcane bagasse was chosen as a first biomass to study for this project. Depithed sugarcane bagasse was obtained from Sustainable Fuels LLC, New Iberia, LA.

2.1.2 Southern yellow pine

As a representative of softwood and native plant in the south, the southern yellow pine (SYP) was chosen as second biomass for this study. The southern yellow pine was obtained from the Starr Memorial Forest of the Forest and Wildlife Research Center, located 10 miles south of Starkville, MS. Both biomass types were ground on model 4 Thomas-Wiley Laboratory Mill (Arthur H. Thomas Company, Philadelphia, USA) and sieved on Ro-Tap Testing Sieve Shaker (The W.S. Tyler Company, Cleveland, OH, USA) through 40-60 mesh.

2.1.3 Chemicals

All chemicals used in this study were purchased from commercial resources and used without further purification. Reagent grade calcium carbonate ≥ 99%, 72% sulfuric acid, 10 N Sodium hydroxide, HPLC grade water, methanol, and sorbitol were purchased from Thermo Fisher Scientific Inc. Sugar standards such as D(+)Glucose, D(+)Xylose,

D(+)Galactose, L(+)Arabinose, D(+)Mannose and Dextran with different molecular weights (1000-80000 Da) were purchased from Sigma Aldrich. Sodium carboxymethylcellulose (D.S. 0.7-0.85; 400-800 mPa s) was purchased from Sigma Aldrich supplied by Fluka Biochemika. Also, 2 mL syringe, 2 µm pore size filter, 2 mL and 4 mL HPLC and GPC sample vials were acquired from Thermo Fisher Scientific Inc. as well. Membranes with molecular cut-off 400, 1000 and 10000 Da were purchased from Alfa-Laval, Sweden.

2.2 Chemical analysis of the biomass

Twenty grams of biomass (sugarcane bagasse or southern yellow pine) was extracted with 300 ml ethanol and benzene mixture (1:1) in a Soxlet apparatus for 6 h to remove water insoluble extractives such as wax, fats, flavonoids and etc. The extracted sample was dried at room temperature for 24 h then stored in plastic bag for moisture conditioning. The chemical composition for extracted bagasse samples was then determined according to the procedure of Sluiter et al. (2006). Briefly, this method is as follows: 0.3 g from extractive-free biomass was hydrolyzed with 3 ml of 72% sulfuric acid for one hour. The sample was agitated and crushed constantly with a glass rod to hydrolyze all holocellulose. Then, 84 g deionized water (DI) was added to obtain 4% sulfuric acid solution and autoclaved at 121 ^{o}C for one hour in a pressure tube to completely hydrolyze all oligomeric sugars into monomers. After hydrolysis, the sugar solution was neutralized with calcium carbonate to pH≈6 and filtered with 0.2 µm syringe nylon membrane (Millex-GN) for the removal of fine particles. The monomeric sugar solution was then analyzed by Agilent 1200 High Performance Liquid Chromatography (HPLC). Acid soluble lignin content was determined by Cary 100 Bio

UV-Visible Spectrophotometer (Varian Australia, Australia) by scanning (200-400 nm) the UV absorbance percentage at 275-278 nm. Klason lignin content was determined by NREL/TP-510-42623 method. Ash content of bagasse was determined by NREL/TP-511-0-42622 technique.

2.3 Pretreatment

Extraction process of HC from biomass was performed by hydrothermal extraction technique. The process was conducted in a Parr Reactor of Series 4550 (Parr Instrument Company, Moline, IL). It has a capacity of 450 mL and is able to attain maximum pressure of 200 bar and maximum temperature 300 ^{o}C. Stirring rate of stirrer was adjusted to 200 revolutions per minute (rmp) to ensure the homogeneity of the reaction medium. The reaction medium was prepared by mixing 100 mL of water and 10 g of biomass (10:1 ratio) and loaded into the reactor (Figure 2.1). Nitrogen gas was applied to keep the water in liquid state at high temperature for enhancing and altering the structure of lignocellulosic material (Alvira et al., 2010) and for preventing possible oxidation process. The system was pressurized to the target pressure by regulating with pressure gauge set on the gas tank. Optimization of the extraction process was investigated at temperatures between 170 ^{o}C and 190 ^{o}C and at pressures between 1 and 5 bar. Duration of this process was between 10 and 50 min. The temperature of the reactor was adjusted with an external heating mantle controlled by a Parr 4848 reactor controller. Once extraction time was completed, the temperature, as well as the pressure of the system, was reduced by quenching the reactor into the cold water bath. When the temperature of the reactor reached 45 ^{o}C or below the valve V-2 was opened to release the pressure. Water soluble extractives dissolved in the water during the hydrothermal

process were mainly HC. This liquid fraction was isolated from the solid woody fraction by vacuum filtration technique and was kept refrigerated for the further analysis.

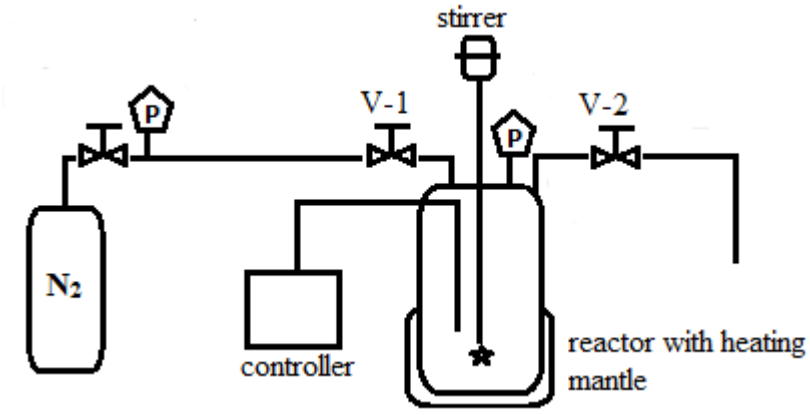


Figure 2.1 Schematic diagram of the reactor unit for the extraction of biomass by hydrothermal technique.

2.4 Analysis of extracted HC

Sugar content of extracted HC was determined according to NREL Laboratory Procedure (Sluiter et al., 2008; Sluiter et al., 2006). Aliquot of 20 mL was placed into the pressure tube. A volume of 697 µL of 72% sulfuric acid was added into the extract to make a 4% solution for converting oligomeric sugars into the monomeric sugars. The amount of sulfuric acid added was determined according to equation 2.1or according to the Appendix I of the NREL/TP-510-42623 procedure (Sluiter et al., 2006). After vortexing a couple of times the pressure tube was placed in the autoclave for 60 min at 121 ^{o}C to complete HC hydrolysis. Then, the content in the pressure tube was transferred into the small beaker and neutralized with calcium carbonate. The addition of calcium carbonate was stopped when the pH reached 4.5 and the excess of calcium carbonate was

allowed to neutralize the excess of sulfuric acid further until pH≈6. Then, the neutral solvent was filtered through a filter with 2 µm pore size, placed in HPLC vial and introduced to HPLC system for determination of sugar content.

$$V_{72\%} = \frac{[(C_{4\%} * V_S) - (V_S * [H^+] * 98.08 g\ H_2SO_4 / 2\ moles\ H^+)]}{C_{72\%}} \quad (2.1)$$

Where:

$V_{72\%}$ is the volume of 72% acid to be added, in mL

V_s is the initial volume of samples or standard, in mL,

$C_{4\%}$ is the concentration of 4% w/w H_2SO_4, 41.0 g/L,

$C_{72\%}$ is the concentration of 72% w/w H_2SO_4, 1176.3 g/L,

$[H^+]$ is the concentration of hydrogen ions, in mol/L.

2.4.1 High Performance Liquid Chromatography analysis

Sugar analysis was conducted by Agilent 1200 Series HPLC. It was equipped with auto sampler, Biorad Aminex-87P column and Refractive Index (RI) detector. The column temperature was 80 ^oC and detector temperature was 45 ^oC, respectively. HPLC grade water was used as a mobile phase. Flow rate of the mobile phase was 0.6 mL/min. Injection volume of the sample was 10 µL.

Hydroxymethylfurfural (5-(hydroxymethyl)-2-furaldehyde) and furfural (furan-2 carboxyaldehyde), sugar degradation products, were determined on Agilent 1100 Series HPLC. It was equipped with manual injection port, Agilent Eclipse-XDB-C18 reverse phase column and Ultra-Violet (UV) detector. The temperature of the detector and column were ambient. The mobile phase constituted of 5% acetic acid solution containing 80/20 ratio of HPLC grade methanol and HPLC grade water. Flow rate of the mobile phase was 1 mL/min and the injection volume of the sample was 10 µL.

2.4.2 Gel Permeation Chromatography (GPC)

Average molecular weight of extracted HC was determined by Waters GPC system. It consists from Waters 600E System controller, Waters 717 plus Auto sampler, Shodex OH pak SB-805HQ and Shodex OH pak SB-804HQ columns, and Waters 410 Differential Refractometer detector. The temperature of the detector was 45 ^{o}C and the temperature of the column was ambient. HPLC grade water was used as mobile phase with 1 mL/min flow rate of the mobile phase is. The injection volume of the sample was 10µL.

2.4.3 Fourier Transform Infrared spectroscopy (FT-IR) analysis

Measurements were performed in Varian 3100 FT-IR spectrometer by direct transmittance using KBr pellet technique. Each spectrum was recorded over 20 scans, in the range from 4000 cm^{-1} to 400 cm^{-1}.

2.4.4 ^{13}C NMR analysis

^{13}C NMR spectra of both HC isolated from sugarcane bagasse and southern yellow pine wood were dissolved in D_2O and introduced on a 400-2 NMR instrument at ambient temperature using a 12-µs pulse-width and 5 s delay time for maximum quantification results (Spectral Data Services, Inc., Champaign, IL) with about 400 scans accumulated. Number of points acquired was 17764. Peaks were integrated and integral values of the two spectra were compared to estimate the characteristics of the HC samples.

2.5 Isolation of HC

2.5.1 Membrane filtration

Extraction liquors obtained from the pretreatment procedure at optimum condition of each biomass type (sugarcane bagasse and southern pine wood) containing polysaccharide oligomers were concentrated on an Alfa Laval M-20 membrane filtration system. In the cross flow filtration system the feed solution was pumped from the feed tank to the membrane module by a Hydra-cell pump that applied trans-membrane pressure in the system. The pressure over the membrane was controlled by a control valve (0-25 bar). The filtration process was continued in the following order. First, ETNA10PP membrane with molecular cut-off 10,000 Da, then ETNA01PP with molecular cut-off 1,000Da and finally, NF membrane with lowest cut-off of 400 Da were used. During the filtration permeates of each type of membrane were collected and used as a feed solution for the next membrane. Retentates from each membrane were collected separately to determine the average molecular weight of polysaccharides, to calculate the yield of HC from each membrane and to determine the total yield of HC. The pressure, applied in the system, was adjusted depending on cut-off of the membranes. Pressure to ETNA10PP, ETNA01PP and NF membranes was 8, 6 and 4 bars (0.8, 0.6 and 0.4 MPa), respectively. The permeate flux was measured in relation between volume and time. The pure water flux tests were performed at the beginning and at the end of the filtration to detect the intensity of the membrane fouling and the effectiveness of the membranes. The reduction of pure water flux was determined by comparing the pure water flux (equation 2.2) before and after the filtration.

$$Fouling\ (\%) = (Aw\ before - Aw\ after)/(Aw\ before) * 100 \qquad (2.2)$$

where:

Aw before- is the pure water flux of membranes before membrane filtration of extracted HC solution,

Aw after-is is the pure water flux of membranes after membrane filtration of extracted HC solution,

Three different membranes ultra-filtration ETNA10PP (composite fluoro polymer, 10000 Da), tight ultra-filtration ETNA01PP (composite fluoro polymer 1000 Da) and nano-filtration NF (thin film composite on polyester 400 Da) were used for this study.

The pH of the feed solution was adjusted to 6-6.5, from the original value of 3.6, with 5N sodium hydroxide prior to membrane filtration to neutralize some carboxylic acids to salts. This helped to decrease their ability to form hydrogen bonds with lignin and stabilized their network (Koivula et al., 2011).

2.5.2 HC concentration

Retentates of membrane filtration with molecular cut-off from 400, 1000 and 10000Da were freeze dried on Freeze Dryer 4.5 Labconco system (Kansas City, Missouri). Dried HC were kept in an air tight container for further analysis such as FT-IR, ^{13}C NMR and for film preparation purposes.

2.6 Preparation and analysis of the films

HC films were prepared from 50 mL aqueous solution containing different ratios of HC (HC), carboxymethyl cellulose (CMC) of sodium salt and fixed amount of sorbitol (Table 2.1).

Initially, water, CMC and HC were placed in an Erlenmeyer flask and stirred under the heat until complete dissolution of CMC followed by addition of sorbitol. Once

the temperature reached 95 ^{o}C heating and stirring was continued for additional 15 min. After cooling, the mixture was poured into 13 cm diameter Petri dish with Teflon insert and dried at ambient condition. Thickness of the prepared films were measured with Fowler & NSK Max Cal electronic digital caliper and used for further analysis such as water vapor permeability, moisture absorption, oxygen permeability and tensile strength tests.

Table 2.1 Composition of mixtures used to prepare films from sugarcane HC and southern yellow pine HC.

HC/CMC (%)	HC (g)	CMC (g)	Sorbitol (g)	Water (mL)
80/20	1.12	0.28	0.49	50
70/30	0.98	0.42	0.49	50
60/40	0.84	0.56	0.49	50
50/50	0.70	0.70	0.49	50

2.6.1 Water Vapor Permeability (WVP)

WVP was measured according to Ghanbarzadeh et al. and Mali et al. modified technique (Ghanbarzadeh et al., 2011; Mali et al., 2006). Initially, films were cut in disks that were larger than the rim of the cups used in the analysis. Approximately 3g of calcium sulfate anhydrous was placed in the cup and covered with films. All cups were placed in the desiccators. RH in the desiccators was 97% which was created by potassium sulfate saturated solution at 25 ^{o}C. Cups were weighed every 2 h for a day and then every

24 h for 48 hr. Amount of water permeated through the film were determined by the calcium sulfate anhydrous weight which gained throughout the time frame between the measurements. The test was conducted twice for each specimen.WVP was calculated by equation 2.3.

$$WVP = slope * \frac{h}{P*RH} \quad (2.3)$$

where,

Slope- water vapor permeability rate (g/h),

h- thickness of the film (m),

P- saturation water vapor pressure (Pa),

RH- relative humidity (RH) in desiccators.

2.6.2 Moisture Absorption

Moisture absorption was measured according to Angles and Dufrense (2000). First, films cut in 2x2 cm dimension were conditioned in the desiccators with anhydrous calcium sulfate for 24 h. Films were then weighed and placed in the desiccators with 55% RH. The RH was created with calcium nitrate saturated solution at 20-25 ^{o}C. Films were weighed while conditioning until an equilibrium state was reached. The test was conducted three times for each specimen. The moisture absorption was calculated by equation 2.4:

$$\text{Moisture absorption } \% = \frac{(Wt-Wo)}{Wo} * 100 \quad (2.4)$$

where,

W_t is the weight of the film when films after t time at 55% of RH desiccators,

W_o is the initial weight of the sample.

2.6.3 Oxygen permeability (OP)

Oxygen Transition Rate (OTR) of the above prepared films was tested on Mocon Oxtran 2/21 oxygen permeability instrument. The inner half of the test cell was routed through with carrier gas which consisted of 98% nitrogen and 2% hydrogen. The outer half of the cell was supplied with 100% oxygen gas at 35% RH. Oxygen permeates were picked up by nitrogen gas flowing through the inner half. The amount of the oxygen carried out by the carrier gas was measured by coulometric sensor (Coulox®) specific for oxygen molecules. The measurements were conducted at 25 ^{o}C and 35% RH. The area of the samples was 5 cm^2 and the oxygen flow was 20 mL/min. OTR measurements were run twice for each specimen. OP (cc μm /(m^2 day kPa) values were derived from OTR (cc/ m^2 day) values by considering the thickness of the films and the pressure of the oxygen gas applied.

2.6.4 Tensile strength

The mechanical properties of the films were determined by a Lloyd universal testing machine (Lloyd Instruments, England) with load cell of 50N. The method used was pull to break. The test is took place at room temperature 25 ^{o}C at 50% RH. Samples were cut in 10 cm long and 1.5 cm wide strips. Thickness of the samples was measured by Fowler & NSK electronic digital caliper. The gauge distance was 30 mm, the rate of grip separation was 50 mm/min. Stress and strain at break were recorded and the Young's modulus at the break was determined. Deflection at maximum load and percentage of strain at break were evaluated. Three specimens were tested and the results were averaged.

CHAPTER III

RESULT AND DISCUSSION

3.1 Sugarcane bagasse

3.1.1 Introduction

Sugarcane is a perennial grass which grows in humid and warm environment. Sugarcane is planted in southern part of United States such as Alabama, Louisiana, Georgia and Mississippi. In industry 1 ton of sugarcane is crushed for sugar production and produces nearly 0.280 tons of bagasse (Sun et al., 2004a). Bagasse is fiber remaining after the extraction of the sugar-bearing juice from sugarcane (Britannica, 2012). Approximately 54 million tons of dry bagasse is produced annually throughout the world (Rodrigues et al., 2003). Generally, bagasse is stored and less than 50% of it utilized for electricity and rest of the bagasse is underutilized (Rocha et al., 2011). Sugarcane bagasse can be used as a raw material for cellulose, HC and lignin derived products. HC can be used as a feedstock in the food industry for sweetener and food additives, in pharmaceutical industries as a precursor of antiviral and antitumoral drugs, and in packaging industries as an oxygen barrier.

3.1.2 Chemical analysis of sugarcane bagasse

Chemical analysis of the sugarcane bagasse was conducted according to the NREL/TP-510-42618 laboratory procedure (Sluiter et al., 2008). The total HC content in 100 g oven dried (OD) biomass was 26.75 g. Chemical analysis showed that HC

consisted mainly from xylose (66.9%) and small amount of galactose (11.77%), arabinose (13.57%) and mannose (7.73%) (Table 3.1).

Table 3.1 Chemical composition of oven dried sugarcane bagasse.

Composition of OD sugarcane bagasse (g/100g)	
Glucose	45.28
Xylose	17.90
Galactose	3.15
Arabinose	3.63
Mannose	2.07
Lignin	24.15
Ash	2.26

3.1.3 Optimization of extraction condition

Hydrothermal extraction technique was employed for extraction of sugarcane bagasse HC. Three parameters have been studied for determining the optimum condition. The effect of time and temperature was determined followed by studying the effect of pressure. The ratio of biomass to pretreatment solution was 1:10 and no catalyst was added.

3.1.3.1 Effect of time and temperature on the extraction of HC

Figure 3.1 shows the result of hydrothermal extraction of sugarcane bagasse HC. Extraction was conducted at 170 ^{o}C and 1MPa pressure for a time ranging from 10 to 50 min. The result exhibits that the yield of the extracted sugars is directly proportional to time. The highest yield for extracted sugars 81.9% xylose, 35% arabinose, 17% galactose, and 7.7% mannose (based on oven dry weight of bagasse HC) was obtained after 50 min. Since the objective of the project is to determine an inexpensive technique

to modify the extraction process, hydrothermal extraction at 170 °C was not continued more than 50 min.

Instead, the extraction was performed at 180 °C and 1MPa for 10 to 50 min. At this temperature, the yield of xylose increased until the time of extraction reached 30 min (Figure 3.2). As the extraction continued for 30, 40 and 50 min, the yield of xylose was relatively stabilized and reached 84.96%, 81.27% and 84.11%, respectively. In the case of mannose, as the extraction time increased the amount of extractable mannose increased and reached a maximum 39.01% at 50 min. The yield of galactose and glucose was increased by small increment. However, the yield of arabinose was inconsistent.

It was observed that increasing the temperature by 10 °C (from 170 to 180 °C) led to extraction of a high amount of xylose in shorter reaction time. For example, 81.9% of xylose was extracted at 170 °C and 50 min. It was similar to the amount of xylose extracted at 180 °C for 30 min. Thus, the extraction was conducted at 190 °C 1MPa for short time 10 to 40 min (Figure 3.3). However, at 190 °C, as the extraction time increased the amount of extracted HC decreased. Maximum amount of sugars, except mannose, was obtained after 10 min. The yields of xylose, galactose and arabinose were 78%, 59.08% and 48.53%, respectively. The yield of glucose was relatively stable in all this different temperatures.

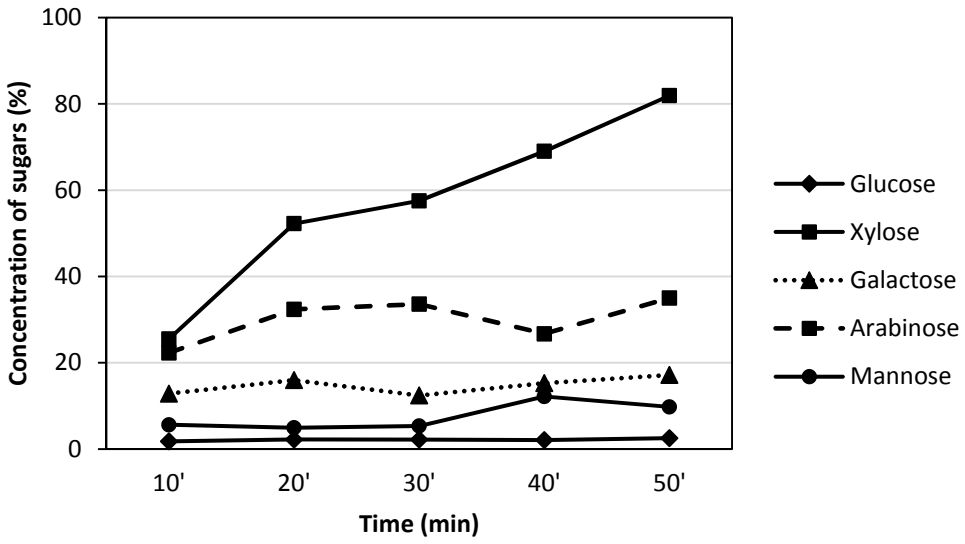


Figure 3.1 Effect of the time on the yield of sugarcane bagasse HC extracted at 170 ^{o}C.

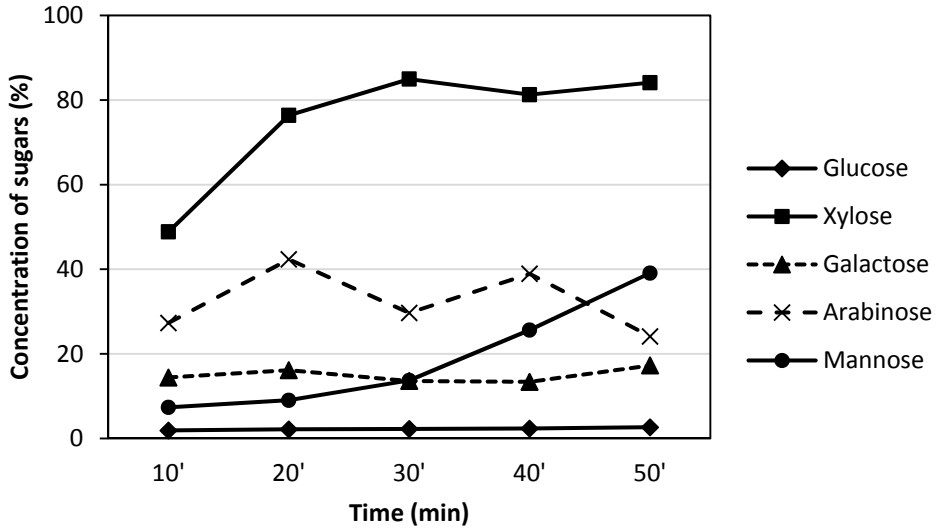


Figure 3.2 Effect of the time on the yield of sugarcane bagasse HC extracted at 180 ^{o}C.

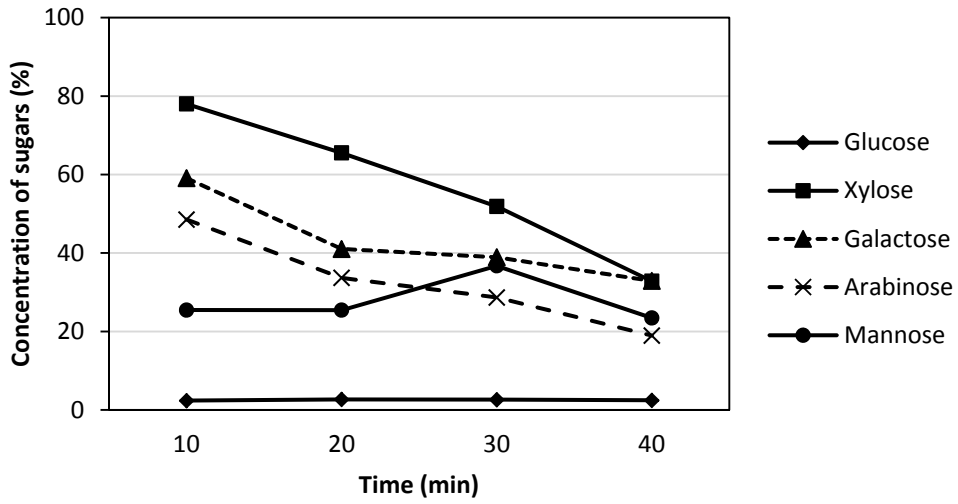


Figure 3.3 Effect of the time on the yield of sugarcane bagasse HC extracted at 190 ^{o}C.

In addition, to temperature and time of the pH value of the aliquot, concentration of HC degradation compounds, and concentration of the soluble lignin were considered.

Figure 3.4 depicts the effect of extraction time and temperature on the pH value of the aliquots. An identical trend was observed at 170, 180 and 190 ^{o}C. The pH values of aliquots were reduced as the extraction time increased. Similarly, the pH values of aliquots decreased as the temperature of extraction increased from 170 to 190 ^{o}C. It appears that high temperature as well as extended period of extraction promotes disintegration of the acetyl group moiety from the xylan backbone producing acetic acid (Carvalheiro et al., 2008).

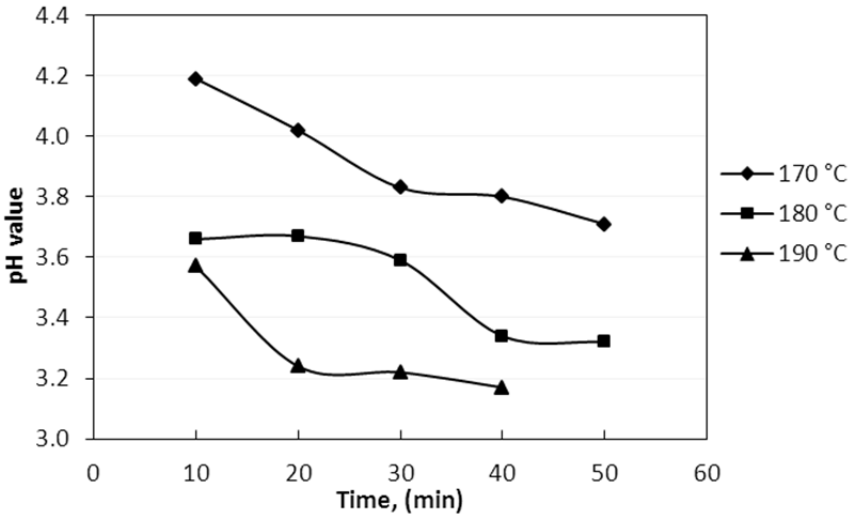


Figure 3.4 Effect of time and temperature on the pH values of the sugarcane bagasse HC extract.

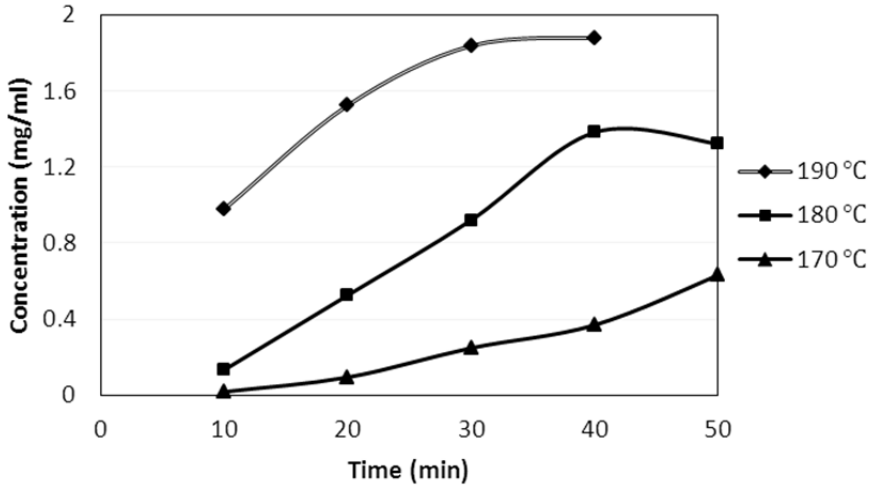


Figure 3.5 Effect of time and temperature on the production of furfural during the extraction of sugarcane bagasse HC.

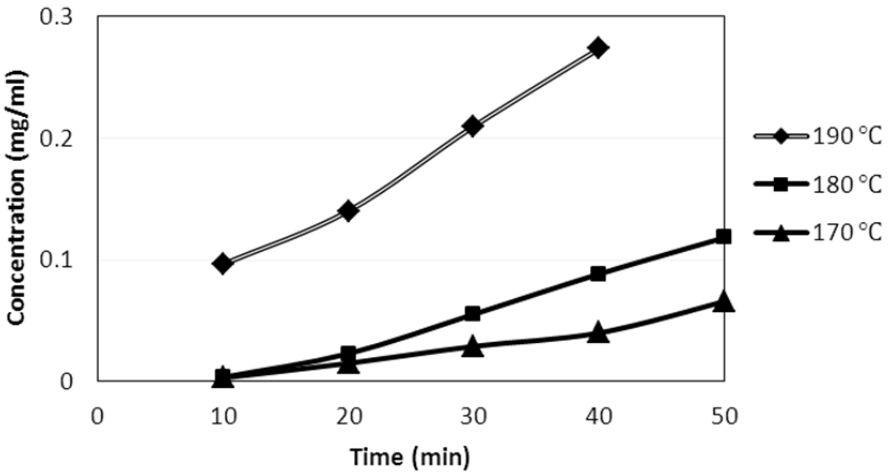


Figure 3.6 Effect of time and temperature on the production of HMF during the extraction of sugarcane bagasse HC.

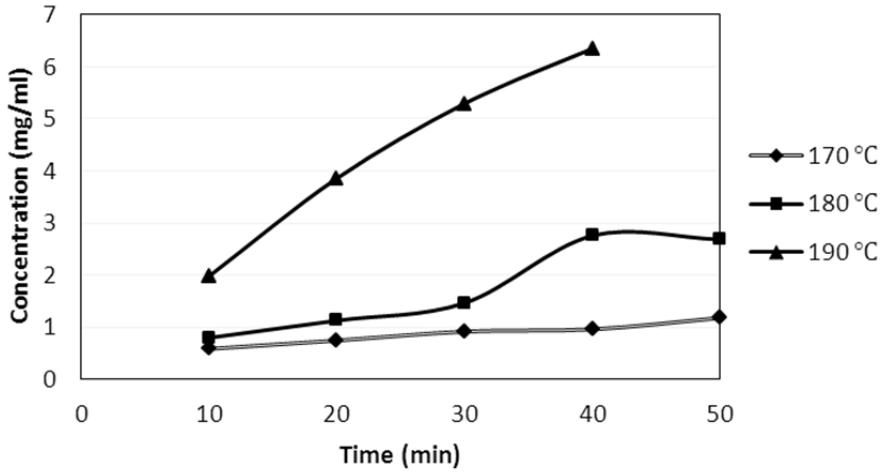


Figure 3.7 Effect of temperature and time to the amount of soluble lignin during the extraction of sugarcane bagasse HC.

Figures 3.5 and 3.6 illustrate the effect of temperature and time on the amount of furfural and 5- hydrohymethylfurfural (HMF). These are the compounds produced due to the degradation of penta- and hexa sugars of HC, respectively. The concentration of furfural and 5- hydrohymethylfurfural was increased as the extraction time increased.

Also, as the extraction temperature increased the concentration of degradation products also increased. At high temperature or at elongated period of extraction, the production of acetic acid increases and further causes the hydrolysis of sugars leading to the degradation of monomeric sugars to furfural and HMF. For the isolation of a high content of HC, the formation of furfural and HMF is not favored. Therefore, the condition gave low furfural and HMF content was taken in consideration as an optimum pretreatment condition.

Figure 3.7 depicts the effect of the time and temperature to the content of soluble lignin. Its content was determined by scanning the aliquot on the UV-Vis spectrophotometer at the wavelength 200-400 nm. The major absorption for lignin occurred at the 275-278 nm (Fukushima and Hatfield, 2001; Yoshida et al., 2002). As the time and temperature increased, the amount of soluble lignin increased as well. It was also noted that as the temperature increased, the increment of the lignin content is increased. For example, at 170 ^{o}C the absorbance at 10, 20, 30, 40 and 50 min was 0.58%, 0.74%, 0.92%, 0.96% and 1.18%, respectively. However, at 190 ^{o}C the absorbance was 1.98%, 3.86%, 5.296% and 6.35% at 10, 20, 30 and 40 min. The increment of the absorption value was much higher at 190 ^{o}C than the absorbance increment at 170 ^{o}C in the same increment of time. Therefore, from the figures 3.4 - 3.7 it can be concluded that the increase of extraction time and temperature leads to production of high sugar degradation products and high dissolution of lignin components.

3.1.3.2 Effect of the applied pressure

The effect of pressure was evaluated for determining the optimum extraction condition. In hydrothermal extraction the pressure was applied to keep the water in liquid

state and incite the alteration of lignocellulosic material structure (Alvira et al., 2010). At 1 MPa pressure, the highest amount of xylose with moderate amount of HC degradation compounds was obtained at 180 ^{o}C and 30 min. Thus, the pressure effect was conducted at this condition. Figure 3.8 exhibits the effect of pressure on the extractability of the HC. When the pressure was increased from 0 to 5 MPa, the xylose content increased by a small increment (Table 3.4). It appears that the pressure change does not significantly affect the extractability of the HC in the chosen range of the pressure. The yields of xylose at pressure 0, 1, 2, 3, 4 and 5 MPa were 80.91%, 84.96%, 83.56%, 84.68%, 85.39% and 87.37%, respectively. However, the HMF and furfural contents were relatively high 1.22 mg/mL and 0.09 mg/mL (Figure 3.9) when no pressure was applied. At 1 MPa the HC degradation compounds production were relatively low 0.95 mg/mL HMF and 0.07 mg/mL furfural comparing to the ones at 2, 3, 4 and 5 MPa.

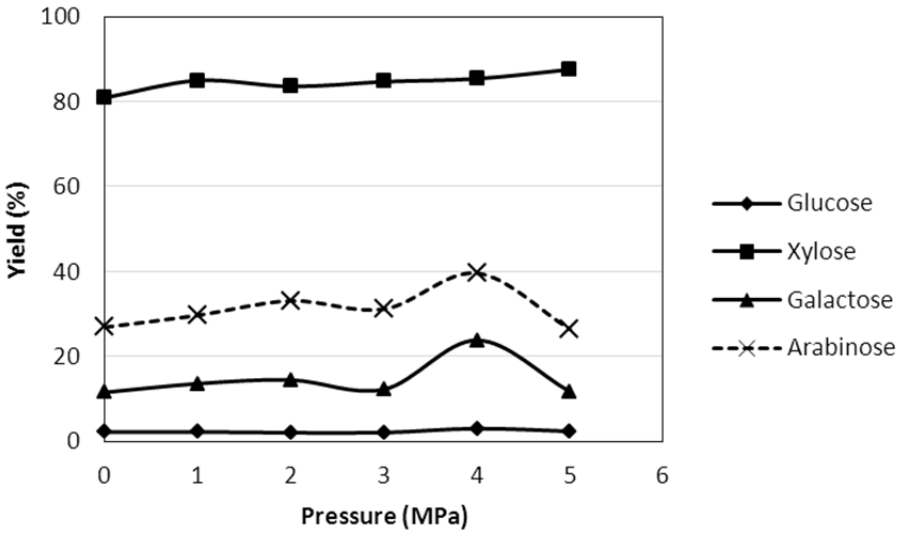


Figure 3.8 Effect of pressure to the yield of sugarcane bagasse HC at the 180 ^{o}C 30 min.

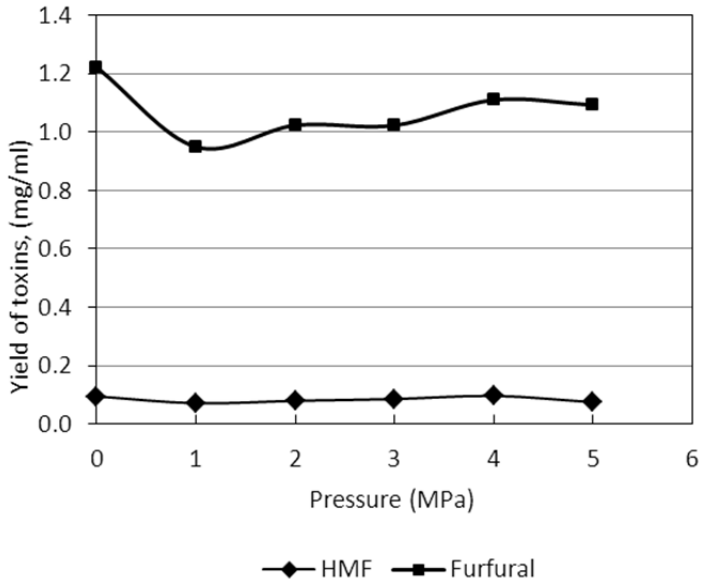


Figure 3.9 Effect of pressure to the yield of the sugar degradation products during the extraction of sugarcane HC at the 180 ^{o}C 30 min.

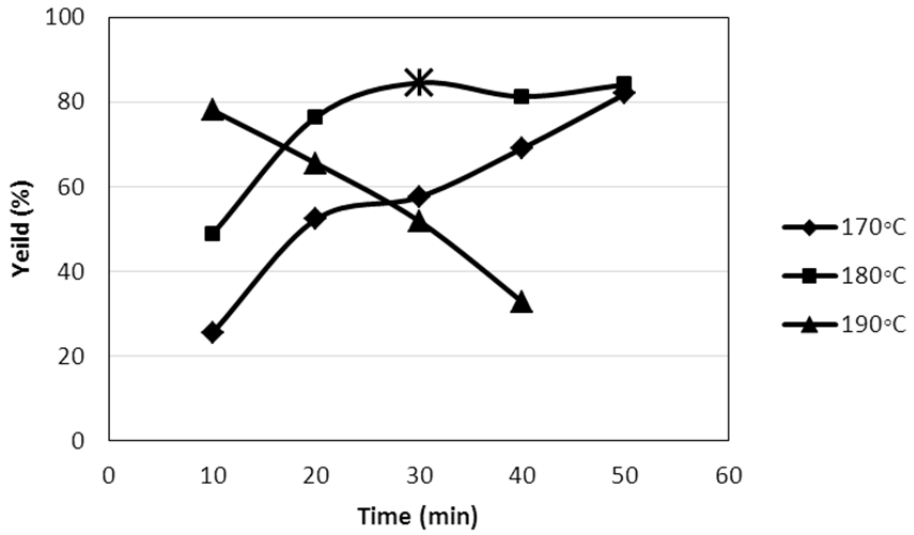


Figure 3.10 The optimum condition for sugarcane bagasse pretreatment.

Xylan is the main HC in plants (Ebringerová and Heinze, 2000; Sjöström, 1993). Chemical content analysis showed that the main sugar in HC of sugarcane bagasse was xylose (Table 3.1). Thus, the optimum condition for extraction of HC (Figure 3.10) was evaluated based on xylose content.

Based on the above results, it can be summarized that the optimum condition for extraction of HC with highest xylose content and lowest degradation compounds was 1 MPa pressure at 180 ^{o}C temperature for 30 min extraction time.

3.1.4 Isolation of HC using membrane filtration

3.1.4.1 Filtration data

The feed solution prepared at the optimum condition contained 14.80 mg/mL (Table 3.2) HC with 27700 Da average molecular weight (MW) (Tables 3.4). Based on the total volume of the feed solution, it was expected to isolate 53.57 g of total HC (Table 3.3). According to the dry content percentage, it was expected to isolate 66.97 g of HC. Since the HPLC system was able to detect only carbohydrates, the difference between the above two values possibly represented the lignin and non-volatile impurity contents. The permeate of ETNA10PP membrane contained 10.20 mg/mL HC with average MW 20540 Da and its retentate contained 22.59 mg/mL HC with average MW 34100 Da. A similar trend was observed during the filtration process with ETNA01PP and NF membranes. (Note: Permeate from ETNA10PP was used as a feed solution for ETNA01PP membrane filtration. Permeate from ETNA01PP was used as a feed solution for NF membrane filtration as well). The concentration of HC in permeate of ETNA01PP was 5.49 mg/mL and in retentate was 16.37 mg/mL. The average MW of ETNA01PP membranes

permeate was 8350 Da, for retentate it was 29100 Da. In the case of NF, the concentration of permeate was 1.14 mg/mL and for retentate it was 14.49 mg/mL. The average MW of permeate was 550 Da and MW for retentate reached 4430 Da.

Table 3.2 Sugarcane bagasse HC concentration in the feed, permeate and retentate fractions of three different membranes.

	Feed		Permeate		Retentate	
	Dry content %	Total sugars* mg/mL	Dry content %	Total sugars* mg/mL	Dry content %	Total sugars* mg/mL
ETNA 10000PP	1.85	14.80	1.20	10.20	2.90	22.59
ETNA 1000PP	1.20	10.20	0.74	5.49	2.07	16.37
NF	0.74	5.49	0.06	1.14	2.00	14.49

*Sugar content was determined by HPLC system.

Table 3.3 Total yield of sugarcane bagasse HC calculated based on dry content and HPLC data.

	Feed		Permeate		Retentate	
	Dry content (g)	HPLC sugar* (g)	Dry content (g)	HPLC sugars* (g)	Dry content (g)	HPLC* (g)
ETNA 10000PP	66.97	53.57	36.00	30.59	19.29	15.03
ETNA 1000PP	35.52	30.59	17.76	13.51	11.36	9.00
NF	17.76	13.51	1.18	2.11	12.80	9.28
Total					43.45	33.10

*Sugar content calculated based on HPLC result.

Table 3.4 Average molecular weight of membrane filtration fractions of HC extract.

	MW (Da)
Feed	27700
ETNA10PP Permeate	20540
ETNA10PP Retentate	34100
ETNA01PP Permeate	8350
ETNA01PP Retentate	29100
NF Permeate	550
NF Retentate	4430

All retentates from different membranes were separately freeze dried. ETNA10PP retentate weighed 19.04 g, the one from ETNA01PP weighed 9.80 g, and the retentate from NF membrane weighed 13.50 g. A total of 42.34 g of HC were isolated by the membrane filtration techniques. According to the HPLC sugar analysis, ETNA10PP retentate contained 15.03g, ETNA01PP retentate contained 9.00 g and NF retentate contained 9.28 g of HC. It was expected the 33.10 g of HC would be isolated. According to the dry content percentage, it was expected to obtain 43.45 g of HC, of which 19.29 g from ETNA10PP retentate, 11.36g from ETNA01PP retentate and 12.80 g of HC from NF retentate. The weight difference (9.24 g) between the values calculated from dry content and HPLC data suggests inclusion of an impurity such as lignin (Table 3.5). From the experimental value and HPLC value it can concluded that approximately 43.3% of dissolved lignin has a size smaller than 10000 Da and higher than 1000 Da. 8.7% of the lignin has a size between 1000 and 400 Da and 45.7% has a size smaller than the 400 Da. Goksu et al. (2007) and Zhu et al. (2011) report that lignin content improves the property of the film. Therefore, lignin content in the isolated HC was kept as it was.

Table 3.5 Calculated and experimentally obtained sugarcane bagasse HC yields.

	Dry content (g)	HPLC (g)	Experiment (g)
ETNA10PP	19.29	15.03	19.04
ETNA01PP	11.36	9.00	9.80
NF	12.80	9.28	13.50
Total	43.35	33.10	42.34

3.1.4.2 Performance of membranes

Efficiency of membranes was determined by Pure Water Flux (PWF) testing. Figures 3.11, 3.12 and 3.13 exhibit the PWF of ETNA10PP, ETNA01PP and NF membranes, respectively. In all three cases PWF before filtration was higher than the PWF after the filtration, meaning the performance of the membranes diminished due to fouling. Also, it can be seen that the PWF of ETNA10PP is higher than the PWF of ETNA01PP and both are higher than the PWF of NF. However, fouling is relatively higher for ETNA10PP than for ETNA01PP and the fouling of ETNA01PP is higher that the fouling of NF membrane.

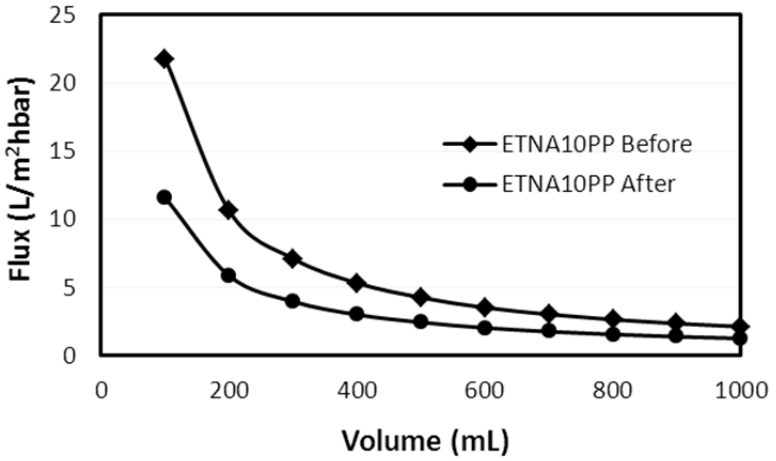


Figure 3.11 Pure water flux of ETNA10PP membrane before and after filtration of sugarcane bagasse HC extract.

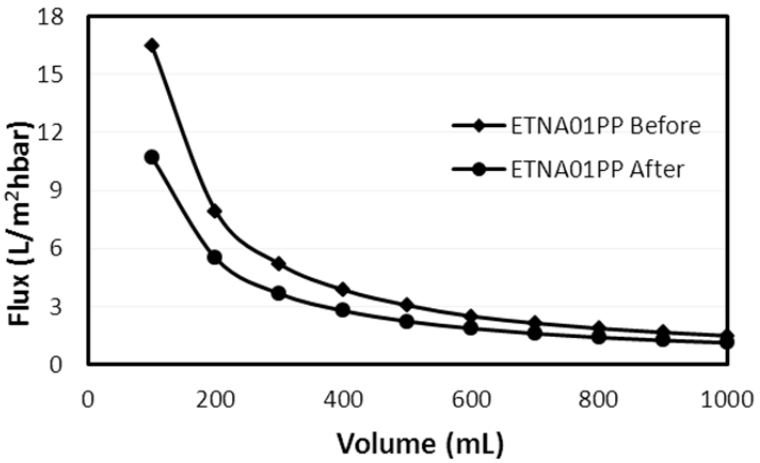


Figure 3.12 Pure water flux of ETNA01PP membrane before and after filtration of sugarcane bagasse HC extract.

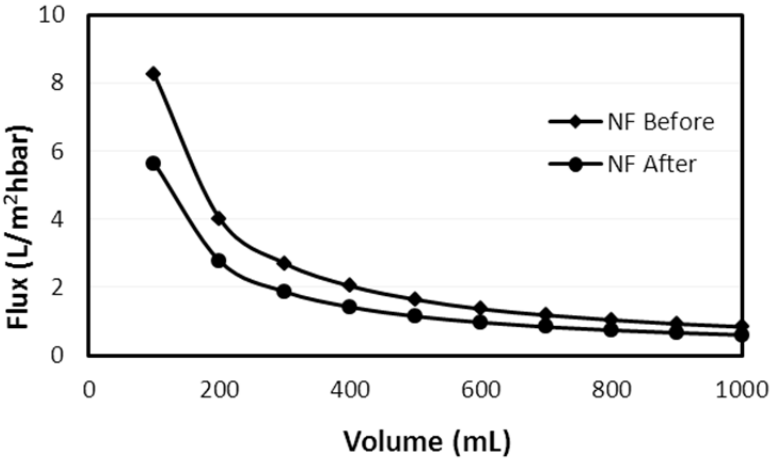


Figure 3.13 Pure water flux of NF membrane before and after filtration of sugarcane bagasse HC extract.

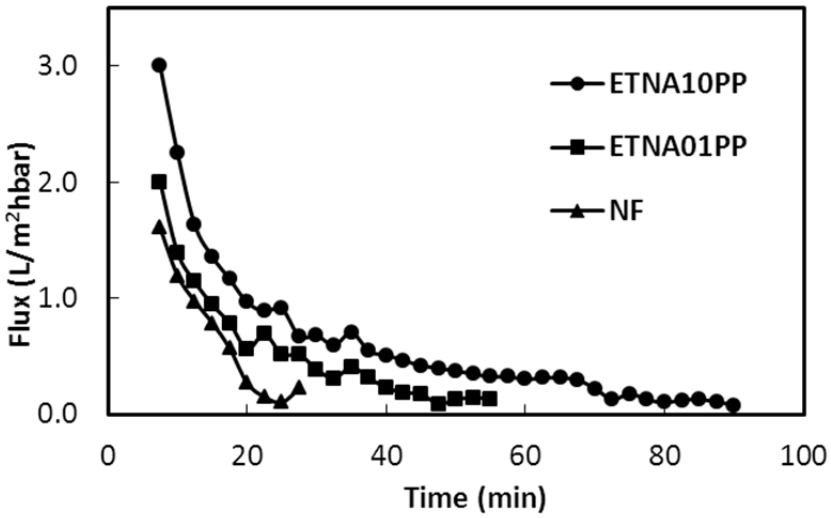


Figure 3.14 Flux of membranes during the membrane filtration of sugarcane bagasse HC extraction.

The performance of the membrane during the concentration of HC extract is depicted in the Figure 3.14. Flux of the membranes was accordingly, ETNA10PP > ETNA01PP > NF and the fouling of the membranes took place in same order.

3.1.4.3 Mass balance

Sugarcane bagasse composition after hydrothermal extraction changed considerably. Total HC content decreased from 26.75 to 8.39% (Table 3.6). Sugarcane bagasse residue mainly consisted of glucose 51.89% and lignin 35.16% compared with 45.28% glucose and 24.15% lignin in the original sample. Most of HC 68.64% representing xylose, mannose, arabinose and galactose were removed by the hydrothermal extraction technique.

Table 3.6 Chemical composition of sugarcane bagasse before and after hydrothermal pretreatment.

Composition of dry bagasse (g/100g)		
	Before treatment	After treatment
Glucose	45.28	51.89
Xylose	17.90	7.29
Galactose	3.15	nd
Arabinose	3.63	0.78
Mannose	2.07	0.32
Lignin	24.15	35.16
Ash	2.26	1.8

nd- not determined

3.1.5 Characterization of isolated sugarcane bagasse HC

3.1.5.1 FT-IR characterization of the isolated HC.

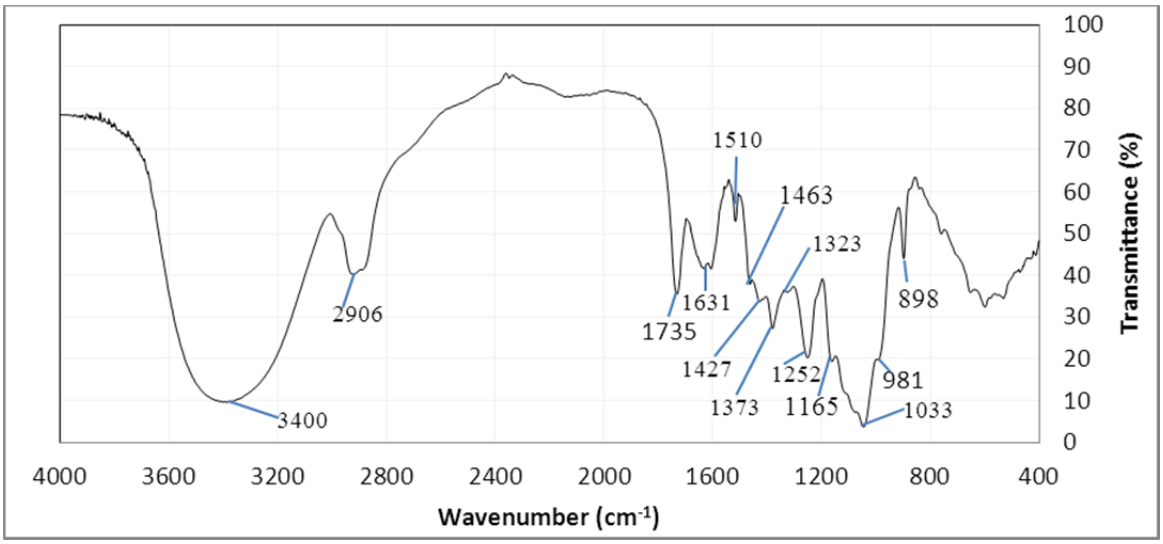


Figure 3.15 FT-IR spectrum of isolated sugarcane bagasse HC.

The FT-IR data showed that the isolated fraction illustrated a typical signal of HC giving a specific band in the 1200 - 1000 cm^{-1} region (Figure 3.15). At 1033 cm^{-1} spectrum has dominant stretching and bending vibration from C-O, C-C, C-OH and C-O-C bonds. Bands between 1165 and 1000 cm^{-1} represents typical xylan. The two low intensity shoulders at 1165 and 981cm^{-1} represent the presence of the arabinosyl side chains (Ma et al., 2012). This is due to the vibration of xylan units at the branched positions of C-2 and C-3. According to Sun and Tomkinson (2002) report, the arabinosyl side chain is only attached to the xylopyranosyl backbone and these two peaks are used for identification of the arabinoxylan structure. Kacuráková et al. (2000) reported that the intensity of these two peaks decrease as the number of branching increases.

Intensive band at 1735 cm^{-1} is represents the carbonyl stretch assigned to the acetyl, glucuronic acid and ferulic ester groups of polysaccharides. Sharp and intense peak at 898 cm^{-1} represents C-1 group frequency or ring frequency that characterize β-glycosidic linkage between the sugar units (Fang et al., 2000; Gupta et al., 1987). Bands at 1463 and 1427 cm^{-1} represent the $–CH_2$ stretching vibration. The bands at 1373, 1323 and 1252 cm^{-1} originate from C-H, OH or $–CH_2$ bending vibration. An insensitive signal at 2906 cm^{-1} is due to the C-H stretching vibration. Another extensive band at 3400 cm^{-1} comes up due to the –OH stretching vibrations of the HC and water. There is a sharp peak at 1510 cm^{-1}, which represents the existence of lignin in the isolated HC (Owen and Thomas, 1989; Sun et al., 2004b).

3.1.5.2 ^{13}C NMR

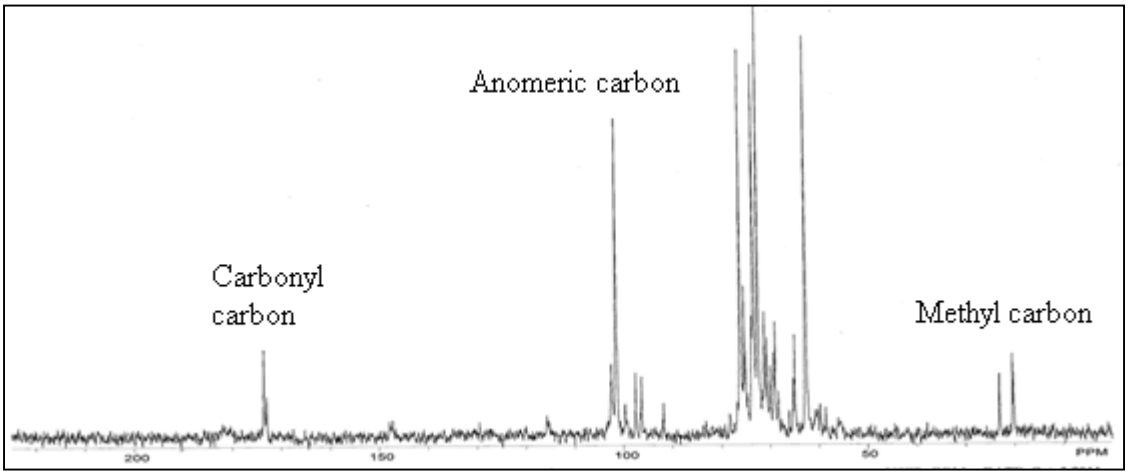


Figure 3.16 ^{13}C NMR of isolated sugarcane bagasse HC fraction.

The isolated hemicellulosic fraction was analyzed by ^{13}C NMR spectroscopy to characterize the structural feature. The spectrum is shown in the Figure 3.16. Five strong

signals at δ 101.49, 76.18, 73.47, 72.53 and 62.796 ppm, which are ascribed respectively to C-1, C-4, C-3, C-2 and C-5 of →4)-β-d-Xyl*p*- (1→ residue substituted at 2 and 3 positions with -OAc and 4-O-Me-GlcA. Signals δ 102.49, 75.07, 75.07 and 65.05 ppm represent C-1, C-2, C-4 and C-5 carbons of →4)-β-d-Xyl*p*- (1→ residue. The carbonyl carbon resonance of uronic acids gives a signal at 173.01 ppm which indicates C-6 in methyl uronic acids. Also anomeric carbon of α-D-GlcA gives the signal at δ 97.54 ppm. The carbonyl carbon resonance of an acetyl group attached to the C-2 and C-3 of xylan backbone gives signal at 173.53 ppm. Signals at 20.22 and 20.45 ppm arise from $–CH_3$ group carbon of -OAc group at C-3 and C-2 carbons of xylan backbone (Ebringerova et al., 1992; Gabrielii et al., 2000; Sun et al., 2004b; Vignon and Gey, 1998). This result implies that the hemicellulosic fraction can be represented by (4-O-methyl-α-D-GlcA) →4)-β-d-Xyl*p*- (1→ with a small amount of lignin impurities. In fact, the lignin impurity in the sample showed signals at δ 173.53 and 56 ppm from carbonyl and methoxyl groups carbons of lignin (Capek et al., 2000).

3.1.6 Film preparation

Films were prepared from the isolated HC with various HC content using water casting method (Table 2.1). Mixture of HC, CMC and sorbitol formed a homogeneous suspension, which upon drying created similar, homogeneous and transparent films. It was observed that as the content of the HC increased the properties of the films changed slightly and it became more moist sensitive and flimsy. The color of the film was slightly brownish-yellow (Figure 3.17). This color is possibly from the lignin content in HC. Thickness of the prepared films was around 0.1 mm.

Figure 3.17 Sugarcane bagasse HC-based film.

3.1.7 Film testing

3.1.7.1 Water absorption

Water absorption isotherm of the sugarcane bagasse HC films at 55% RH and 25°C is presented in the Figure 3.18. The water content differences of the films were not very large between the samples. Generally, water uptake of the films was rapid in the first 8 h and continued increasing until 24 h. The water uptake was small during 36 and 48 h. The increment of absorption tended to decrease and further stabilize. At 36 h the film containing 80% HC absorbed the highest content of water (24.63%). Next maximum water absorption (23.63%) determined with the 70% HC containing film. The film with 60% HC appeared to absorb slightly low content of water (20.86%) than the one with lowest content 50% HC (21.99%) during the 36 h measurement. Overall, the water absorption of the sugarcane bagasse based films increased as HC content was increased.

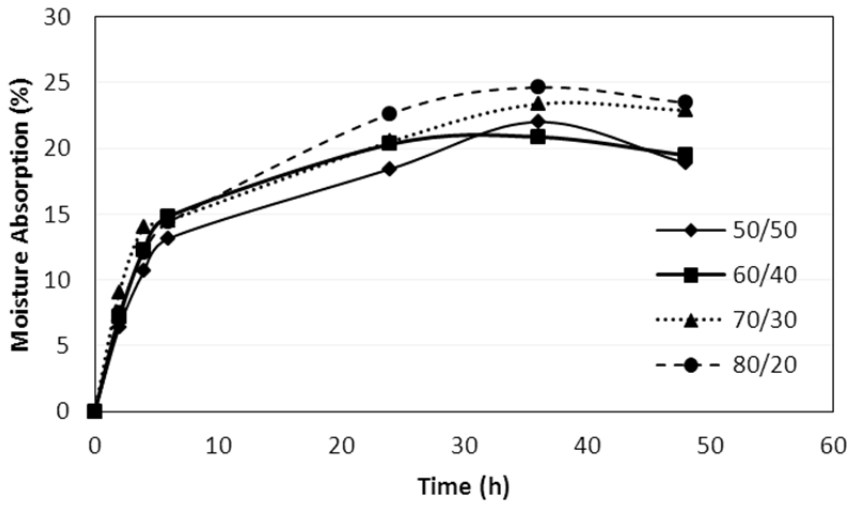


Figure 3.18 Water absorption trend of sugarcane bagasse HC-based films.

Note: tests were run in duplicate and the values were averaged.

3.1.7.2 Water vapor permeability

Figure 3.19 exhibits the water vapor permeability trend of sugarcane bagasse HC based films. The film containing 60/40% of HC /CMC shows the lowest ($3.84e^{-12}$ g/Pa h m) water vapor permeability than the films containing 50/50, 70/30 and 80/20% HC and CMC. It is possible that the mixture ratio was the optimum that permeates less water vapor. On the other hand, as Goksu et al. (2007) noted, the WVP is an important parameter in food packaging films.

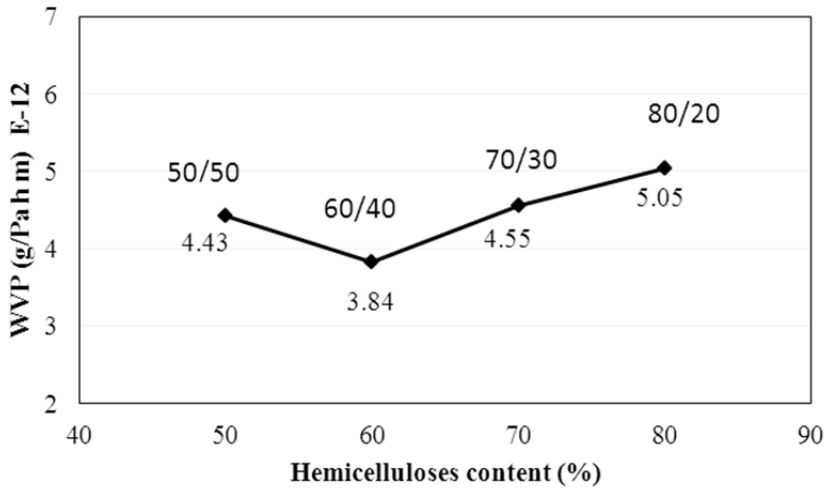


Figure 3.19 Water vapor permeability of the sugarcane bagasse HC-based films.

Note: tests were run in duplicate and the values were averaged.

Depending on the purpose of the usage of the films, the desirability of the WVP of the films needed to be different. For example, for vegetable packaging high water vapor permeable material is desired. However, food materials need to be kept dry but ones faced in a humid environment. This use requires low WVP films. In general, the films produced from sugarcane bagasse HC have relatively low WVP than the films in the reference literature (Hansen and Plackett, 2008; Hartman et al., 2006). This can be explained by the lignin content, which provided stronger interactions between HC (Goksu et al., 2007).

3.1.7.3 Oxygen permeability

Table 3.7 Oxygen permeability (OP) and oxygen transmission rate (OTR) of sugarcane bagasse HC-based films at 35% relative humidity.

HC/CMC (%)	Avg. thickness (µm)	OTR (cc/(m^2 day))	OP (cc µm /(m^2 day kPa))
0/100	97.0	0.0095	0.009415
50/50	112.0	0.0130	0.014606
60/40	104.5	0.0050	0.005265
70/30	100.0	1.6530	1.583212
80/20	91.0	0.8235	0.642887

Note: tests were run in duplicate and the values were averaged.

Oxygen permeability data of the SCBH films are shown on the Table 3.7(tests were run in duplicate and the values were averaged). The OP of the system with ratio 60/40 CH/CMC had the lowest value 0.005265 cc µm / (m^2 day kPa) than the other films. The film 50/50 HC/CMC had relatively high OP 0.014606 cc µm / (m^2 day kPa). Compared to the measured OP values of some commercialized polymers such as ethylene vinyl alcohol (0.1-12 cc µm / (m^2 day kPa) at 0-95% RH) and low-density polyethylene (1870 cc µm / (m^2 day kPa) at 50% RH) (McHugh and Krochta, 1994), the OP values of sugarcane bagasse HC based films at the above ratios have comparable values. This indicates that the SCBH films are suitable for using as an oxygen barrier.

3.1.7.4 Tensile strength

Table 3.8 Mechanical properties of sugarcane bagasse HC-based films.

HC/CMC (%)	Young's modulus (MPa)	Deflection at max load (mm)	% Strain at break
0/100	794,90	4,85	16,17
50/50	58,76	14,87	49,55
60/40	22,00	20,96	70,01
70/30	18,97	20,91	78,85
80/20	3,77	23,86	93,22

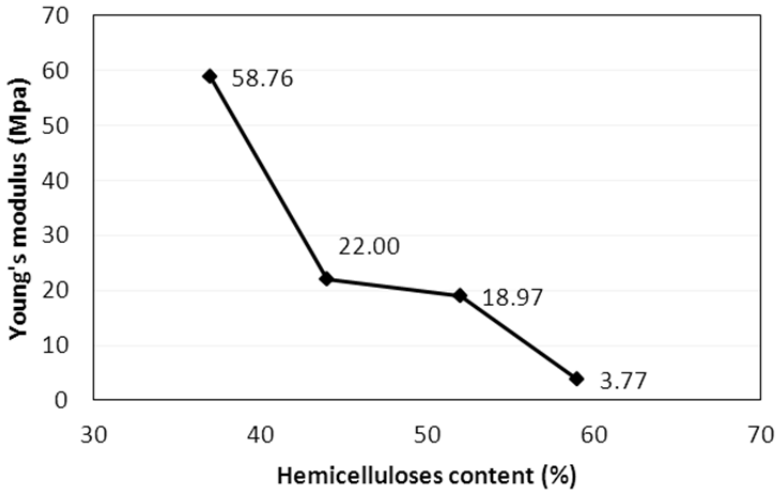


Figure 3.20 Young's modulus of sugarcane bagasse HC-based films.

Note: 0% sugarcane bagasse HC based films Young's modulus was not included in the graph because the value was incomparably high.

The mechanical properties of the films were evaluated using tensile testing at 50% RH and ambient temperature. All tests were run in duplicate and the values were averaged. Table 3.8 shows the results in the form of Young's modulus, deflection at maximum load and percentage of the strain at break. Young's modulus also known as the

tensile modulus that measures of the stiffness of an elastic material. Figure 3.20 illustrates how the amount of HC affects the Young's modulus of the films. The value of Young's modulus was decreasing as the sugarcane bagasse HC content is increasing. It means the material property is changing from stiff to elastic as the HC content increases. In reverse, deflection and % strain at break values of the films were increasing as the HC content increasing.

3.2 Southern yellow pine

3.2.1 Introduction

Southern yellow pine is native to the Southern United States. Southern yellow pine is referred to a group of species which include loblolly, longleaf, shortleaf and slash pines. They are mainly used in dimensional lumber and plywood which is extensively used in home construction, paper manufacturing and treated wood products including decking, utility poles, flooring and paneling (Weimann, 2010).

3.2.2 Chemical composition analysis

Chemical analysis of the southern yellow pine was conducted according to the NREL/TP-510-42618 laboratory procedure (Sluiter et al., 2008). Total HC content was 27.17 g of 100g of oven dried biomass. Southern yellow pine HC mainly consists of mannose (43.01%), xylose (31.29%), galactose (16.23%), arabinose (9.38%) and a minute amount of other sugars (Table 3.9).

Table 3.9 Chemical composition of oven dried southern yellow pine.

Composition of dry pine (g/100g)	
Glucose	44.75
Xylose	8.5
Galactose	4.41
Arabinose	2.55
Mannose	11.71
Lignin	26.47
Ash	1.01

3.2.3 Optimizing the extraction condition

Hydrothermal extraction technique was employed for extraction of southern yellow pine HC. Similar to sugarcane bagasse HC extraction, three parameters have been studied for determining the optimum condition. The effect of time and temperature was determined first followed by studying the effect of pressure. The ratio of biomass to pretreatment solution was kept constant in all experiments (1:10) and no catalyst was added. Since mannan is the main HC in softwood (Ebringerová and Heinze, 2000; Sjöström, 1993), the optimum condition for extraction of HC was evaluated based on mannose content.

3.2.3.1 Effect of time and temperature on the extraction of southern yellow pine HC

Figure 3.21 shows the result of hydrothermal extraction of southern yellow pine HC. Extraction was conducted at 170 ^{o}C and 1MPa pressure for a time ranging from 10 to 50 min. The result exhibits that the yield of the HC increased with the increase of the extraction time. The highest yields for the extracted mannose, xylose and galactose were obtained at 50 min. The yields of extracted sugars were 70.17%, 41.10% and 49.70% (dry basis) for mannose, xylose and galactose, respectively. In the case of arabinose, an opposite trend was observed. The difference in the yield of glucose between 10 and

50 min was not significant. Since the target of this work is to determine an inexpensive technique to modify the extraction process, hydrothermal extraction at 170 ^{o}C was not continued more than 50 min.

At 180 ^{o}C, the yield of mannose kept increasing in small increments as the extraction time increased (Figure 3.22). The yields of xylose, arabinose and galactose started to decline after 30 min. It was observed as the temperature increased the amount of mannose increased. Since mannose is the main content of southern yellow pine HC, the extraction was conducted at 190 ^{o}C to determine the possibility of increasing the yield of mannose over a short extraction time.

The extraction at 190 ^{o}C was conducted at the same pressure but for shorter reaction time ranging from 10 to 40 min. Figure 3.23 shows that the HC content start decreasing as extraction time increased. The highest yield of mannose (79.86%), galactose (46.98%), and arabinose (35.35%) was obtained after 10 min of extraction. In the case of xylose, the yield after 10 min (34.82%) was slightly lower than the yield after 20 min (38.02%). The yield of glucose was relatively stable at all studied times and this could be related to the moderate extraction conditions that were not long enough to alternate the cellulose structure.

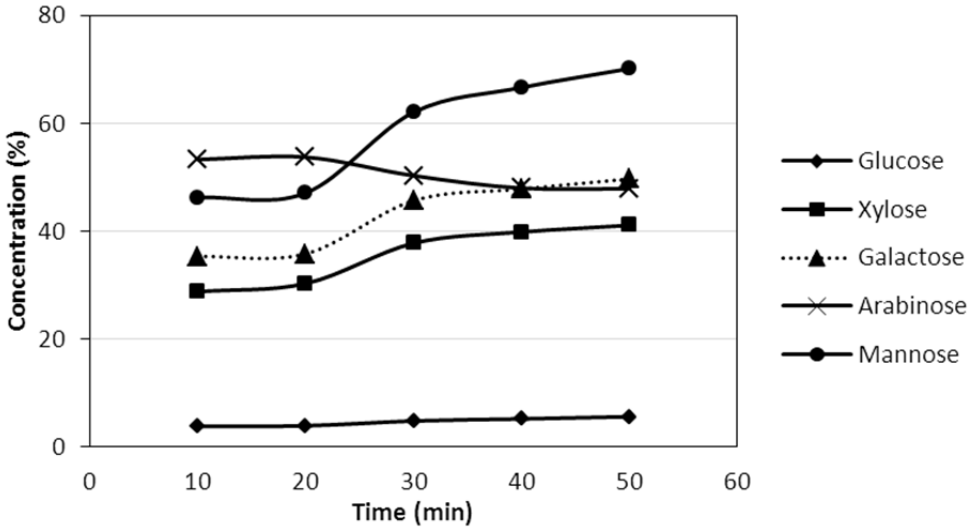


Figure 3.21 Effect of the time on the yield of southern yellow pine HC extracted at 170 °C.

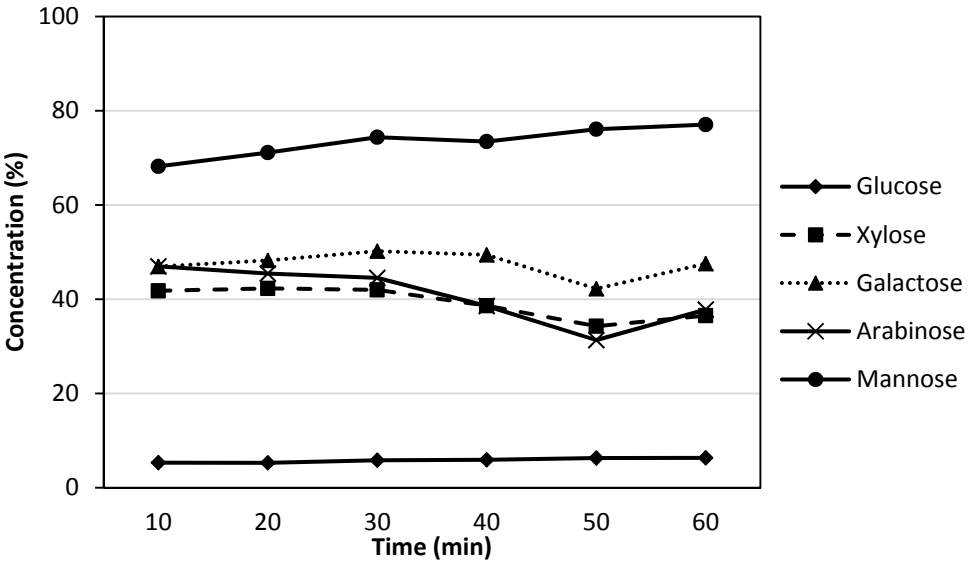


Figure 3.22 Effect of the time on the yield of southern yellow pine HC extracted at 180 °C.

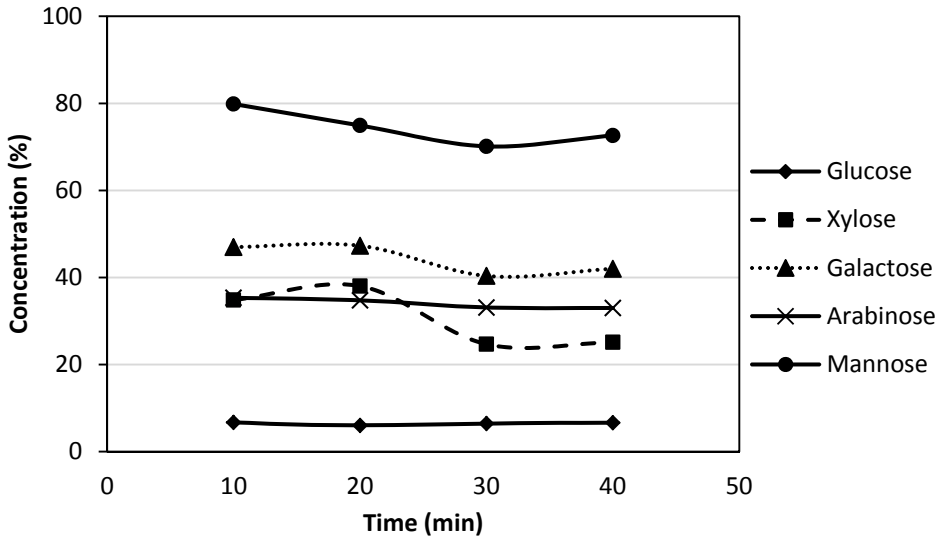


Figure 3.23 Effect of the time on the yield of southern yellow pine HC extracted at 190 ^{o}C.

In addition, to choose the optimum condition, couple of parameters such as temperature or time effect on the pH value of the aliquot, temperature and time effect on the concentration of sugar degradation compounds and temperature and time effect on the concentration of the soluble lignin was considered as well.

Figures 3.24 and 3.25 relate the effect of extraction time or temperature to the pH value of the aliquots. An identical trend was observed for the pH value of aliquots when the temperature or time of the extraction increased. This was due to the cleavage of acetyl group moiety into acetic acid (Carvalheiro et al., 2008) resulting in low pH values.

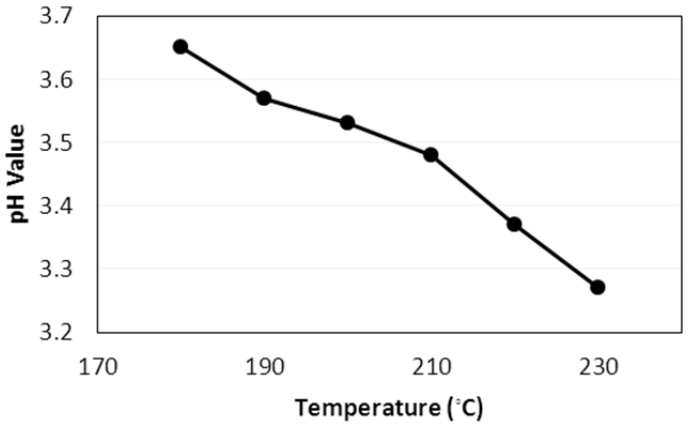


Figure 3.24 Effect of temperature on the pH values of southern yellow pine HC extracts.

At all temperatures the extraction time were 60 min.

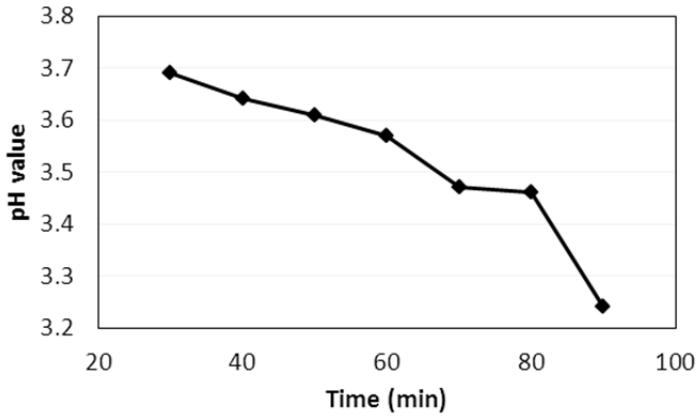


Figure 3.25 Effect of time on the pH values of southern yellow pine HC extracts.

At all time the extraction temperature was 180 ^{o}C and the pressure was 1 MPa.

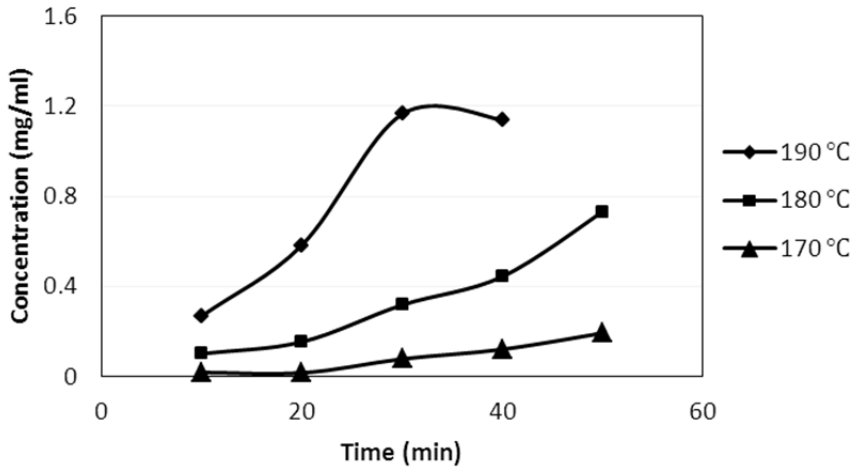


Figure 3.26 Effect of time and temperature on HMF content of southern yellow pine HC extract.

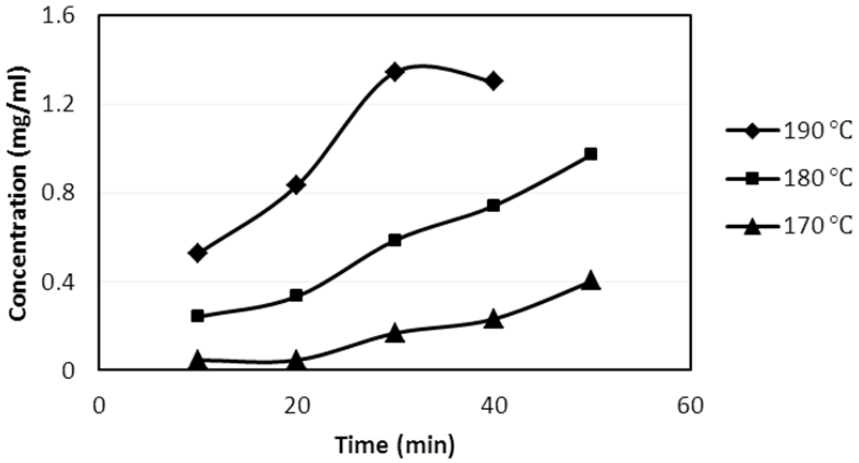


Figure 3.27 Effect of time and temperature on the furfural content of southern yellow pine HC extract.

Figures 3.26 and 3.27 illustrate the effect of temperature and time on the amount of furfural and HMF produced from the degradation of pentoses and hexoses of HC. The concentrations of both furfural and HMF were increased as the extraction time and temperature increases. This is because at high temperature or at prolonged extraction

time, the production of acetic acid increases and further causes the hydrolysis and degradation of monomeric sugars to furfural and HMF. Since the objective is to isolate high HC content, the degradation into furfural and HMF was not favored. Therefore, the condition with low furfural and HMF content was considered as an optimum pretreatment condition.

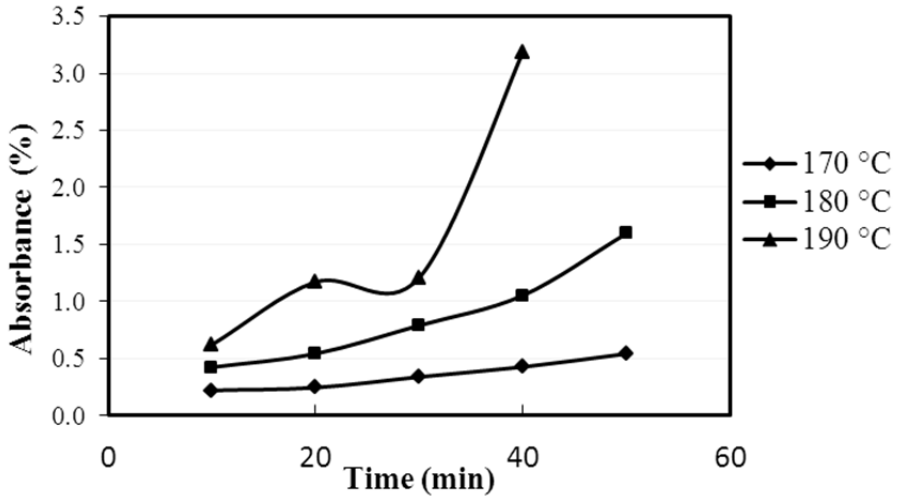


Figure 3.28 Effect of time and temperature to the amount of soluble lignin during the hydrothermal extraction of southern yellow pine HC.

Figure 3.28 depicts the effect of the time and temperature to the extractability of soluble lignin. Lignin content was determined by scanning the aliquot on the UV-Vis spectrophotometer at the wavelength 200-400 nm. The major absorption for lignin occurred at 275-278 nm (Fukushima and Hatfield, 2001; Yoshida et al., 2002). As the time and temperature increased, the absorption of soluble lignin increased as well. According to Lambert's law the absorption percentage is directly proportional to the concentration (Skoog et al., 2007). It was also noted that as the temperature increased, the increment of the lignin content is increased. For example, at 170 ^{o}C the absorbance at 10,

20, 30, 40 and 50 min was 0.22%, 0.24%, 0.34%, 0.43% and 0.54%, respectively. At 190 °C the absorbance was 0.62%, 1.17%, 1.21% and 3.18% at 10, 20, 30 and 40 min, respectively. In fact, the increment of the absorption value was much higher at 190 °C than the absorbance increment at 170 and 180 °C during the same extraction time.

From the results depicted in Figures 3.21-3.23, three conditions 170 °C, 50 min, 180 °C, 50 min and 190 °C, 10 min were considered as the optimum condition for southern yellow pine HC extraction. The yields of mannose at the above optimum conditions were 70.17%, 77.05% and 79.86%, respectively. The yields of degradation products at 170 °C, 50 min were 0.19 mg/ml for HMF and 0.40 mg/ml for furfural, at 180 °C, 50 min were 0.74 mg/ml for HMF and 1.01 mg/ml for furfural, and at 190 °C, 10 min yields were 0.27 mg/ml for HMF and 0.53 mg/ml for furfural (Figures 3.26 and 3.27). Finally, soluble lignin absorbance at the above conditions was 0.54%, 1.59% and 0.62%. Based on above results, the optimum extraction time and temperature was considered to be 190 °C and 10 min (Figure 3.29).

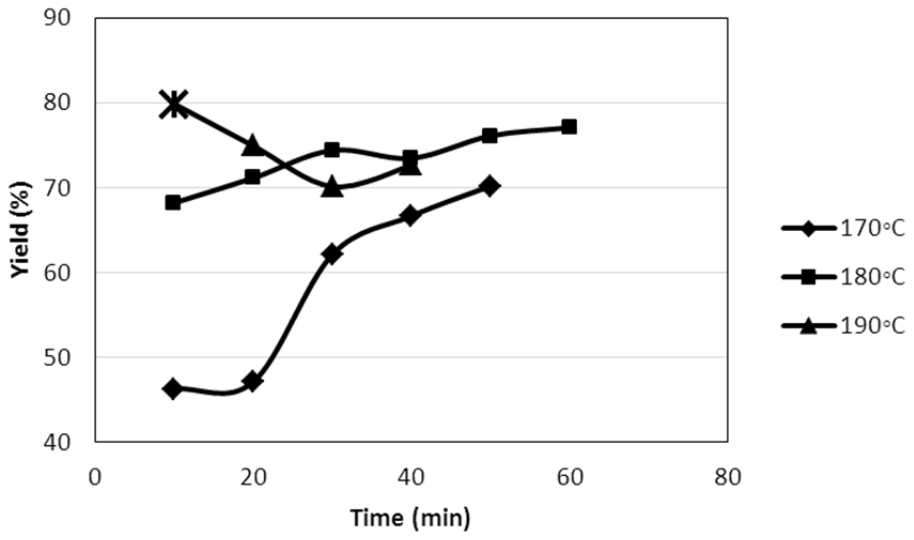


Figure 3.29 Optimum condition for extraction of southern pine HC.

3.2.3.2 Effect of applied pressure

Effect of the pressure on extractability of HC was conducted at 190 ^{o}C for 10 min at pressures from 1 to 5 MPa. Figure 3.30 depicts the pressure effect on the yield of HC. At 2 MPa the yield of mannose increased compared to the yield of mannose at 1 MPa. Further pressure increase caused a slight decrease in the yield of total HC. It appears that the pressure change does not significantly affect the extractability of the sugars in the chosen range of pressure. Yields of mannose at pressures 1, 2, 3, 4, and 5 MPa were 79.86%, 87.78%, 82.42%, 82.25% and 76.38%, respectively. In the case of sugar degradation compounds, as the pressure increases the production of toxins decrease (Figure 3.31). At 1 MPa the concentrations of HMF and furfural were 0.42 mg/ml and 0.56 mg/ml. By increasing the pressure to 2, 3, 4 and 5 MPa, the yield of HC degradation products slightly decreased. For example, at 2, 3, 4 and 5 MPa the concentration of HMF was 0.14, 0.12, 0.11 and 0.01 mg/ml and the concentration of furfural was 0.23, 0.21, 0.18 and 0.20 mg/ml.

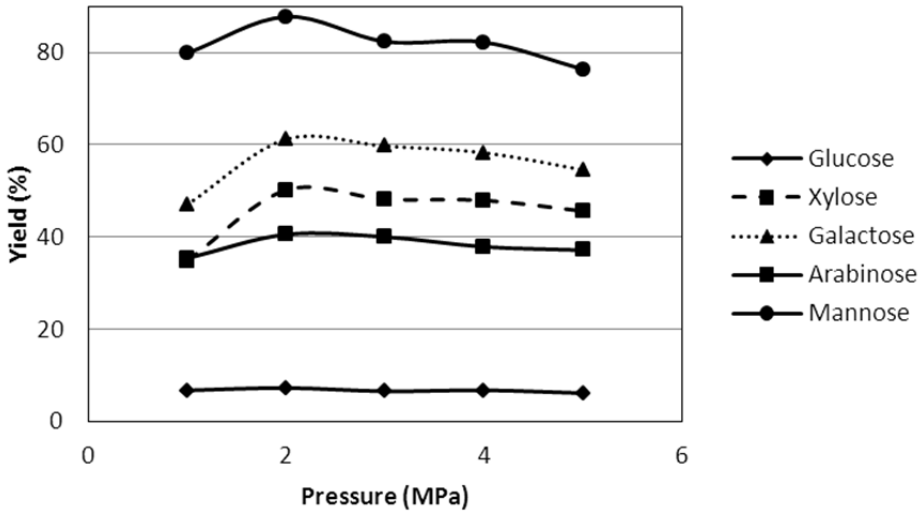


Figure 3.30 Effect of pressure to the yield of southern yellow pine HC.

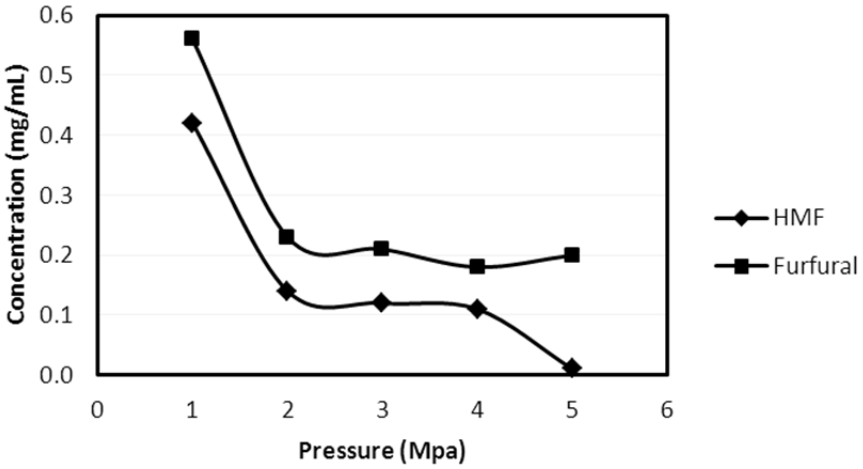


Figure 3.31 Effect of pressure to the yield of HMF and furfural production during the hydrothermal extraction of southern yellow pine HC.

In summary, the effect of temperature, time and pressure on the extraction of mannose revealed that the maximum amount of mannose can be obtained at 190 ^{o}C 10 min when the applied pressure was 2 MPa. Therefore, this condition was chosen as the optimum condition for extraction of HC from southern yellow pine.

3.2.4 Isolation of HC using membrane filtration

3.2.4.1 Filtration of pine wood HC

The feed solution prepared at the optimum condition contained 16.01 mg/mL HC. The average molecular weight (MW) was 19500 Da (Tables 3.10, 3.11 and 3.12). Based on the HPLC data of the feed solution, it was expected to isolate totally 57.63 g of HC. According to the dry content percentage, it was expected to isolate 54 g of HC. The concentration of permeate from ETNA10PP membrane was 14.22 mg/mL HC with 3740 Da average MW. The concentration of the retentate reached to 21.47 mg/mL and average MW was 9650 Da. A similar trend was observed during the filtration process with ETNA01PP and NF membranes. (Note: Permeate from ETNA10PP was used as a feed solution for ETNA01PP membrane filtration. Permeate from ETNA01PP was used as a feed solution for NF membrane filtration as well). The concentration of HC in permeate of ETNA01PP was 9.95 mg/mL and the concentration of HC in retentate was 19.62 mg/mL. The average MW of permeate from ETNA01PP membranes was 2390 Da and the MW for retentate was 7810 Da. In the case of NF, the concentration of permeate was 0.64 mg/mL and for retentate it was 26.04 mg/mL. The average MW of permeate was 330 Da and the MW for retentate reached 4660 Da.

Table 3.10 Southern yellow pine HC content in feed, permeate and retentate fractions of three different membranes.

	Feed		Permeate		Retentate	
	Dry content %	Total sugars* mg/mL	Dry content %	Total sugars* mg/mL	Dry content %	Total sugars* mg/mL
ETNA 10000PP	1.5	16.01	1.24	14.22	2.17	21.47
ETNA 1000PP	1.24	14.22	0.94	9.95	1.91	19.62
NF	0.94	9.95	0.014	0.64	2.6	26.04

*HC content was determined by HPLC system.

Table 3.11 Total yield of southern yellow pine HC determined based on dry content or HPLC data.

	Feed		Permeate		Retentate	
	Dry content (g)	HPLC sugar* (g)	Dry content (g)	HPLC sugars* (g)	Dry content (g)	HPLC* (g)
ETNA 10000PP	54.00	57.63	39.15	44.89	12.80	12.66
ETNA 1000PP	39.15	44.89	25.15	26.62	9.45	9.72
NF	25.15	26.62	0.28	1.28	16.90	16.93
Total					39.16	39.31

*HC content calculated based on HPLC result.

Table 3.12 Average molecular weight of membrane filtration fractions of southern yellow pine HC extract.

	MW (Da)
Feed	19500
ETNA10PP Permeate	3740
ETNA10PP Retentate	9650
ETNA01PP Permeate	2390
ETNA01PP Retentate	7810
NF Permeate	nd
NF Retentate	4660

nd- not determined

All retentates from the tested membranes were collected separately and freeze dried. The weights from ETNA10PP, ETNA01PP and NF membranes were 7.57 g 15.06 g and 15.00 g, respectively (Table 3.13). A total of 37.63 g of HC were isolated by hydrothermal extraction and membrane filtration techniques. According to the HPLC sugar analysis, ETNA10PP, ETNA01PP and NF retentate contained 12.66 g, 9.72 g and 16.93 g of HC, respectively. It was expected to isolate a total of 39.31 g of HC. On the other hand, according to the dry content percentage, it was expected to obtain 39.15 g of

HC, with 12.80 g from ETNA10PP retentate, 9.45 g from ETNA01PP retentate and 16.90 g of HC from NF retentate.

Table 3.13 Calculated and experimentally obtained southern yellow pine HC.

	Dry content (g)	HPLC (g)	Experiment (g)
ETNA10PP	12.80	12.66	7.57
ETNA01PP	9.45	9.72	15.06
NF	16.90	16.93	15.00
Total	39.15	39.31	37.63

3.2.4.2 Membrane filtration efficiency

The efficiency of membranes was determined by pure water flux (PWF) testing. Figures 3.32, 3.33 and 3.34 exhibit the PWF of ETNA10PP, ETNA01PP and NF membranes before and after the filtration, respectively. PWF before filtration in ETNA10PP and NF membranes was higher than the PWF after the filtration, meaning the performance of membranes diminished due to the fouling. In the case of ETNA01PP membrane, the PWF before and after filtration was almost the same (Figure 3.33) indicating a small fouling effect. Also, it can be seen that the PWF of ETNA10PP was higher than the PWF of ETNA01PP and PWF of ETNA01PP was higher than the PWF of NF membrane. However, fouling of the ETNA10PP was relatively higher than the fouling of ETNA01PP and the fouling of ETNA01PP was higher than the one of NF membrane.

In summary, using different membrane with different sizes for concentration and isolation purposes is beneficial.

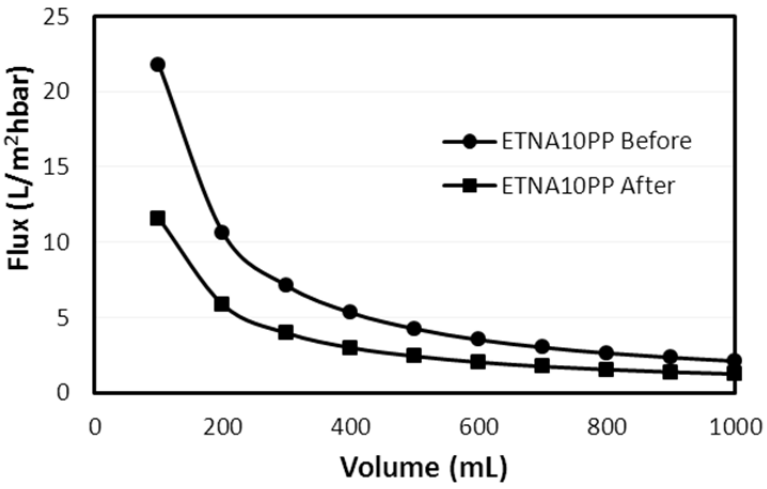


Figure 3.32 PWF of ETNA10PP membrane before and after filtration of southern yellow pine HC extract.

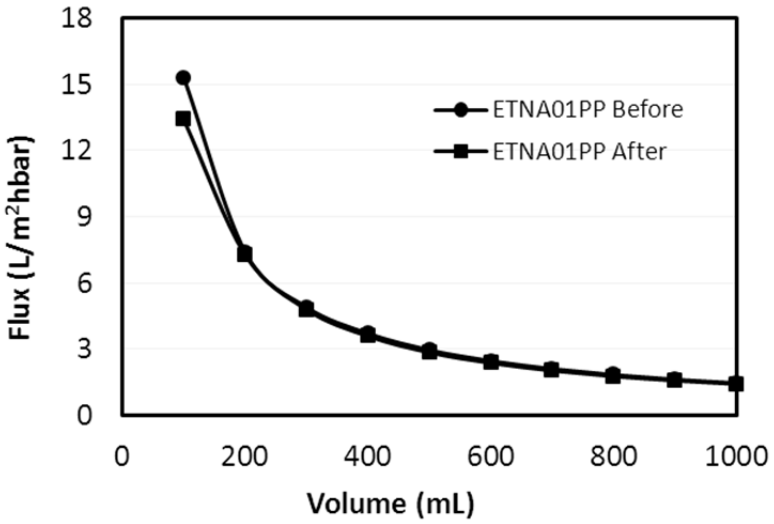


Figure 3.33 PWF of ETNA01PP membrane before and after filtration of southern yellow pine HC extract.

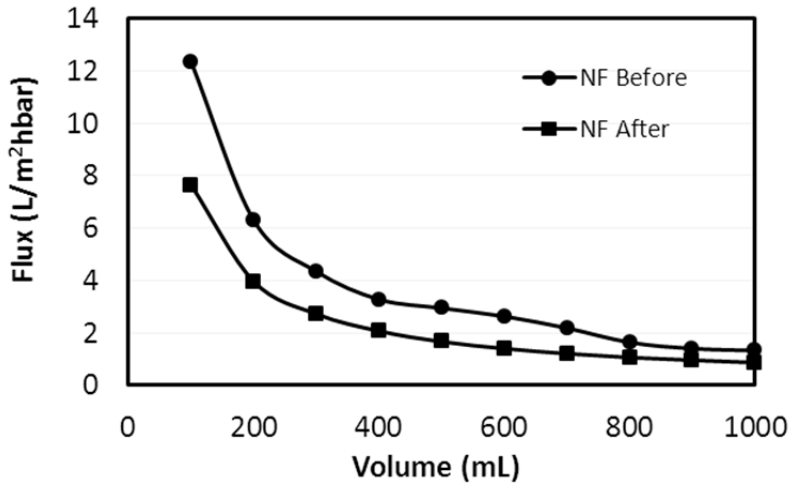


Figure 3.34 PWF of NF membrane before and after filtration of southern yellow pine HC extract.

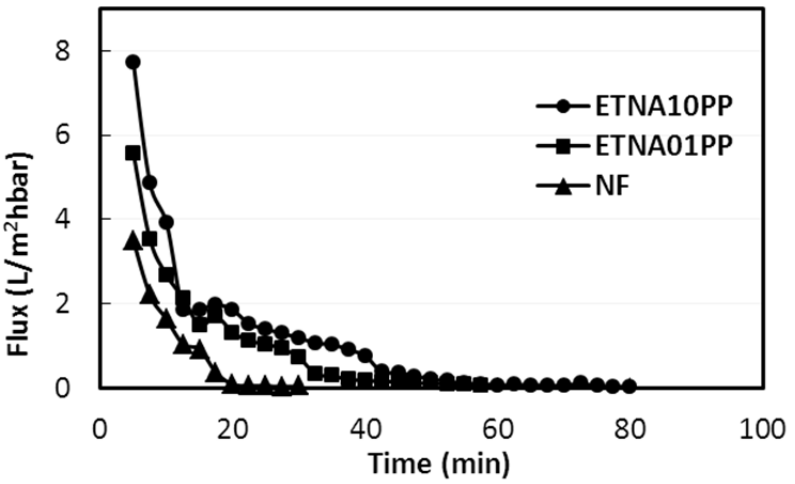


Figure 3.35 Flux of different membranes during the extraction of southern yellow pine HC extract.

The performance of the membrane during the concentration of southern yellow pine HC extract is depicted in the Figure 3.35. Flux of the membranes was accordingly, ETNA10PP > ETNA01PP > NF and the fouling of the membranes took place in same order.

3.2.4.3 Mass Balance

Southern yellow pine composition has changed considerably after hydrothermal extraction. HC content decreased from 27.17% before pretreatment to 9.41% after the pretreatment step (Table 3.14). Also, glucose and lignin contents were increased from 44.75 g and 26.47 g to 50.94 g and 36.28 g after the pretreatment step.

Table 3.14 Chemical composition of southern yellow pine before and after hydrothermal pretreatment.

Composition of dry pine (g/100g)		
	Before treatment	After treatment
Glucose	44.75	50.94
Xylose	8.5	2.11
Galactose	4.41	0.60
Arabinose	2.55	2.17
Mannose	11.71	4.53
Lignin	26.47	36.23
Ash	1.01	0.11

3.2.5 Characterization of isolated southern yellow pinewood HC

3.2.5.1 FT-IR

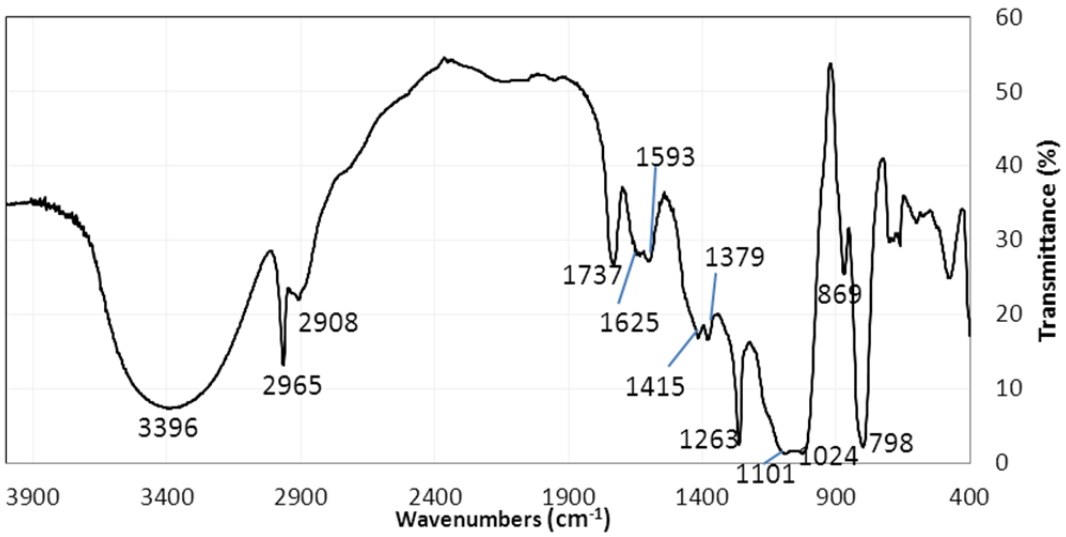


Figure 3.36 FT-IR spectrum of isolated southern yellow pine HC.

FT-IR spectroscopy was used to identify the main functional groups of isolated HC. The spectrum was collected from 4000 to 400 cm^{-1} wavenumber (Figure 3.36). At 3396 cm^{-1} the spectrum has an extensive band coming from –OH stretching vibration of HC and water. Another prominent peak at 2965 and 2908 is come from C-H stretching vibration. An intensive band at 1737 cm^{-1} is represented the free carbonyl stretch assigned to the acetyl, glucuronic acid and ferulic ester groups of polysaccharides. The band at 1625 cm^{-1} represents the presence of absorbed water. At 1593 cm^{-1} the absorbance was attributed to the C-H deformation and at 1379 cm^{-1} the band was related to –CH bending vibration. On the other hand, the band at 1415 cm^{-1} represented the $–CH_2$ stretching vibration. A strong signal at 1263 cm^{-1} was indicative of C-O vibration of carboxylic acids, which was due to the 4-O-methyl-a-D-glucuronic acid side group. In the region

1200-800 cm^{-1}HC polysaccharides gives specific band. Nikonenko et al. (2005) reported that the peaks at the range 1175-1140 cm^{-1} represent HC at different conformational states. Also, intensive bands at 1101 and 1024 cm^{-1} is derived from –OH stretch in glucose units at mixture of GGM and arabinoglucuronoxylan in softwood. Sharp intense peak at 869 cm^{-1} represents C-1 group frequency or ring frequency that characterizes β-D-glycosidic linkage between sugar units (Xue et al., 2012).

3.2.5.2 ^{13}C NMR study of isolated southern yellow pine HC

Generally, the ^{13}C NMR spectrum (Figure 3.37) showed signals of several different carbonyl carbon signals at δ 173.69-172.43 ppm, anomeric carbon (C-1) signals in the range from δ 104.44 ppm to 96.56 ppm showing the presence of mannopyranose and glycopyranose units and C-2 to C-5 carbon signals in the range from δ 85 to 61 ppm. In addition, the lignin impurity in the sample showed signals at δ 173.69 and 56 ppm from carbonyl and methoxyl groups of lignin. Also, acetyl groups attached to mannose showed signals at δ 20.70-20.47 ppm. There were two extensive peaks representing the presence of isopropanol. It was used to prevent extensive water absorption, because southern yellow pine HC were sensitive to the moisture becoming a gooey and sticky substance after exposure to the air for an elongated time. Assignments of the signals are listed on the Table 3.15. The main signal in the spectrum assigned to the C-atoms of →4)-β-D-Man*p*-(1→ and →4)-β-D-Glc*p*-(1→. Peaks at δ 100.06, 71.55, 72.85, 76.43, 75.60 and 60.38 ppm are attributed to the C-1, C-2, C-3, C-4, C-5 and C-6 of 4- linked β-D-Man*p* units. Capek et al. (2000) reported that mannose and glucose units give several sets of signals due to the positions at the backbone such as at reducing or non- reducing terminal or at internal positions. An intensive peak at δ 96.71 represents the β-D-Man*p*

reducing end (Capek et al., 2000; Willför et al., 2003). Assigning those type of peaks based solely on ^{13}C NMR is complicated. Thus the ^{13}C NMR assignments were made based on literature data (Capek et al., 2000; Escalante et al., 2012; Hannuksela and Hervé du Penhoat, 2004; Owen and Thomas, 1989; Willför et al., 2003).

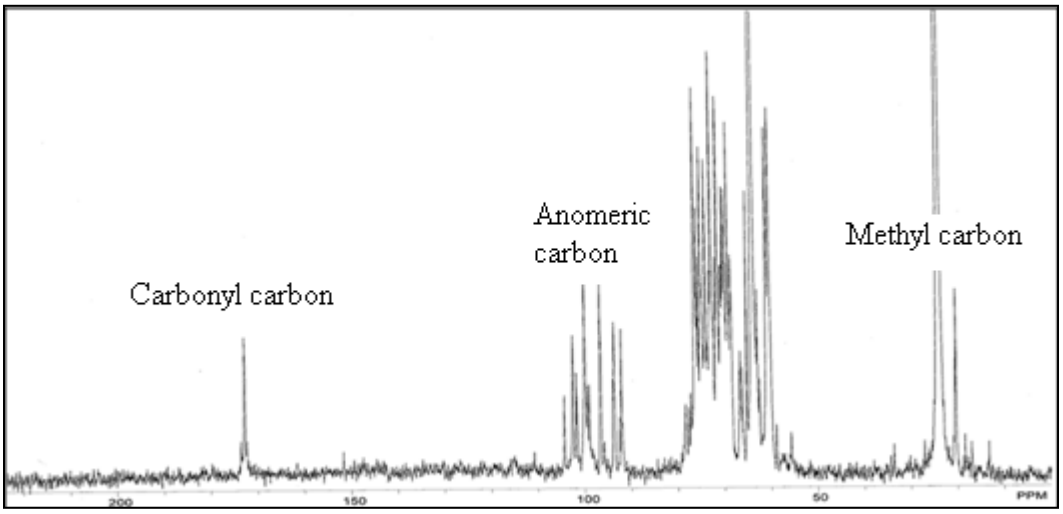


Figure 3.37 ^{13}C NMR spectrum of isolated southern yellow pine HC.

Table 3.15 ^{13}C NMR data of the isolated southern yellow pine HC (δ/ppm).

Residue	C-1	C-2	C-3	C-4	C-5	C-6
Man (n.r.)	100.065	71.55	72.85	76.43	75.60	60.38
Man (r.)	96.70					
Xyl	102.55	nd	75.04	75.60	61.02	nd
Glc	104.43	74.09	75.07	78.50	77.50	62.60
Gal	101.67	75-71	75-71	75-71	nd	60.38
Ara	111.20	81.7	79.30	84.50	60.38	

According to literature, acetyl groups are attached to the mannopyranose units at C-2 or C-3 positions (Capek et al., 2000; Lundqvist et al., 2002; Willför et al., 2003). In this case, methyl carbons of acetyl groups attached to the mannan backbone at C-2 or C-3 positions gave signals at δ 20.70-20.47 ppm. The ratio of 2-O-Ac-Man to 3-O-Ac-Man was about 1:1.6.

The signals at lower intensities at δ 104.44, 74.09, 75.07, 78.50, 77.50 and 62.60 ppm are assigned to the C-1, C-2, C-3, C-4, C-5 and C-6 of the non-reducing β-D-Glc*p* units. For the galactose units, a weak signal at δ 101.666 can be assigned to anomeric carbon of β-units. Willför et al. (2003) reported the presence of β-Gal*p* units in softwood (Norway spruce). Lundqvist et al. (2002) reported, softwood (Norway spruce) GGM contains only α-Gal*p* units. However, in the current study, the signal around δ 107 ppm that can be assigned to α-Gal*p* units was not detected.

A medium intensity signals at δ 104.44, 75.044, 75.604 and 61.02 assigned to C-1, C-3, C-4 and C-5 of β-D-Xyl*p* units. Based on FT-IR and ^{13}C NMR assignments the isolated southern yellow pine HC mainly consists of galactoglucomannan. In addition, considerable amount of glucuronoxylan exists in southern yellow pine HC.

3.2.6 Film preparation

Films were prepared from the isolated HC with various HC content using water casting method (Table 2.2). Mixture of southern yellow pine HC, CMC and sorbitol formed a homogeneous suspension, which upon drying created similar, homogeneous and transparent films. It was observed when the content of the CMC decreases the properties of the films slightly changed, namely it become more moisture sensitive and flimsy. The color of the film was slightly yellowish (Figure 3.38). This color is possibly from lignin

content in HC. Thickness of the prepared films was slightly different from each other and it was around 100 μm.

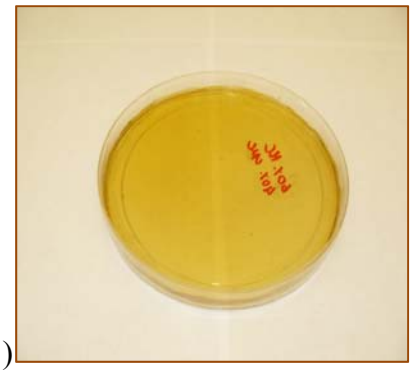
1)  2) 

Figure 3.38 Film made from southern yellow pine HC (1) film casting; (2) film after drying.

3.2.7 Film testing

3.2.7.1 Water absorption

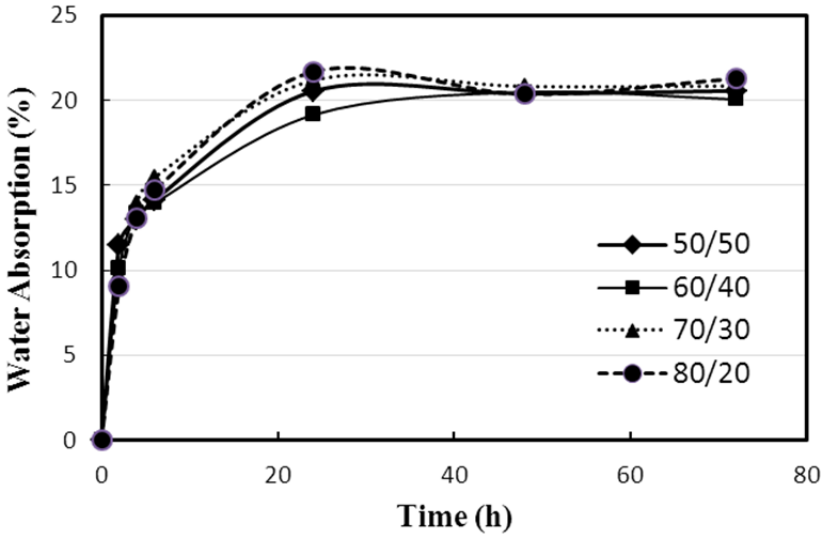


Figure 3.39 Moisture absorption feature of southern pine HC-based films at 55% RH.

Water absorption isotherm of the southern yellow pine HC films at 55% RH and 25°C environment is presented in Figure 3.39. The water content differences of the films were not very large between the samples. Generally, water uptake of the films was rapid in the first 8 h. It continued increasing and reached the absorption maximum at 24 h. The water uptake was small during 48 and 72 h. The increment of absorption tended to decrease and further stabilize. The amount of absorbed water was directly proportional to the amount of HC included in the film. Accordingly, the film containing 80% HC absorbed the highest amount of water, and the films containing lower amounts from HC (50%) absorbed the least amount of water.

3.2.7.2 Water vapor permeability

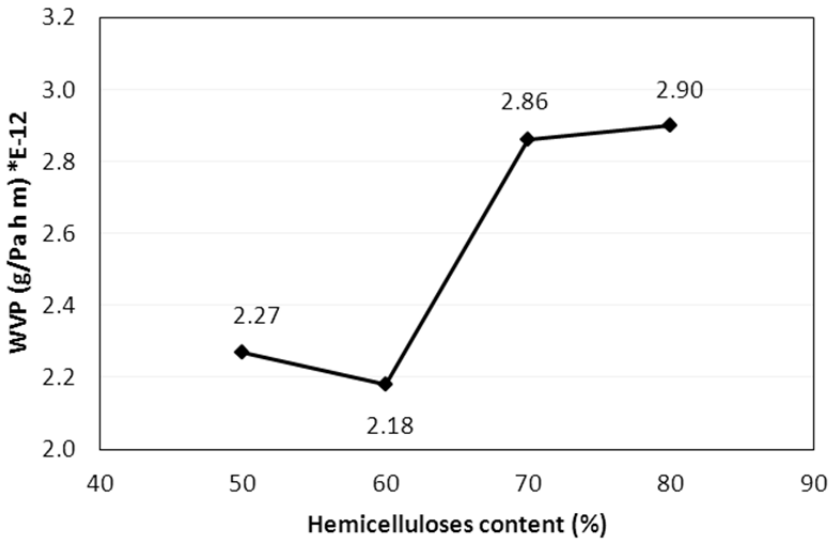


Figure 3.40 Water vapor permeability of southern yellow pine HC-based films.

Figure 3.40 exhibits the water vapor permeability (WVP) trend of southern yellow pine HC and CMC system with varying ratio of southern yellow pine HC and CMC content. The film containing 60/40 % of HC / CMC shows lower ($2.18*e^{-12}$ g/Pa h m) water vapor permeability than that in the films containing 50/50, 70/30 and 80/20 HC and CMC. It is possible that the ratio of the blend was at an optimum that permeates less water vapor. It appears that increasing the amount of HC increases the water vapor permeability. A similar trend was reported in Ghanbarzadeh et al. (2011) and Ma et al. (2008) studies. The film with ratio 50/50 HC/CMC had slightly higher WVP than the film 60/40 HC/CMC had. However, when the content of HC increased to 70 or 80%, the WVP of the films began to increase. On the other hand, as Goksu et al. (2007) noted, the WVP is an important parameter in food packaging films. Depending on the purpose of the use, the desirability of the WVP of the films needed to be different. For example, for

vegetable packaging high water vapor permeable material is desired. However, for food materials those are needed to be kept dry but stand in humid environment require low WVP films. In general, the films produced from southern yellow pine HC have relatively low WVP than the films in the reference literature (Ghanbarzadeh et al., 2011; Mikkonen et al., 2012). It can be explained by the presence of a small amount of lignin content, which provided stronger interactions between HC (Goksu et al., 2007).

3.2.7.3 Oxygen permeability

Oxygen permeability (OP) data of the southern yellow pine HC films are shown in Table 3.16. Southern yellow pine HC /CMC film with ratio 70/30 had the lowest value of oxygen permeability 0.007570 cc µm / (m^2 day kPa). The film with 60% had relatively low oxygen permeability 0.040923 cc µm / (m^2 day kPa) than the films totally made from CMC. It implies that the southern yellow pine HC improves the oxygen permeability property of the film. Compared to the measured oxygen permeability values of some commercialized polymers such as ethylene vinyl alcohol (0.1-12 cc µm / (m^2 day kPa) at 0-95% RH) and low-density polyethylene (1870 cc µm / (m^2 day kPa) at 50% RH) (McHugh and Krochta, 1994), the oxygen permeability values of southern yellow pine HC based films at the above ratios had comparable values. This indicates that the southern yellow pine HC films are suitable as an oxygen barrier.

Table 3.16 Oxygen permeability (OP) and oxygen transmission rate (OTR) of southern yellow pine HC-based films at 35% relative humidity.

HC/CMC (%)	Avg. thickness (µm)	OTR (cc/(m^2 day))	OP (cc µm /(m^2 day kPa))
0/100	97.0	0.0095	0.009415
50/50	104.5	1.0715	1.047076
60/40	97.0	0.0395	0.040923
70/30	116.5	0.0065	0.007570
80/20	108.5	0.1365	0.145739

3.2.7.4 Tensile strength

Table 3.17 Mechanical properties of southern yellow pine HC-based films.

HC/CMC (%)	Young's modulus (MPa)	Deflection at max load (mm)	% Strain at break
0/100	794,90	4,85	16,17
50/50	17,42	20,55	68,62
60/40	12,42	27,06	86,43
70/30	3,88	34,34	116,94
80/20	0,61	78,01	318,58

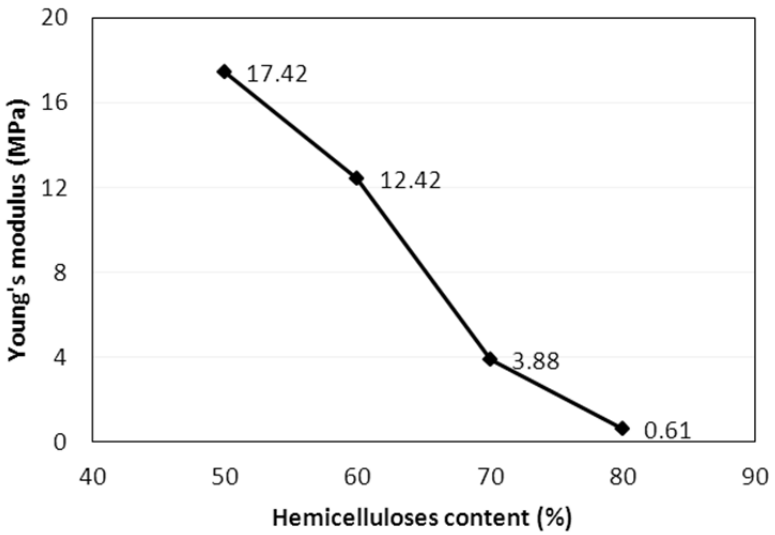


Figure 3.41 Tensile strength of southern yellow pine HC-based films.

Note: 0% southern yellow pine HC film's Young's modulus was not included in the graph because the value was incomparably high.

The mechanical properties of the films were evaluated using tensile testing at 50% RH and ambient temperature. Table 3.17 shows the results in the form of Young's modulus, deflection at maximum load and percentage of the strain at break. Young's modulus also known as the tensile modulus that measures of the stiffness of an elastic material. Figure 3.41 illustrates how the amount of HC affects the Young's modulus of the films. The value of Young's modulus decreased as the southern yellow pine HC content increased. Therefore, the material property was changing from stiff to elastic as the HC content increased. By contrast, deflection and percentage of strain at break values of the films increased as the HC content increased.

CHAPTER IV

CONCLUSION

4.1 Sugarcane bagasse

Extraction of sugarcane bagasse HC was conducted by hydrothermal extraction technique at relatively mild conditions of time, temperature and pressure effects. The optimum condition for extracting HC from sugarcane bagasse was achieved at 180 ^{o}C for 30 min and 1MPa pressure. At this condition, the yield of xylan reached 84% and the concentration of the sugar degradation products such as HMF and furfural were minimal 0.95 and 0.07 mg/mL, respectively.

Isolation and concentration of extracted HC was approached by membrane filtration technique. The content of HC in 100 g dry biomass was reduced from 26.75 g to 8.39 g. Meantime, the content of glucose and lignin was increased from 45.28 g and 24.15 g to 51.89 g and 35.16 g, respectively. FT-IR and ^{13}C NMR spectrum of isolated HC showed the typical spectrum of xylan-based HC which were consistent with references. The FT-IR spectrum revealed typical xylan bands in the 1200 – 1000 cm^{-1} region. Also, a sharp and intensive band at 898 cm^{-1} represents C-1 group frequency or ring frequency that characterize β-glycosidic linkage between the sugar units. An intensive band at 1735 cm^{-1} represented the carbonyl stretch showing the existence of acetyl, glucuronic acid and ferulic ester groups of polysaccharides. Another extensive band at 3400 cm^{-1} which is from –OH stretching vibration suggested the incidence of HC.

^{13}C NMR spectrum showed five strong signals at δ 101.49, 76.18, 73.47, 72.53 and 62.796 ppm, which are depicted C-1, C-4, C-3, C-2 and C-5 of 4)-β-d-Xyl*p*- (1→ residue substituted at 2 and 3 positions with -OAc and at 4-position 4-O-Me-GlcA. Signals δ 102.49, 75.07, 75.07 and 65.05 ppm are represents C-1, C-2, C-2, C-4 and C-5 carbons of →4)-β-d-Xyl*p*- (1→ residue. As a typical glucuronoxylan it was substituted with an OAc group at the C-2 and C-3 position, which can be seen by methyl group carbon signals at *δ* 20.22 and 20.45 ppm. Also, substitution of the HC backbone with glucuronic acid confirmed with existence of an uronic acid signal at δ 173.01 ppm and anomeric carbon signal of GlcA. Moreover, the hydrolysis product of isolated HC revealed that the HC consists mainly from xylan. Thus, it can be concluded that the isolated material is glucuronoxylan.

Properties of the films prepared from isolated HC with addition of CMC in different ratios and sorbitol were evaluated in terms of tensile properties, water absorption, water vapor permeability and oxygen permeability. Young's modulus of the films showed that the stiffness of the films decreased from 794.90 to 3.77 MPa as the HC content in the films increased from 0 to 80%. In contrast, deflection at maximum load and percentage of strain at break were decreasing from 4.85 to 23.86 mm and from 16.17 to 93.22 %, respectively, as HC content in the film increased from 0 to 80%. This implies that the HC important elastic feature to the films which suggests the opportunity of preparing the films with variable elasticity features depending on the requirements of the market. Meantime, HC content influences the moisture absorption and moisture permeability features of the films as well. Test results suggested that the HC is responsible for the moisture absorbing feature. As HC content in the film increased the moisture absorption of the films increased. A similar trend was observed for the WVP of

the films except for the 60/40 HC/CMC film. WVP of current film was slightly lower (3.84 e^{-12} g/Pa h m) than the film containing 50/50 HC/CMC (4.43 e^{-12} g/Pa h m). In case of 70/30 and 80/20 HC/CMC films, the WVP was slightly high (4.55 and 5.05 e^{-12} g/Pa h m, respectively). Importantly, these WVP values were relatively lower than the WVP values of the reference literature films.

The oxygen barrier property of the HC based films is one of the important features of biodegradable films. The OP test showed that the OP of the system with ratio 60/40 CH/CMC had the lowest value 0.005265 cc µm / (m^2 day kPa) than the other films 0/100, 50/50, 70/30, and 80/20 HC/CMC films (0.009415, 0.014606, 1.583212 and 0.642887 cc µm / (m^2 day kPa), respectively). WVP and OP test results suggested that the 60/40 ratio of HC and CMC can be the optimum proportion for making xylan based biodegradable films with relatively low WVP and OP. Therefore, for preparation of HC based films this finding may help.

4.2 Southern yellow pine

Extraction of southern yellow pine HC was conducted by hydrothermal extraction technique at relatively mild conditions with respect to time, temperature and pressure effects. The optimum condition for extracting HC from SYP was achieved at 190 ^oC for 10 min and 2 MPa pressure. At this condition, the yield of mannose reached to 79.85% and the concentration of the sugar degradation products (HMF (0.14 mg/ mL) and furfural 0.23 (mg/mL)) was minimal.

Isolation and concentration of extracted HC was approached by membrane filtration technique. After the hydrothermal extraction the content of HC in 100 g dry biomass was reduced from 27.17 g to 9.41 g. Meantime, the content of glucose and lignin

was increased from 44.75 g and 26.47 g to 50.94 g and 36.28 g, respectively. FT-IR and ^{13}C NMR spectrum of isolated HC showed a typical spectrum of galactoglucomannan which were consistent with references. The FT-IR spectrum revealed typical HC bands in the 1200 –800 cm^{-1} region. Also, a sharp and intensive band at 869 cm^{-1} represented a C-1 carbon frequency or ring frequency that characterizes β-glycosidic linkage between the sugar units. An intensive band at 1737 cm^{-1} represented the carbonyl stretch showing the existence of acetyl, glucuronic acid and ferulic ester groups of polysaccharides. Another extensive band at 3396 cm^{-1} which is from –OH stretching vibration suggests the incidence of HC. A strong signal at 1263 cm^{-1} was indicative of C-O vibration of carboxylic acids, which was due to 4-O-methyl-a-D-glucuronic acid side group.

^{13}C NMR spectrum showed six strong signals at δ 100.06, 71.55, 72.85, 76.43, 75.60 and 60.38 ppm, which are depicted C-1, C-2, C-3, C-4, C-5 and C-6 of mannan in →4)-β-D-Man*p*-(1→ and →4)-β-D-Glc*p*-(1→. The signals at lower intensities at δ 104.44, 74.09, 75.07, 78.50, 77.50 and 62.60 were assigned to the C-1, C-2, C-3, C-4, C-5 and C-6 of the non-reducing β-D-Glc*p* units in →4)-β-D-Man*p*-(1→ and →4)-β-D-Glc*p*-(1→. Existence of galactose units verified with a weak signal at δ 101.67 ppm of anomeric carbon of β-units and the existence of uronic acids was confirmed with the signal at δ 172.43 ppm. Methyl carbons of acetyl groups attached to the mannose backbone at C-2 or C-3 positions gave signals at δ 20.70-20.47 ppm. The ratio of 2-O-Ac-Man to 3-O-Ac-Man was about 1:1.6. A medium intensity signals at δ 104.44, 75.044, 75.604 and 61.02 assigned to C-1, C-3, C-4 and C-5 of β-D-Xyl*p* units. Based on FT-IR and ^{13}C NMR assignments, the isolated southern yellow pine HC consisted mainly of from galactoglucomannan. In addition, a considerable amount of glucuronoxylan exists in southern yellow pine HC.

Properties of the films prepared from isolated HC with addition of CMC (in different ratios) and sorbitol were evaluated in terms of tensile strength properties, water absorption, water vapor permeability and oxygen permeability. Young's modulus of the films showed that the stiffness of the film decreased from 794.90 to 0.61MPa as the HC content in the films increased from 0 to 80%. In contrast, deflection at maximum load and percentage of strain at break decreased from 4.85 to 78.01 mm and from 16.17 to 318.58 %, respectively, as HC content in the film increased from 0 to 80%. This implies that the HC contributed significant input into the elasticity of the films. Meantime, HC content influences the moisture absorption and moisture permeability features of the films as well. Test results suggested that the HC is responsible for the moisture absorbing feature. As HC content in the film increased the moisture absorption of the films is increasing. A similar trend was observed for the WVP of the films except for the 60/40 HC/CMC film. WVP of the current film was slightly lower (2.18 e^{-12} g/Pa h m) than the film containing 50/50 HC/CMC (2.27 e^{-12} g/Pa h m). In case of 70/30 and 80/20 HC/CMC films, the WVP was slightly high (2.86 and 2.90 e^{-12} g/Pa h m, respectively). Importantly, these WVP values were relatively lower than the WVP values of the reference literature films.

Oxygen barrier property of the HC based films is one of the important features of biodegradable films. The OP test shows that the OP of the system with ratio 70/30 CH/CMC had the lowest value 0.007570 cc µm / (m^2 day kPa) than the other films 0/100, 50/50, 60/40, and 80/20 HC/CMC films (0.009415, 1.047076, 0.040923 and 0.145739 cc µm / (m^2 day kPa), respectively.

4.3 Future work suggestions

Production of oxygen barrier material from HC of lignocellulosic materials in food packaging industries appears to be a promising value added product. Based on this study, several ideas for future work have been suggested. For example, prepare films from different MW of HC to determine the effect of MW to the property of the films. It can be conducted by preparing films from the retentate fractions of different cut-off membranes. Also, modify the plasticizers' ratio and HC to plasticizer ratio to improve the property of the film such as water absorption feature. In addition, conduct an economical evaluation of HC based film compared to the petroleum based films.

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